EU COST Action FA0701 Summit meeting

Arthropod Symbiosis: from fundamental research to pest and disease management

St. Pierre d' Oléron, France

12-13 of June, 2012

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June 11th : Arrival day

June 12th

8:30-9:00 **Kostas Bourtzis & Einat Zchori-Fein** (Chair & Vice Chair of the COST Action): Welcome address and summary of COST Action

Honoring Lynn Margulis

9:00-9:40 **Antonio Lazcano:** Symbiosis and evolution: on the origin of cells

9:40-10:00 **Claudio Bandi:** Journeys with a free mind: life, symbiogenesis and our personal memories of Lynn Margulis

10:00-10:25 : coffee break

WP1 Arthropod Symbiont Diversity

10:25-10:40 WG leaders (D**affonchio & Horn**): Overview of WG1

10:40-11:10 **Takema Fukatsu:** Novel experimental model systems for insect symbiosis studies

11:10-11:30 **Koos Boomsma:** On ant symbiotic associations

11:30-11:50 **Patrick Mavingui:** Symbiotic diversity in mosquito vectors from Madagascar and Reunion islands, Indian Ocean

11:50-12:10 **Olle Terenius:** *Thorsellia anophelis* and *Aedes aegypti* symbionts

12:10-12:30 **Rok Kostanjsek:** Recent advances and future perspectives of *Rhabochlamydia porcellionis* research

12:30-12:50 **Hans Breeuwer:** Symbionts in mites

13:00-14:00 : Lunch

WP2 Arthropod-Symbiont Metagenomes

14:00-14:15	WG leaders (**Anderson & Lattore**): Overview of WG2
14:15-14:45	**Serap Aksoy:** Tsetse symbiosis with genus *Wigglesworthia*
14:45-15:05	**Lisa Klasson:** Comparative genomics of insect endosymbionts
15:05-15:25	**Matthias Horn:** '*Candidatus Cardinium hertigii*' - a reproductive manipulator with a role in host nutrition
15:25-15:45	**Alistair Darby:** *Arsenophonus* genomes

15:45-16:00 : coffee break

16:00-16:20	**Fabrice Vavre:** The story of communities affecting communities
16:20-16:40	**Sandy Macdonald:** Nitrogen recycling in the aphid/*Buchnera* symbiosis: the role of the host cell

WP3 Host-Symbiont Interactions

16:40-16:55	WG leaders (**Gross, Hurst & Vavre**): Overview of WG3
16:55-17:25	**Jan van den Abbeele**: *Sodalis glossinidius*, a symbiotic Trojan horse against a tsetse fly transmitted parasite – the trypanosome
17:25-17:55	**Abdelaziz Heddi:** Immunity and symbiosis in weevils

18:00-20:00 : Final MC meeting

20:00 :Dinner

June 13th

8:30-8:50 **Amparo Lattore:** From insect pathogen to obligate symbionts: what's in between?

8:50-9:10 **Carolin Ratzka:** Immune response of the ant *Camponotus floridanus* against pathogens and its obligate mutualistic endosymbiont

9:10-9:30 **Christoph Vorburger:** Symbionts mediate coevolution between aphid hosts and parasitoids

9:30-9:50 **Greg Hurst:** Disease epidemiology in arthropods is altered by the presence of non-protective symbionts

9:50-10:10 **Mathieu Sicard:** Experimental evolution of virulence in a symbiosis: from vertical to horizontal transmission

10:10-10:30 : coffee break

WP4 Symbiont-Based Control Strategies

10:30-10:45 WG4 leaders (**Bandi & Sinkins**): Overview of WG4

10:45-11:15 **Horacio Frydman:** *Wolbachia* as a tool for stem cell biology studies

11:15-11:35 **Daniele Daffonchio**: Is the "multicoloured" microbiome associated with insect vectors of plant pathogens a potential resource for disease control?

11:35-11:55 **Guido Favia:** Mosquitoes-symbionts interactions: from complex relationships to biotechnological applications

11:55-12:15 **Ameur Cherif:** Symbiotic control of honeybee pathogens

12:15-12:35 **Boaz Yuval:** Tephritid symbionts and their applied potential

12:35-12:55 **Tom Groot**: The potential of symbionts in improving biological control agents

13:00-14:10 : Lunch

WP5 Regulations

14:10-14:40	**Jaime Aguilera:** Regulatory aspects and safety considerations in the EU
14:40 15:00	**Maria Alejandra Perotti:** Mites, symbionts and commercial implications
15:00-15:20	**Henk Braig:** Is it mine? Ethical, regulatory and other considerations regarding the manipulation of the human microbiome or human symbionts

15:20-15:55 : coffee break

National projects of COST partners

15:55-16:10	**Liga Jankevica:** Research on arthropod symbiosis in Latvia
16:10-16:25	**Sandra Breum Andersen:** Dynamic Wolbachia prevalence in Acromyrmex leaf-cutting ants

ESRs presenting STSMs

16:25-16:40	STSM Coordinator (**Feldhaar & Wilkinson**): Overview of STSMs
16:40-16:55	**Renate Zindel:** The microbiome of the pest mite *Rhizoglyphus robini*: implications for the pest's status
16:55-17:10	**Davide Sassera:** The genome sequence of *Midichloria mitochondrii* and the origin of mitochondria
17:10-17:25	**Evangelos Doudoumis:** On tsetse flies-*Wolbachia* symbiotic associations
17:25-17:40	**Marta Moreira:** Detection of spiders microbial communities in Madeira Island
17:40-17:55	**Anton Strunov:** Effect of high temperature on pathogenic *Wolbachia* strain wMelPop in *Drosophila melanogaster* brain cells
17:55-18:10	**Daniela Schneider:** Manipulation of mating behavior
18:10-18:25	**Thijs Machtelinckx:** Effects of bacterial endosymbionts on the use of predatory bugs in biological pest control
18:30- 20:00	Poster session

18:30- 20:00 Evaluation meeting (Participants: Prof. Anna Pretova, Dr. Ioanna Stavridou, Prof. Kostas Bourtzis, Dr. Einat Zchori-Fein and External Evaluator)

20:00 : Dinner

June 14th : Departure day

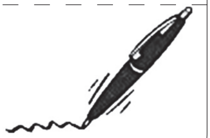
Oral Presentations

EU COST Action FA0701 «Arthropod Symbiosis: from fundamental research to pest and disease management»: a synopsis of a four-year-long journey

Bourtzis K.[1,2] and Zchori-Fein E.[3]

[1]Department of Environmental and Natural Resources Management, University of Western Greece, 2 Seferi St, 30100 Agrinio, Greece; [2] Biomedical Sciences Research Center Al. Fleming, 16672 Vari, Greece; [3]Agricultural Research Organization, Entomology, Newe Ya'ar Research Center 30095 Ramat Yishay Israel - kbourtz@uoi.gr

During the last decades, there has been growing concern in the medical and agricultural communities about the many risks associated with chemical pest and disease control. Certain arthropods are devastating agricultural pests, others are exceptionally efficient vectors of plant, animal and human pathogens. Bacterial symbionts are present in many of these arthropod pests. Manipulation of the symbiotic relationships could provide novel and revolutionary biotechnological means for improving pest control for food production, public health and environment protection. The COST Action FA0701 "Arthropod Symbiosis: from fundamental research to pest and disease management" was funded by the EU with the goal of exploring and harnessing arthropod symbiotic associations, from the level of single genes through genomics to populations, towards the development and commercialization of symbiont-based control strategies (SCS) that are environmentally friendly and have the potential to replace chemical control methods. Major aspects of the progress made during the four years of our Action (26 March 2008 - 25 March 2012) towards achieving these goals will be presented.



Symbiosis and evolution: on the origin of cells

Lazcano A.

Facultad de Ciencias, UNAM, Mexico - alar@ciencias.unam.mx

In 1967 Lynn Margulis published a now classical paper in the *Journal of Theoretical Biology* advocating the endosymbiotic origin of eukaryotic cells. While it can be argued that some of these ideas had been suggested since the late 19th century, it is equally true that most of them were largely speculative proposals part of the non Darwinian explanations prevalent during such period. In contrast, Lynn Margulis provided a testable hypothesis that made specific predictions in a broader context, and that placed the evolution of cells within a wider context. It is little appreciated that when Lynn Margulis’ first published her proposal, the molecularization of life sciences was undergoing an explosive development. Much was gained, but often at the expense of other approaches including biochemistry, organismal biology, and an evolutionary perspective. Equally significant, neodarwinism was at its height, and it became difficult for many not only to accept the idea of endosymbiosis but also to acknowledge that microbes where not only pathogens, but also the ancestors of all extant life.

Lynn Margulis’s first book is remarkable. It discusses not only prokaryotic evolution and the origin of nucleated cells, but also the deep interaction between the biosphere and the planet itself. This volume is a sample of impressive scholarship and intellectual versatility, in which she traced in authoritative detail the development of microbial life and the emergence of eukaryotes as the outcome of symbiosis. One of the one of the most notable aspects of the books its is multidisciplinary approach, linking biological evolution to the transformation of the Earth into a planet endowed with an oxygen atmosphere and the subsequent changes in the chemistry of the oceans and sediments.

Her views do not contradict Darwinian evolution by natural selection. Endosymbiosis represents a different form of genetic variation, and like conjugation and meiotic sex followed by fertilization, it is a combinatorial mode of evolutionary change. The statement that symbiosis is a Lamarckian event is certainly incorrect. Indeed, the acquisition of genes and genomes due to endosymbiosis is a random event, i.e., it is independent of the advantage it may confer on the organisms in their specific environment, and the biological traits acquired by endosymbiotic events have a previous history, which has been shaped by the sieve of natural selection. What Lynn Margulis did was not only to document symbiosis as a major source of biological variation and evolution, but also to change our views of the biosphere itself.

Keywords: endosymbiosis, Margulis, Darwinian evolution

Journeys with a free mind: life, symbiogenesis and our personal memories of Lynn Margulis

Bandi C.[1], Pajoro M.[2], Sassera D.[1] and Sacchi L.[3]

[1]Università degli Studi di Milano; [2]IZSLER, Sezione di Pavia; [1]Università degli Studi di Pavia - claudio.bandi@unimi.it

In the field of evolutionary biology, dominated by mathematical models and equations, aimed at describing the dynamics of micromutations within populations, the study of symbiosis remained neglected for years. It was useful to provide students with nice examples and curiosities, but was not regarded as a field capable of generating general concepts on evolution. In this framework, till about 20 years ago, Lynn Margulis' seminal papers and books on the symbiotic origin of eukaryotic cells seem to have been considered only in piecemeal fashion, limited to the origin of mitochondria and chloroplasts. Studies published in different fields in the last 20/30 years have however lead to a profound re-evaluation of symbiosis, and have convinced most biologists that symbiosis acted and still acts as a central force in evolution. Lynn Margulis is now part of the history of biology, for the contributions she gave to symbiosis and to the understanding of the origin of eukaryotic cells. But there are other aspects of the science and culture and of Lynn Margulis that are less known, and we will devote part of the seminar to these aspects. Lynn Margulis had a profound knowledge and passion for a variety of microorganisms, from prokaryotes to eukaryotic algae, and for a variety of aspects of organismal biology and ecology. From the details of a specific biological system, she had an unsurpassed capacity to derive general concepts, and to integrate the different tiles of the mosaic into a unique, coherent picture. It is thus not a case that she loved pointillist painters, like Seurat, Signac, Pelizza da Volpedo, that portrayed humans, animals, plants and their environment using collections of tiny points. The stories, or the 'points', that formed the evolving and living paint of Lynn Margulis were *Mastotermes darwiniensis, Mixotricha paradoxa, Treponema pallidum, Pectinatella magnifica*, and many others. We learned some of these stories during a trip by car with Lynn Margulis, from Milano to Pisa. She was going receive a Laurea ad Honorem at the University of Pisa, and she asked one of us to translate into Italian the talk she had prepared for the ceremony. The four hours of driving in the car, while translating a talk and explaining Italian pronunciation, had been very intense, and we had a unique, crazy and funny occasion for understanding a little piece of Lynn Margulis' view of life.

Keywords: symbiogenesis, eukaryotic cell, spirochetes, Midichloria, syphilis

Novel experimental model systems for insect symbiosis studies

Fukatsu T.

National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 305-8566, Japan - t-fukatsu@aist.go.jp

There are several well-established insect models for symbiosis studies, represented by aphid-*Buchnera* for obligate symbiotic association and *Drosophila-Wolbachia* for facultative symbiotic association, with both host and symbiont genomic data available. In addition, powerful molecular and genetic tools are available for the *Drosophila* system. Meanwhile, symbiont culturing and genetic manipulation are practically impossible in both the systems. In the *Drosophila* system, highly-developed symbiotic organs are absent. In the aphid system, even RNA interference does not work, which makes functional analyses quite difficult. To overcome these difficulties, we are recently developing several new insect models with the following features: (i) the host insects are easily maintainable and experimentally tractable in the laboratory, (ii) the symbiotic bacteria are easily cultivable on standard microbiological media, (iii) genetic transformation and gene-knockout are applicable to the symbiotic bacteria, (iv) the genetically manipulated symbiotic bacteria are easily re-introduced into the host insects, (v) the complete symbiont genomes are being determined, (vi) the RNA sequencing data are being accumulated for their well-developed symbiotic organs, and (vii) expression of the host insect genes can be efficiently suppressed by RNA interference. We present several remarkable achievements with these new insect models for understanding of molecular mechanisms underlying the insect-microbe associations.

Keywords : new insect models, symbiont culturing and genetic manipulation, symbiont genomics, host transcriptomics, RNA interference

Ant bacterial symbionts: how specific can they be and do they need to be?

Boomsma Jacobus J. (Koos), Andersen S.B. and Sapountzis P.

Centre for Social Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen – JJBoomsma@bio.ku.dk

In recent years, many remarkable bacterial symbionts have been discovered to be associated with ants. They are usually related to diet deficiencies, particularly for ants that abandoned their original wasp-like predatory life-style to become dependent on homopteran secretions, but the protective association between fungus-growing ants and cuticular cultures of actinomycetes has also attracted considerable interest and debate. Evolutionary theory predicts that symbionts with the same host niche should end up being kept in monocultures, but selection in this direction will continue to be challenged by infections with competing strains in spite of expected countermeasures by hosts and resident symbionts. Recent results from 454 sequencing show that the cuticular ectosymbionts of Acromyrmex leaf-cutting ants are much more specific and monocultural than recent challenges of this idea have suggested. We hypothesize that the unusually constant and possibly partially deficient diet of leaf-cutting ants should have provided interesting additional niches for bacterial symbionts. The first findings confirming this idea will be presented and discussed in light of the title of this presentation.

Keywords: fungus-growing ants, symbiosis, actinomycetes, genome sequencing, diet

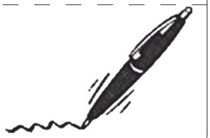
Symbotic diversity in mosquito vectors from Madagascar and Reunion islands, Indian Ocean

Zouache K., Raharimalala F., Raveloson L., Tran-Van V., Failloux A-B., Ravelonandro P. and Mavingui P.

Ecologie Microbienne Lyon, UMR CNRS 5557, Université Lyon 1, 69622 VILLEURBANNE FRANCE - patrick.mavingui@univ-lyon1.fr

Symbiotic bacteria have a considerable impact on the behavior of many arthropods hosts. Indeed, it is well known that microbial communities associated with arthropods can contribute to host reproduction and survival, community interactions, protection against natural enemies and vectorial competence. However, such extended phenotypes were mostly shown in phytophagous insects, whereas research on hematophagous insects has been limited. For instance, the current knowledge of bacterial communities associated with mosquitoes is very limited and consequently their contribution to host behaviors is mostly unknown. As recent works have demonstrated that some *Wolbachia* strains can inhibit mosquito-borne pathogens in some circumstances, characterizing the microbiote of mosquitoes in their environment is a step forward in understanding the ecology and the multipartite interactions occurring in these vectors. In this research we surveyed the composition of bacterial communities associated with wild mosquitoes and explored whether the bacterial diversity can be related to host ecology and pathogen infection. To that purpose, we have chosen two insular ecosystems, Madagascar and Reunion islands, that are prone to invasion by mosquito vectors and where severe epidemics of dengue and chikungunya occurred recently involving high incidences in human populations.

Keywords: Aedes aegypti, Aedes albopictus, Microbial ecology, Symbiosis



Thorsellia anophelis and *Aedes aegypti* symbionts

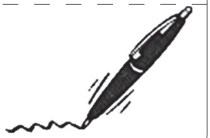
Terenius O.[1], Linda J.M.[2], Guy L.[3], Eriksson-Gonzales K.[4], Bussière L.[5], Laugen A.T.[1], Titanji K.[6] and Faye I.[4]

[1]Department of Ecology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden; [2]Department of Chemistry, Royal Institute of Technology, Stockholm, Sweden; [3]Department of Molecular Evolution, EBC, Uppsala University, Uppsala, Sweden; [4]Department of Genetics, Microbiology and Toxicology, Stockholm University, Sweden; [5]Institute of Biological and Environmental Sciences, University of Stirling, United Kingdom; [6]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA, USA - olle.terenius@slu.se

In our research we have focused on the possible co-adaptation of gut bacteria to their mosquito host. We have previously isolated a unique species of bacteria, *Thorsellia anophelis*, from Anopheles *gambiae* in Kenya. *T. anophelis* has also been found to dominate the gut flora of *An. gambiae* in two other locations in Kenya. Recent data show that *Thorsellia* species also exist in the major malaria vectors of South America and Asia, but so far it has not been found outside malaria mosquitoes or their breeding waters. We have genome sequenced the type isolate from Kenya discovering that it belongs to a new family of bacteria within gamma-proteobacteria.

We have also experimentally analyzed midgut bacteria growth in *Aedes aegypti*. To quantify the dynamics of midgut bacteria growth we experimentally infected *Ae. aegypti* with four different bacterial species isolated from five different host species. We show that the four bacterial species differ in dynamics over time and both the dynamics over time as well as the differences between the species depend on host sex. Our results can at least partly be explained by co-adaptation between host and bacteria and suggest that even closely related species of bacteria differ in their ability to remain in the mosquitoes.

Keywords : Thorsellia, Anopheles, Pantoea, Enterobacter, Aedes aegypti



Recent advances and future perspectives of *Rhabochlamydia porcellionis* research

Kostanjsek R.

Department of Biology, Biotechnical Faculty, University of Ljubljana, Vecna pot 111, 1000 Ljubljana, Slovenia - rok.kostanjsek@bf.uni-lj.si

Rhabdochlamydia porcellionis is intracellular pathogen initially described in digestive glands or hepatopancreas of terrestrial isopod crustacean *Porcellio scaber*. Beside the later, the infections with the members of the family Rhabdochlamydiaceae are described in other arthropods, as well as in amoebe from water, soil and sponges. At the same time recent description of Rhabdochlamydia-like sequences in clinical isolates of vertebrates, including humans indicate their pathogenic potential in higher animals as well. In order to obtain additional data on their transmission, host range and interactions with host, it is essential to maintain the rhabdochlamydia in pure culture, which is, according to their intracellular nature, possible only with their co-cultivation within cell lines.

In recent studies we combined the expertise of recognition and isolation of infected tissues in isopods, with co-cultivation of environmental chlamydiae in insect cell lines. Successfully infected cell lines were used to optimize the stringency of hybridization conditions by *R. porcellionis*-specific probe in FISH, develop the protocol for fixation and preservation of *R. porcellionis* morphology in cell lines for further ultrastructural analysis by electron microscopy, and to optimize a protocol for isolation of high molecular weight DNA from co-cultivated *Rhabdochlamydia*, required for its further genome sequencing analysis. Successful isolation of *R. porcellionis* in insect cell lines therefore provides important step towards understanding the biology of these intracellular bacteria, which will initially include comparative genomics and proteomics, as well as ultrastructural description of *R. porcellionis* developmental cycle, which is already in progress.

Keywords : Rhabdochlamydia porcellionis, cell lines, isolation, Porcellio scaber

Symbionts in mites

Breeuwer J.A.J.

Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, The Netherlands - J.A.J.Breeuwer@uva.nl

Mites can be infected with bacteria that can alter the normal mode of reproduction. These bacteria are *Wolbachia* and *Cardinium*, but more may be found since only a small number of mite species have been studied. Also mite endosymbionts seem to be more difficult to detect by specific PCR, because they either occur at lower density or are slightly different from similar symbionts in insect hosts. I will discuss two examples of symbiont induced parthenogenesis in mites: the genus *Brevipalpus*, infected with *Cardninium*, and the genus *Bryobia* infected with *Wolbachia*. The genus *Brevipalpus* is characterized by a unique feature: they are the only known animal species with haploid females. This situation is caused by an infection with intracellular *Cardinium* bacteria. These bacteria feminize haploid males, thereby ensuring that they can be vertically transmitted to the next generation. An mtDNA phylogeny shows that Brevipalpus mites can be divided into a limited number of clades, representing the most abundant species. Three species are infected and feminized by *Cardinium* symbionts. In the fourth species the feminization appeared to be a genetic trait of the mite itself, since no *Cardinium* is present. Comparing mitochondrial and nuclear genetic markers showed that two species have reproduced strictly clonally for quite some time. In the other two species evidence was found for sexual reproduction in the relatively recent past. In the genus *Bryobia*, several sexual and parthenogenetic species have been found. Parthenogenesis is induced by *Wolbachia*, but double infections with *Cardinium* do occur. Also, double infections are found in sexual species that induce incompatibility. I will argue that asexuality originated multiple times within *Bryobia*. *Wolbachia* bacteria cause asexuality in at least two *Bryobia* species and may have infected different species independently. Although asexual reproduction is expected to result in low levels of genetic variation, we found a large number of clones in each species. Incongruencies between the mitochondrial phylogeny of the host and bacterial phylogeny, suggests that there may be occasional sexual reproduction or horizontal transfer and recombination of symbionts. Both suggestions will generate genetic variation.

Tsetse symbiosis with genus *Wigglesworthia*

Brian W.[1], Wang J.[1], Brelsfoard C.[1,2] and Aksoy S.[1]

[1]Yale University School of Public Health, 60 College St., 626 LEPH, New Haven, CT 06520 USA; [2]Current address: Department of Entomology, University of Kentucky, S-225 Ag. Science Center North, Lexington, KY 40546 USA - serap.aksoy@yale.edu

Tsetse flies (*Glossina* spp.) are the sole vectors of *Trypanosoma* spp., the causative agents of sleeping sickness in humans (human African trypanosomosis, HAT) and of nagana (African animal trypanosomosis, AAT) in livestock. Although epidemics have significantly declined during the last years, still tens of millions of people in Africa continue to be at risk of contracting sleeping sickness. While control of disease in the mammalian host is difficult, reduction of tsetse populations is an effective approach for disease control. In this regards, tsetse's dependence on its microbiome provides novel opportunities for control.

Wigglesworthia is an obligate inherited symbiont of tsetse flies. *Wigglesworthia* resides a group cells (bacteriocytes) forming the midgut bacteriome organ. In addition, extracellular forms of the bacterium are present in the milk secretions of the accessary milk gland, which provides the route of transmission to tsetse's intrauterine larva. The genome sequence indicates retension of vitamin pathways, suggestions a nutritional role for this organism to supplement tsetse's vertebrate specific bloodmeal diet.

Our study has indicated that elimination of *Wigglesworthia* by antibiotic treatment coupled with dietary supplementation of yeast extract gives rise to female progeny that are infertile but that also lack immunity functions, particularly cellular immunity. Supplementation of tsetse's diet with antibiotics, yeast and *Wigglesworthia* extracts can rescue loss of immunity as detected by increased presence of hemocytes in sterile progeny. A tsetse protein, Peptidoglycan Recognition Protein (PGRP)-LB, apparently plays an important role in the density regulation of *Wigglesworthia* in the tsetse host.

Keywords : Wigglesworthia, tsetse flies, mutualism, vector control

Comparative genomics of insect endosymbionts

Klasson L.[1], Tamarit D., Ellegaard K.M.[1], Näslund K.[1], Bourtzis K.[2,3] and Andersson S.G.E.[1]

[1]Molecular Evolution, Department of Cell and Molecular Biology, Uppsala University, Sweden; [2]Department of Environmental and Natural Resources Management, University of Western Greece, Greece; [3]Biomedical Sciences Research Center Al. Fleming, 16672 Vari, Greece - lisa.klasson@icm.uu.se

Many endosymbionts cannot be cultivated in artificial media and their DNA can only be obtained after isolation of large numbers of bacterial cells directly from the hosts. Not surprisingly, it may take years to get enough DNA for sequencing. We have developed a method to isolate, purify and amplify endosymbiont DNA from small quantities of starting material. We applied the method to the isolation, amplification and sequencing of *Wolbachia* strains infecting *Drosophila simulans*. Endosymbionts were isolated from the eggs of flies reared in our laboratory and after DNA amplification we sequenced the genomes of *Wolbachia* strains wHa and wNo, which belong to supergroup A and B, respectively. The comparative analysis of these two genomes with those already sequenced from supergroups A and B has revealed a massive exchange of genes among strains within supergroups as well as a few examples of genetic exchange across the two supergroups. Additionally, we have applied these methods to the study of endosymbionts isolated from aphids and other insects collected from the environment. The endosymbionts contained inside these insects were dissected under microscopy, and their DNA was amplified and sequenced. We have identified both primary and secondary endosymbionts. We have selected samples from aphids containing the secondary endosymbiont *Hamiltonella defensae* for a more detailed comparative genomics study, with a focus on putative virulence genes. We discuss the role of recombination and gene exchange for the evolution of insect endosymbionts.

'*Candidatus Cardinium* hertigii' - a reproductive manipulator with a role in host nutrition

Penz T.[1], Schmitz-Esser S.[2], Kelly S.E.[3], Müller A.[2], Woyke T.[4], Malfatti S.A., Hunter M.S.[3] and Horn M.[1]

[1]Department of Microbial Ecology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria; [2]Institute for Milk Hygiene, University of Veterinary Medicine Vienna Veterinärplatz 1, 1210 Vienna, Austria; [3]Department of Entomology, University of Arizona, 85721 Tucson, AZ, USA; [4]DOE Joint Genome Institute, 94598 Walnut Creek, CA, USA - horn@microbial-ecology.net

Bacterial symbionts of insects have evolved sophisticated strategies to manipulate their host's reproduction to ensure survival and spread within the host population. This may lead to sex role reversal and might even contribute to host speciation. *Cardinium hertigii*, a member of the *Bacteroidetes* and maternally transmitted symbiont of the parasitic wasp *Encarsia pergandiella* is the only known reproductive manipulator outside of the *Alphaproteobacteria*, which causes cytoplasmic incompatibility (CI) constraining crosses between uninfected females and infected males. Here we report the complete genome sequence of *Cardinium hertigii* strain Texas. Comparative analysis with the next closest sequenced relative, the amoeba symbiont *Amoebophilus asiaticus*, provides insights into the evolution of symbiosis in the *Bacteroidetes*. The metabolically restricted genome of *Cardinium* lacks all major biosynthetic pathways but harbors a complete biotin biosynthesis pathway, which suggests a role of *Cardinium* in host nutrition. *Cardinium* lacks known protein secretion systems but encodes a putative new, phage derived secretion system distantly related to the antifeeding prophage of the entomopathogen *Serratia entomophila*. Comparison of the *Cardinium* genome with the genomes of other CI inducing reproductive manipulators, the *Wolbachia pipientis* strains wMel, wRi and wPip, provides a unique opportunity to pinpoint conserved proteins mediating host cell interaction. This allowed us to propose a set of candidate proteins possibly involved in CI. Taken together, the reproductive manipulator *Cardinium* shows genome characteristics of known obligate, mutualistic symbionts of insects as well as of parasitic CI inducing *Wolbachia* strains, but seems to be on the path to become a long-term obligate symbiont.

Keywords : cytoplasmic incompatibility, evolution, phage

Arsenophonus genomes

Novakova E.[1,2], Hypsa V.[1], Moran N.A.[2], Lehane[3] M., Hurst G.D.D.[3] and Darby A.C. [3]

[1]Faculty of Science, University of South Bohemia, and Institute of Parasitology, Biology Centre, ASCR, v.v.i., Branisovka 31, 37005 Ceske Budejovice, Czech Republic; [2]Department of Ecology and Evolutionary Biology, Yale University, 300 Heffernan Dr, West Haven, CT 06516-4150, USA; [3] Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool, L69 7ZB, United Kingdom - acdarby@liv.ac.uk

Arthropods commonly have symbiotic relationship with maternally inherited bacteria. These relationships are diverse, and include obligate mutualism, facultative benefit to the host, and reproductive parasitism. Our understanding of patterns of genomic evolution under different forms of symbiosis has generally relied on contrast to either free living relatives or distantly related symbionts. The genus *Arsenophonus* is unusual in containing symbionts whose interactions with their hosts are diverse in themselves, and therefore allows comparison of genome form between closely related symbionts that share a recent common ancestor but display very different interactions with their host.

We demonstrate that Arsenophonus lineages adapted to different lifestyles also show substantial differences in important genomic traits. These differences reveal general tendencies along the hypothetical evolutionary continuum from almost "standard" bacterial genomes in parasites and facultative symbionts to highly modified and degenerated genomes in obligate mutualists. The most typical manifestations of these tendencies are massive gene loss, compositional bias and economization of the genome with decreasing frequency of horizontal gene transfer.

Keywords: Arsenophonus, Genome Evolution, transmission

The story of communities affecting communities

Vavre F.

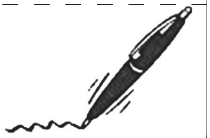
Laboratory of Biometry and Evolutionary Biology, UMR CNRS 5558, University Lyon 1, Villeurbanne, FRANCE. fabrice.vavre@univ-lyon1.fr

Arthropod symbiosis has long been studied by considering only a "one host-one symbiont" approach. However, bacterial symbionts directly or indirectly affect the interactions of the host with other species within a community, and multiple symbionts often share the same hosts. Integrating these multiple interactions at different levels of integration is a challenging task, but is clearly a way to pursue to better understand the functioning and evolutionary trajectory of host-symbiont interactions and to use symbionts for applied perspectives.

In this talk, I will briefly review some concepts showing that the community level may play a fundamental role in shaping symbiosis at different levels, from populations to genomes. The whitefly *Bemisia tabaci* will be used to illustrate this and to show progresses that have been made on this system by gathering different teams from the COST action.

One conclusion is that insects' symbiotic complements are dynamic communities that affect and are affected by the communities in which they are embedded.

Keywords: Multiple infections, competition, cooperation, genomics, Bemisia tabaci



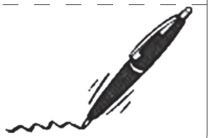
Nitrogen recycling in the aphid/*Buchnera* symbiosis: the role of the host cell

Macdonald S.J.[1], Lin G.G.[2], Russell C.W.[2], Douglas A.E.[2] and Thomas G.H.[1]

[1]Department of Biology (Area 10), University of York, York, UK, & [2]Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY, USA. sandyjmacdonald@gmail.com

The pea aphid/*Buchnera* system is a well characterized symbiosis, where the process of nitrogen recycling operates to enable the aphid to reuse a limited supply of nitrogen via its bacterial partner for synthesis of essential amino acid (EAA). Using *in silico* metabolic modelling with complementary transcriptomic, proteomic and metabolite profiling we have investigated nitrogen recycling in this model symbiotic partnership. The data suggest that, contrary to expectations, aphid waste ammonia is recycled predominantly by the host cell (bacteriocyte) and not *Buchnera*. This recycling of host waste ammonia is mediated through the terminal transaminase reactions of four EAA biosynthesis pathways which occur within the bacteriocytes and not the *Buchnera*. Also, our data suggest that this ammonia is generated directly within the bacteriocytes as a consequence of the adaptation of bacteriocyte metabolism to provide metabolic precursors to the *Buchnera* using non-essential amino acids as substrates. The modeling also reinforces additional metabolic interactions between the two partners over and above EAA biosynthesis such as complementary purine metabolism and pantothenate biosynthesis.

Keywords: Aphid, Buchnera aphidicola, flux balance analysis, bacteriocytes, essential amino acids



Sodalis ***glossinidius***, a symbiotic Trojan horse against a tsetse fly transmitted parasite - the trypanosome

Van Den Abbeele J.

Institute of Tropical Medicine Antwerp, Dept. Biomedical Sciences, Nationalestraat 155, B-2000 Antwerp, Belgium, jvdabbeele@itg.be

African trypanosomiasis is a devastating vector-borne parasitic diseases in human and animals caused by protozoan parasites of the genus *Trypanosoma*. These parasites are biologically transmitted by an obligatory blood feeding insect, the tsetse fly (*Glossina* sp.) in which the parasite undergoes a complex life cycle with several rounds of differentiation and proliferation, orchestrated by a largely unknown molecular parasite-vector crosstalk. *Sodalis glossinidius* is a maternally inherited gram-negative bacterial endosymbiont of the tsetse fly that can be found both inter- and intracellularly in the tsetse fly midgut, muscle, fat body, milk glands, and salivary glands. Given the close proximity of *S. glossinidius* to the different insect tissues where the trypanosome parasites reside and the fact that this symbiotic bacteria can be cultured and genetically modified in vitro, this bacterium is considered as a potential in vivo drug delivery vehicle to control *T.congolense* and *T.brucei* development in the fly. In this context, we recently demonstrated the expression and extracellular release of functional trypanosome-targeting Nanobodies by genetically modified *Sodalis* symbionts. Furthermore, these *Sodalis* strains were not affected in their growth, suggesting that they may be competitive with endogenous microbiota in the midgut environment of the tsetse fly. These data strengthen the view that a paratransgenic approach using *Sodalis* as a Trojan horse for trypanosome-targeting Nanobody delivery has a realistic potential to generate parasite-resistant tsetse flies. Moreover, this approach could also open a new avenue to unravel the molecular determinants of the specific parasite-vector crosstalk during development in the tsetse fly. This innovative Nanobody delivery through bacterial symbionts could also be applicable for paratransgenesis in other vector-pathogen systems.

Keywords: tsetse fly, trypanosome, Sodalis glossinidius, paratransgenesis, nanobodies

Immunity and symbiosis in weevils

Heddi A.

INSA-Lyon, UMR203 BF2I, INRA, Biologie Fonctionnelle Insectes et Interactions, Bâtiment Louis-Pasteur 20 Avenue Albert Einstein, F-69621 Villeurbanne, France
Abdelaziz.Heddi@insa-lyon.fr

Mutualistic associations between animals and bacteria are widespread in nature and are particularly common in insects that exploit unusually restricted nutritional resources. Among symbiotic insects, many are serious pests of crops or vector of animal diseases. Insect intracellular bacteria (endosymbionts) are transmitted vertically for millions of years and provide nutrient supplementation to their hosts, thereby improving their physiology, their adaptive capabilities and their invasive power.

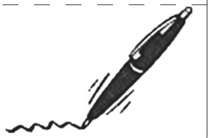
However, maintaining the beneficial nature of this long-term relationship constrains both the host and the symbiont to adaptive changes in their interaction. Evolutionary data have shown that upon establishment, insect endosymbionts experience drastic gene deletions and mutations, which affect among other genes involved in bacterial virulence and host tolerance. Contrariwise, knowledge on how host immune system is regulated to tolerate cooperative bacteria remains scarce and mainly limited to intestinal lumen microbiota and few extracellular bacterial associations.

Insect obligate associations have selected an "endosymbiont compartmentalization" strategy to control endosymbiont space and number. In addition to germ cells, from which endosymbionts are maternally transmitted to offspring, bacteriocytes are the only cells that tolerate endosymbionts and permit their growth and division. Bacteria escaping bacteriocytes face in the hemolymph the activation of a systemic immune response that produce effectors similar to those expressed against pathogens.

Specific molecular features of the weevil bacteriocytes were approached with transcriptomic analyses. Bacteriocyte cells were shown to undergo a modulated immune response. Except the coleoptericinA (ColA) antimicrobial peptide (AMP), AMP encoding genes are slightly (thought not) expressed within the bacteriocytes, probably to allow symbiont maintenance. Functional investigations showed that ColA selectively targets endosymbionts within the bacteriocytes and regulates their growth through the inhibition of cell division. Silencing the colA gene with RNAi resulted in a decrease in size of the giant filamentous endosymbionts, which then escaped from the bacteriocytes and spread to insect tissues.

While AMPs have commonly been linked with microbe clearance, these findings demonstrate that endosymbiosis benefits from ColA and suggest that long-term host-symbiont coevolution might have shaped immune effectors for symbiont maintenance.

Keywords: Symbiosis, immune system, endosymbiont control, evolution



From insect pathogen to obligate symbionts: what's in between?

Latorre A.

Instituto Cavanilles de Biodiversidad y Biología Evolutiva. Grupo de Genética Evolutiva. C/ Catedrático José Beltrán nº 2. 46980 Paterna,Valencia, Spain. amparo.latorre@uv.es

A critical issue in evolutionary biology is to find traits or organisms that provide evidence of the transition from one lifestyle to another, no matter how gradual the process may be. The evolutionary history of intracellular symbiosis involving the transition of bacteria from free-living to obligate lifestyle reveals the existence of different types of bacteria, ranging from mere facultative to obligatory symbionts. Bacterial species with different strains having both types of traits, as it is the case of *Serratia symbiotica* in aphids, represent an important "missing link" in the history of this process.

Aphids are insects that feed on plant-sap, an unbalanced nitrogen/carbon diet deficient in a number of nutrients, such as essential amino acids, which aphids cannot synthesize and are provided by *Buchnera aphidicola*, their primary endosymbiont. In addition to B. aphidicola, some aphid populations harbor additional facultative (or secondary) symbionts that are not required for growth or reproduction, being *S. symbiotica* the most occurring in aphids. Nowadays, the genomes of *B. aphidicola* from five different aphid species have been fully sequenced, being the one from C. cedri, with only 416 kb, the smallest known *Buchnera* genome[1]. Its sequencing and functional analysis revealed that this bacterium had lost part of its symbiotic role as it was not able to synthesize tryptophan. More recent studies showed that the biosynthesis of tryptophan is shared with the symbiont *S. symbiotica* SCc, which coexists with *B. aphidicola* in this aphid, and thus cannot be considered as facultative. The whole genome sequencing of *S. symbiotica* SCc[2] reveals an endosymbiont in a stage that is closer to an obligate endosymbiont, such as *B. aphidicola* from the *aphid Acyrthosiphon pisum* (with a genome size of 640 kb), than to the facultative *S. symbiotica* from the same aphid. Moreover, the comparative genomic study of five strains of *Serratia*, three free-living and two endosymbiotic ones, has let us to disentangle the evolutionary steps undergone from an insect pathogen to an obligate symbiont.

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[2] Lamelas, A. et al. 2011, PLoS Genetics 7(11) e1002357

Immune response of the ant *Camponotus floridanus* against pathogens and its obligate mutualistic endosymbiont

Ratzka C.[1], Feldhaar H.[2] and Gross R.[1]

[1]University of Wuerzburg, Department for Microbiology, Germany; [2]University of Bayreuth, Department for Animal Ecology, Germany - carolin.ratzka@biozentrum.uni-wuerzburg.de

Bacterial endosymbionts are very common in insects and can confer several fitness benefits to their hosts. Insect hosts face the problem of having to maintain such mutualistic bacteria while staging an immune response towards pathogens upon infection. *Camponotus floridanus* ants harbour the obligate endosymbiont *Blochmannia floridanus* in specialized midgut cells and ovaries. The distribution of bacteriocytes and of *Blochmannia* endosymbionts varies strongly during the holometabolous life cycle of the ant hosts [1]. Especially, during metamorphosis the proportion of endosymbiont-bearing midgut cells increases strongly and peaks in late pupal stages, where the entire midgut is transformed into a symbiotic organ. After eclosion of workers the symbiosis degenerates as bacterial numbers decrease with age of workers. It was hypothesized that hosts could regulate the number of endosymbionts present in their tissues via the innate immune system [2]. A quantitative gene expression analysis revealed distinct expression patterns of some immune genes according to developmental stage and tissue. Particularly, two pattern recognition receptor genes, *PGRP-LB* and *PGRP-SC2*, reached maximal expression levels in the pupal midgut tissue at the time, when the highest number of *Blochmannia* is present in this tissue.

A quantitative analysis of immune gene expression revealed different expression kinetics of individual factors and also characteristic expression profiles after injection of gram-negative and gram-positive bacteria. Likewise, *B. floridanus* injected into the hemocoel elicited a comparable immune response of its host *C. floridanus*. Thus, the host´s immune system may prevent colonization of tissues other than the bacteriocytes by *Blochmannia* and may play a role in the regulation of the number of bacteria present within bacteriocytes.

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2. Ratzka C, Liang C, Dandekar T, Gross R, Feldhaar H (2011) Immune response of the ant Camponotus floridanus against pathogens and its obligate mutualistic endosymbiont. Insect Biochem Mol Biol.

Symbionts mediate coevolution between aphid hosts and parasitoids

Vorburger C.

Institute of Integrative Biology, ETH Zürich, 8092 Zürich, Switzerland & EAWAG, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland - christoph.vorburger@eawag.ch

The present understanding of host-parasite coevolution is based on models that assume a direct genetic interaction between host and parasite. This assumption has been challenged by recent empirical findings, showing that hosts often rely on 'helpers' in the form of microbial endosymbionts for defence against parasites. The best-studied case are aphids, in which much of the variation in resistance to parasitoids is due to facultative endosymbiotic bacteria. Using the example of the black bean aphid, *Aphis fabae*, and its parasitoid *Lysiphlebus fabarum*, I illustrate how defensive endosymbionts alter the reciprocal selection between host and parasitoid and thus mediate coevolution. They increase the variation available to selection and make the host-parasite interaction more specific as a consequence of strong genotype-by-genotype interactions between the parasitoids and the aphids' endosymbionts. Protection by symbionts does not come for free to the host. Infected aphids suffer from a reduced lifespan and lifetime reproduction in the absence of parasitoids, and the magnitude of this costs is again determined by a genotype-by-genotype interaction, here between aphids and their symbionts. I refer to this complex three-way interaction as 'symbiont-mediated coevolution', and I highlight the need for new models to understand this intriguing process.

Keywords: Coevolution, genotype x genotype interaction, Hamiltonella defensa, resistance, symbiosis

Disease epidemiology in arthropods is altered by the presence of non-protective symbionts

Ryder J.J.[1,2], Hoare M.J.[1], Pastok D.[1], Bottery M.[1], Boots M.[2], Atkinson D.[1], Knell R.J.[3] and Hurst G.D.D.[1]

[1]Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, U.K.; [2]Centre for Ecology & Conservation, Biosciences, College of Life & Environmental Sciences University of Exeter, TR10 9EZ, U.K.; [3]School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, U.K. - G.Hurst@liverpool.ac.uk

Inherited microbial symbionts can profoundly alter the susceptibility of their arthropod hosts to pathogen and parasite infection. A wider range of symbionts influence host demography, potentially modifying host disease risk through altering transmission opportunities. We tested whether the demographic effects produced by symbionts were sufficient to alter disease dynamics, examining the impact of a sex ratio distorter on the dynamics of a sexually transmitted infection (STI). We examined this in the two spot ladybird *Adalia bipunctata*, which carries a mite STI and a range of male-killing bacteria, and compared STI dynamics between populations where the male-killer was common and ones where it was rare. We observed that presence of *Spiroplasma* male-killers was associated with male-biased epidemic spread of the STI, driven by the strongly female-biased sex ratios produced by *Spiroplasma* male-killers. There were no individual effects of *Spiroplasma* infection on susceptibility to mite infection, and conclude therefore that the differences in STI epidemiological differences arose solely as a consequence of the male-killer on population sex ratio. We conclude that understanding and predicting parasite dynamics in arthropods requires incorporation of the effects of symbionts and that the range of symbionts that are likely to alter parasite epidemiology is much wider than previously envisaged, because it will include those that impact host demography without altering susceptibility.

Experimental evolution of virulence in a symbiosis: from vertical to horizontal transmission

Le Clec'h W., Bertaux J., Bouchon D. and Sicard M.

Laboratoire Ecologie et Biologie des interactions UMR 7267 CNRS, Université de Poitiers, France. mathieu.sicard@univ-poitiers.fr

Wolbachia, may be the most widespread endosymbiotic bacteria in arthropods, are vertically transmitted from mother to offspring. In their native hosts, they are weakly virulent except for one virulent strain in *Drosophila* called wMelpop. This global feature in term of virulence is coherent with theoretical works predicting evolution towards limited virulence for vertically transmitted symbionts. In this talk, I will present data that indicate that *Wolbachia* can evolve quickly towards strong virulence when horizontally transmitted. By using *Armadillidum vulgare*'s hemolymph, that naturally contains immune cells infected by *Wolbachia* (wVulC), as a vector of infection from one individual to another, we forced *Wolbachia* to evolve under the major constraint of horizontal transmission. After only few horizontal passages via hemolymph injection, the number of *Wolbachia* recorded in the receiving animals increased dramatically compared to the beginning of the experiment. Then, the number of bacteria infecting receiving hosts still increased at each passage. In parallel, the mortality of the naïve animals that received the bacteria also strongly increased starting from 0% at the first passage to reach 90% after only 6 selection passages. Our results indicate that *Wolbachia* can rapidly adapt their virulence due to a new transmission mode and become deadly pathogens.

Keywords: Virulence, Evolution, Transmission mode, Symbiont, Pathogens

Using *Wolbachia* infections to control dengue transmission

O'Neill S.L.

School of Biological Sciences, Monash University, VIC 3800 - scott.oneill@monash.edu

Dengue fever is an emerging insect-transmitted disease causing illness in more than 50 million people each year. Currently there are no effective treatments beyond prevention measures targeting the main mosquito vector. Our group is examining the potential use of *Wolbachia* as a novel method to interfere with dengue transmission. This work is now moving from basic bench studies into open field trials in Australia, Vietnam and Indonesia. The presentation will give an overview of current understanding of *Wolbachia*-mosquitio-pathogen interactions as well as the current status of the applied program.

Is the "multicoloured" microbiome associated with insect vectors of plant pathogens a potential resource for disease control?

Daffonchio D.

DISTAM, University of Milan, via Celoria 2, 20133 Milan Italy, daniele.daffonchio@unimi.it

Different types of symbiotic microorganisms have been reported in sap-feeding insects like leafhoppers and planthoppers that are important for their capability of transmitting plant pathogens to plants. Such symbionts have different prevalence and topology in the host body, undergo complex transmission pathways both vertical and horizontal and support many important functions for the hosts. By considering as examples the planthopper Hyalesthes *obsoletus* (Hemiptera: Cixiidae) and the leafhopper Scaphoideus titanus (Hemiptera: Cicadellidae) both vectors of phytoplamas pathogenic to grapevine, we show different features of these symbionts and discuss their potential for blocking disease transmission. In H. obsoletus the high infection rates of the Bacteroidetes "*Candidatus* Sulcia muelleri" the Gammaproteobacterium "*Candidatus* Purcelliella pentastirinorum" and the Betaproteobacterium "*Candidatus* Vidania fulgoroideae" suggest a tripartite primary symbiosis with the host. These symbionts are not alone being associated to the intracellular bacteria "*Candidatus Wolbachia pipientis*", "*Candidatus Rickettsia* sp.", and "*Candidatus Cardinium* hertigii" that are localized in different organs and tissues as revealed by fluorescent in situ hybridization. The diversity of the symbiotic processes is highlighted in *S. titanus* where *Cardinium* is capable of colonizing very different tissues besides the female gonads. From the salivary glands *Cardinium* can be released in the feeding medium and the plant tissues, to be up taken by other individuals in an horizontal transmission mode associated to a co-feeding habit. The diversity of the biological processes mediated by such a diverse range of symbionts is a potential source of novel approaches for blocking pathogenic microorganisms during their transmission to the plant.

Keywords: mutualistic symbiosis, Scaphoideus titanus, Hyalesthes obsoletus, symbiont transmission

Mosquitoes-symbionts interactions: from complex relationships to biotechnological applications

Guido F.

School of Biosciences & Biotechnology, University of Camerino, Camerino (MC) Italy - guido.favia@unicam.it

Several tens of mosquitoes out of around 3500 species described to date are vectors of pathogens of great interest in public health. Mosquitoes show a great ability to adapt to very different habitats where they play relevant ecological roles. In fact, they are present all around the world. It is very likely that the mosquito microbiota have provided the mosquito a great capacity to adapt to many different environments. Thus, a better understanding of the mosquito–microbiota relationships may have a great impact in a better knowledge of some traits of mosquito biology and in the development of innovative mosquito-borne disease-control strategies aimed to reduce mosquito vectorial capacity and/or inhibiting pathogen transmission. In this context, since some years the unit of Molecular Entomology of the University of Camerino has started a study for the identification of mosquito symbionts. In particular we have focused our interest on two of them: 1) The acetic acid bacterium *Asaia* and 2) The yeast *Wickeramomyces anomalus*. *Asaia* is a Gram-negative, found in some mosquito species including several malaria vectors and dengue virus vectors. *Asaia* is the most prevalent bacteria in both natural and lab mosquito populations and its prevalence often reaches 100%, detected in high numbers in the midgut, salivary glands, and reproductive organs. For all these reasons *Asaia* could has been proposed to express anti-pathogen(s) molecules directly in the midgut of the mosquito to exert an inhibitory effect against pathogens. Furthermore, its presence in the reproductive organs permits a vertical transmission route that occurs both maternally and paternally thus providing the basis for the introduction of engineered bacteria into mosquito populations in the field. Experiments performed with engineered bacteria expressing fluorescent proteins has provided a proof of principle to the feasibility of using *Asaia* to express anti-parasite effectors to control malaria and others mosquito-borne diseases At the present we have focusing on the "function(s) that *Asaia* is exerting on the mosquito biology. *Asaia* in mosquito larval development has been previously shown to reduce the time required from the larvae to develop in pupae. Currently we are assessing the impact of *Asaia* on adult mosquito vitality. *W. anomalus* was detected at all the developmental stages of both malaria and dengue mosquito vectors where it localizes in the midgut and reproductive organs. The gut and gonads may provide nutrients for yeasts, thus representing optimal niches for symbionts. The finding that *W. anomalus* associates with some mosquito vectors of several human parasites led to the proposition to use *W. anomalus* to control mosquito-borne diseases. Moreover, some strains of *W. anomalus* produce killer toxins with antimicrobial activities against several human pathogens including arthropod-transmitted parasites such as *Leishmania* spp.. Preliminary observations indicate that the yeast strains isolated from a mosquito also produce killer toxins thus suggesting the possible use of *W. anomalus* in the control of mosquito-borne diseases. Studies for the biochemical characterisation of this protein are in due course. In conclusion, the field of mosquito symbiosis is providing an important contribution to the knowledge of the biology of mosquitoes. Curiosity-driven studies have lead to new insight into the biology of facultative symbionts likely affecting relevant traits of mosquito vectors. Genetic transformation of the symbionts with genes encoding for anti-pathogen effector molecules as well as the use of killer toxins produced by mosquito are interesting field of application, that could contribute to the set-up of integrated control strategies.

Symbiotic control of honeybee pathogens

Hamdi C.[1,2], Balloi A.[3], Essanaa J.[1], Abdi K.[2], Crotti E.[3], Najjari A.[1], Belghuith K.[2], Barbouch N.[4], Alma A.[5], Daffonchio D.[3], Boudabous A.[1] and Cherif A.[1,2]

[1]Laboratoire Microorganismes et Biomolécules Actives, Faculté des Sciences de Tunis, Université de Tunis El Manar, 2092, Tunis, Tunisia; [2]Laboratooire Biotechnologie et Valorisation des Bio-Géo Ressources, Institut Supérieur de Biotechnologie de Sidi Thabet, Université de la Manouba, 2020, Ariana, Tunisia; [3]Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi, 20133, Milano, Italy ; [4]Laboratoire de Zoologie et Apicultures, Institut National Agronomique de Tunis, 43 Avenue Charles Nicolle, 1082 Tunis Mahrajène, Tunisia; and [5]Di.Va.P.R.A. Entomologia e Zoologia applicate all'Ambiente, Università degli studi di Torino, Italy - cherif.ameur@gmail.com

Honeybees are hosts for a large diversity of microorganisms, including commensal, probiotic and pathogenic bacteria. *Paenibacillus larvae* is the primary *bacterial pathogen* that cause a *serious disease* called American foulbrood (AFB). For controlling AFB and other bacterial brood diseases, antibiotics were extensively used which has led the appearance of pathogen resistance and the risk of contamination of hive products. For these reasons, the pressing public concern pointed out the need of finding alternative control strategies; one of these could be based on the symbiotic control approach.

In our laboratory, *we* are *interested in studying* the composition of bacterial communities in honeybee with cultivation-dependent (isolation experiments) and independent techniques (DGGE and ARISA). Ribosomal genes-based methods including ARISA and PCR-DGGE on total DNA from the honeybee gut showed remarkable differences between AFB-diseased and healthy larvae. In AFB-diseased larvae a community less complex than in the healthy ones occurred, in which *P. larvae* appeared to thrive and dominate. In healthy individuals, bacteria mainly represented by *Lactobacillus* and the acetic acid bacteria (AAB) *Saccharibacter*, *Acetobacter* and *Gluconobacter* have been detected. Isolation experiments conducted on AFB-diseased honeybees allowed the establishment of a large collection of *P. larvae* (210 isolates) that showed a previously unrecognized high intraspecific diversity. The isolation of bacterial symbionts from healthy larvae left a large collection of *Bacillaceae*, *Lactobacilli* and AAB of the genus *Saccharibacter*.

Several of these honeybee-associated bacteria showed high capacity to inhibit the growth of P. larvae strains in-vitro. These antagonists belonged to the Bacillus, *Brevibacillus* and *Lactobacillus* genera. The efficiency of the active bacteria was tested on in-vitro reared larvae and then at colony level (in the field). Our overall results showed the ability of the active strains in preventing from P. larvae infestation in larval tissues (in vivo) and to counteract the development of AFB disease at colony level after several applications on bee hives. For other bee diseases, further symbiotic control experiments are currently in progress.

Tephritid symbionts and their applied potential

Yuval B., Gavriel S. and Jurkevich E.

Department of Entomology, Hebrew University of Jerusalem. Rehovot, Israel - yuval@agri.huji.ac.il

Tephritid fruit flies (Diptera: Tephritidae), harbor a diverse community of bacteria in their digestive system. Members of the Enterobacteriaceae form the dominant populations in the medfly (*Ceratitis capitata*) gut, and contribute to various physiological and behavioral parameters related to fitness. Microbial communities in mass rearing strains used for the sterile insect technique (SIT) differ from wild flies, and irradiation further impoverishes the microbial community. We tested the hypothesis that inoculation of sterile flies with members of the original bacterial community results in enhanced competitiveness. Specifically we determined how feeding on diets enriched with *Klebsiella oxytoca* affect the ability of sterile males to compete for wild females against wild males, their ability to inhibit female receptivity, and their survival. We found that enriching the sterile male diet with *K. oxytoca* significantly improved mating competitiveness in the laboratory and in field cages. In addition, bacterially enriched sterile males inhibited female receptivity more efficiently than sugar fed males and survived longer duration of starvation. We conclude that inoculating mass reared sterile flies with bacteria prior to their release is a valid approach to improve the efficacy of the SIT.

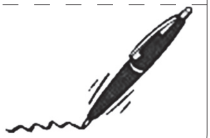
Keywords: Symbiosis, Tephritidae, Sterile Insect Technique

The potential of symbionts in improving biological control agents

T. Groot and K. Bolckmans

Research & development, Entomology department, Koppert B.V.
Veilingweg 17, P.O. Box 155, 2650 AD Berkel en Rodenrijs, Nederland

With the increased interest in the interactions between arthropods and their symbionts has come the realization that symbionts can be used to control arthropod pests. Several symbiont based control strategies are currently being developed, but in most cases still quite some time will pass before these methods will be implemented on a large scale. However, even if symbionts aren't the basis of the control strategy, they still can be important in biological control strategies. For example, symbionts that induce parthenogenesis will allow more efficient production of a parasitoids and better control in the field. In contrast, cytoplasmic incompatibility inducing symbionts may have negative effects in mass-rearing and field applications. The symbionts living in the digestive tracts of the beneficials may be of special interest as many beneficials are reared on artificial diets that contain antimicrobial preservatives. Biological control in general will benefit from a better understanding of the symbionts involved in both pests species and their natural enemies. Unfortunately these subject receive only limited attention leaving the potential benefits unexplored.



Regulatory aspects and safety considerations in the EU

Aguilera J., Gomes A.R. and Olaru I.

European Food Safety Authority. Via Carlo Magno 1/a, 43126 Parma, Italy - Jaime.Aguilera@efsa.europa.eu

Paratransgenesis, or the genetic modification of microbial symbionts to confer new traits to their hosts, has been identified as a promising tool to fight arthropods which are pests or vectors of infectious diseases. The applied use of paratransgenesis would involve the deliberate release into the environment of genetically modified micro-organisms (GMMs) or arthropods carrying them, thereby raising questions about health and environmental safety. The European Union has different legislative instruments in place to ensure the safe use of pesticides and insecticides (including those of microbial origin) as well as GMMs. Depending on their purpose, paratransgenic insects or their GM symbionts may fall under one or more of those legislations. A common requirement to be fulfilled by those who intend to commercialise such products is to conduct a scientific risk assessment to determine whether the product is safe for humans, animals and the environment. The European Food Safety Authority (EFSA) has a key role in the evaluation of applications for marketing genetically modified organisms and plant pesticides, and therefore would assess the safety of GMMs to be used as plant pest controllers via paratransgenesis. EFSA has published guidance which explains the strategy and the scientific data necessary to conduct the risk assessment of GMMs. Future EFSA guidance on the risk assessment of GM insects will provide elements that may also be applicable for paratransgenic insects. EFSA opinions are taken into account by the different European regulatory authorities prior to a decision regarding authorisation to commercialise the product.

Keywords: Paratransgenesis, GMM, biocontrol, biopesticide, risk assessment

Mites, symbionts and commercial implications

Perotti M.A.

School of Biological Sciences, University of Reading, Reading, RG6 6AS, UK. - m.a.perotti@reading.ac.uk

Acarine Biocontrol Agents or ABAs are of extraordinary importance for the control of pest and vectors. ABAs are mass-produced and commercialised worldwide. Good examples are mites of the family Phytoseiidae, which have become regular components of agricultural systems where, as predators, they assist with suppressing pest mite/insect populations that affect crops.

ABAs, like many other arthropods, also engage in a variety of symbiotic associations with microorganisms that induce a wide diversity of phenotypes. For example, mite-bacteria interactions can be manifold and may involve manipulations of the mites rearing conditions to enhance productivity. Therefore, symbionts are not longer exclusive to symbiont-based pest control strategies because they can also be used to manipulate and increase the production of beneficial arthropods. The use of these mite-microbial interactions is leading to a new way of commercialization of ABAs: All-in-one ABAs, where the beneficial hosts (ABAs) are produced following new symbiont-based enhanced production systems.

Commercialization procedures must be reconsidered. New rules for accessing, patenting, exporting, importing and/or releasing All-in-one ABAs are required and these must follow rules of utilization of these genetic resources in accordance with international conventions and protocols.

Keywords: acari, mites, acarology, biological control, pest control

Is it mine? Ethical and other considerations regarding the manipulation of the (human) microbiome or (human) symbionts

Braig H. R.

School of Biological Sciences, Bangor University, Bangor, Wales, UK; h.braig@bangor.ac.uk

Humans have 10-times more bacterial than human cells accounting for roughly 100-times more bacterial genes than human genes, which suggests that the human microbiome might contribute significantly to the definition of a human self. These bacteria can be found on the skin, in the mouth, nose, esophagus, digestive tract and vagina. Such association is neither unique to humans as it applies to arthropods as well, nor is its discovery new. In 1942, Edward A Steinhaus already produced a Catalog of Bacteria Associated Extracellularly with Insects and Ticks. And it is not only bacteria; it can be archaea, fungi, parabasalids, heterokonts, even mites. Some of the associations are very short-lived and much influenced by the environment leading to an ephemeral bacterial fingerprint while others constitute a core microbiome.

Ethics or moral philosophy is the basis from which the general legal system has evolved in a very slow evolutionary process driven by thinkers and the populace. However, matching the processes characteristic of a punctuated equilibrium, the ethics and law of dental hygiene, the ethics and law of genomes and environmental regulation, just to name a few examples, have rather suddenly arisen. This no longer seems to be driven by the populace but by a few academics and a few associations, examples being the International Society for Environmental Ethics, The Animal Welfare Science, Ethics & Law Veterinary Association, and so on. This raises the questions of who is leading the discussion on the ethics of microbiomes and symbionts.

Issues concerning microbial transplantation, pharmacomicobiomics and microbial profiling are most prominent. Ethical questions of privacy and confidentiality, consent, ownership and governance emerge. Microbial profiling might not only reveal information about diet (vegetarian or not) or shape (fat or slim) of an individual, it also might hold information about predisposition for caries, inflammatory bowel disease, Crohn's disease, asthma, type 1 diabetes, rheumatoid arthritis; life expectancy, cultural identity, caste or social characteristics. For insects and mites, the microbiome might determine fitness, the ability to vector diseases as important as dengue and malaria, and the ability to mass rear insects for sterile insect release or as predatory mites for biological control.

Ethical issues of bioprospecting (biopiracy) and return of results are interrogated from the point of patentability of microbial associations. Using caries-preventing Streptococcus bacteria and insect-symbiont associations as examples, the current legal interpretation making naturally occurring bacteria even in new combination unpatentable and returnless for its discoverer is questioned. Lawmakers and judges in the last century could not have imagined the impact natural symbiotic associations might have on human health and economic productivity. What in the past has been considered as dust, debris, dirt or waste and feces, has become an important and defining commodity. The question whether or not sequence variations in natural genes are patentable is still open, but should this evolve as being the case, variations in naturally occurring symbionts might be the next challenge; in terms of legal interpretation, a very slippery slope. Would a change in law benefit the research community?

Keywords : ethics, ownership, legal protection, beneficial bacterial, naturally occurring bacteria

Research on arthrophod symbioses in Latvia

Jankevica L.[1], Metla Z.[1] and Bormane A.[2]

[1]Institute of Biology, University of Latvia, Miera street 3, Salaspils, LV – 2169, e-mail: jankevica.liga@inbox.lv, [2]Centre of Disease Prophylaxis and Control, Klijanu street 7, Riga, LV 1012, - antra.bormane@gmail.com

Research on biological control has been carried out in the Institute of Biology since 1967. The Laboratory of experimental entomology deals with all basic and practical aspects of biological control. The main attention is paid to ecology of pest organisms and their pathogens (viruses, bacteria and fungi). In recent years microflora of pollinator honey bee (*Apis mellifera*) and different forest pests (Lepidoptera, Hymenoptera) was investigated. The impact of different biotic and abiotic factors on associations of microorganisms was observed. Our interest is based on symbiotic control using associated microorganisms. The laboratory studies interactions between insect pathogenic bacteria and bacteria, that belong to the genera *Bacillus* and *Lactobacillus*. The efficiency of the active bacteria is also being tested.

Research on vectors has been going on for more than twenty years in Latvia. Tick-borne encephalitis (TBE) and Lyme borreliosis (LB), the latter caused by spirochaete bacteria vectored by the same tick species, are significant vector-borne diseases in Latvia. Abundance and sesonal dynamics of ticks *Ixodes ricinus* and *Ixodes* persulcatus and infection prevalence in tick populations with pathogenic agents were regulary monitored by scientists from Centre of Disease Prophylaxis and Control. There are registred clinical cases of borreliosis and cases of erlihiosis. In reecent years infections in tick populations with *Borrelia burgdorferi* was in average 30 %; with *Anaplasma phagocitophylum* – in 5%, TBE infection (varies by the year) in range 2-7%. In future attention will be paid to elaboration of new methods of diagnostic and control of nonviral tick-born infections.

Keywords: associations of microorganisms, ticks, vectors

Dynamic *Wolbachia* infection of *Acromyrmex leaf-cutter* ants: potential for a nutritional symbiosis

Andersen S.B.[1], Boye M.[2], Nash D.R[1]. and Boomsma J.J.[1]

[1]Centre for Social Evolution, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark SBAndersen@bio.ku.dk
[2]National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, DK-1790 Copenhagen, Denmark

Wolbachia are renowned as reproductive parasites, but their phenotypic effects in eusocial insects are not well understood. We used a combination of qrt-PCR, fluorescence *in situ* hybridisation, and laser scanning confocal microscopy to evaluate the dynamics of *Wolbachia* infections in the leaf-cutting ant *Acromyrmex octospinosus* across developmental stages of sterile workers. We confirm that workers are infected with one or two widespread wsp genotypes of *Wolbachia*, show that colony prevalences are always 100%, and characterize two rare recombinant genotypes. One dominant genotype is always present and most abundant while another only proliferates in adult workers of some colonies and is barely detectable in larvae and pupae. An explanation may be that Wolbachia genotypes compete for host resources in immature stages while adult tissues provide substantially more niche space. Tissue-specific prevalences of the two genotypes differ, with the rarer genotype being overrepresented in the adult foregut and thoracic muscles. Both genotypes occur extracellularly in the foregut, suggesting an unknown mutualistic function in worker ant nutrition. Both genotypes are also abundant in the faecal fluid of the ants, suggesting that they may have extended functional phenotypes in the fungus garden that the ants manure with their own faeces.

Keywords : esocial ant host, extracellular Wolbachia, FISH, nutritional symbiosis, conflict

The microbiome of the pest mite Rhizoglyphus robini: implications for the pest's status

Zindel R.[1], Ofek M.[2], Minz D.[2], Palevsky E.[3], Zchori-Fein E.[3] and Aebi A.[1]

[1]Agroscope Reckenholz-Tänikon, Research Station ART, Reckenholzstr. 191, 8046 Zürich, Switzerland; [2]Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization, Bet Dagan, Israel. [3]Department of Entomology, Newe Ya'ar Research Center, Agricultural Research Organization, Ramat Yishay, Israel - renate.zindel@art.admin.ch

The biology of many arthropods can only be understood when the associated microbiome is also considered. The bulb mite *Rhizoglyphus robini* Claparede (Acari: Astigmata: Acaridae), a pest on Liliacean cultures, has previously been observed on fungus infected plant cultures and preferred infected plants over uninfected onion seedlings in choice tests. We investigated the composition of its microbial associates and were able to link it to its nutritional preferences. We described the bacterial community using 454 sequencing of 16S rRNA gene fragments and DGGE, finding three genera to be dominant: *Myroides* (41.4% of total sequences), *Serratia* (11.4%) and *Alcaligenes* (4.5%), of which *Serratia* and *Alcaligenes* are known chitinase producers. We showed that mite fecundity was indeed higher when feeding on fungi and that chitin was digested by mite homogenate. Bacteria were shown to be responsible for chitinase production. We conclude that *R. robini*, at least to a certain extent uses fungal chitin as a food source with the help of its associated bacteria. This knowledge on the mite's biology supports the general concept of multi genome organisms.

Keywords: bacterial symbionts, chitin digestion, 16S rRNA pyrosequencing, DGGE

The genome sequence of *Midichloria mitochondrii* and the origin of mitochondria

Sassera D.[1], Lo N.[2], Epis S.[2], D'Auria G.[3], Montagna M.[1], Comandatore F.[1], Horner D.[1], Peretó J.[3], Luciano AM.[1], Franciosi F.[1], Ferri E.[4], Crotti E.[1], Bazzocchi C.[1], Daffonchio D.[1], Sacchi L.[5], Moya A.[3], Latorre A.[3] and Bandi C.[1]

[1]Università degli Studi di Milano. Via Celoria 10, Milano, Italy; [2]University of Sydney, Australia; [3]Universidad de Valencia, Spain; [4] Università di Milano Bicocca, Italy; [5]Università degli Studi di Pavia, Italy - davide.sassera@unimi.it

The sheep tick *Ixodes ricinus*, an arthropod of high medical and veterinary importance, harbours a peculiar intracellular symbiont, the alphaproteobacterium *Midichloria mitochondrii*. This symbiont is a member of the order *Rickettsia*les, bacteria which are considered, based on phylogenetic analyses, to be the closest extant relatives of mitochondria. Furthermore, *M. mitochondrii* is not only phylogenetically close to the mitochondrion, it also presents a unique type of interaction with it. *M. mitochondrii* is in fact able to invade the mitochondria of the ovary cells of its tick host, specifically localizing in the intramembrane space, between the inner and outer membranes of the organelles.

We decided to perform whole genome sequencing of *M. mitochondrii* in order to shed light on this unique symbiosis.We developed a specific method, consisting of single oocyte isolation by micromanipulation followed by multiple displacement amplification and sequencing with 454 and Sanger technologies. The genome was assembled using Mira assembler, Bambus scaffolder, Gap4 for contig viewing and ad-hoc bioinformatics tools for finishing. Inverse-PCRs were performed to close gaps and disambiguate repetitive regions.

Genome annotation revealed the presence of genes coding for a complete flagellar apparatus and for the *cbb3* cytochrome oxidase, both previously unreported in *Rickettsia*les. Phylogenomics analyses showed these genes to be vertically inherited from the common ancestor of *Rickettsia*les and mitochondria. These findings suggest a novel scenario on the origin of eukaryotes, with the ancestor of mitochondria playing a more active, parasitic role, in a microaerophilic habitat.

Keywords: Ixodes ricinus, Rickettsiales, mitochondria, eukaryogenesis, flagellum

On tsetse flies-*Wolbachia* symbiotic associations

Doudoumis V.[1], Tsiamis G.[1], Wamwiri F.[2,6], Brelsfoard C.[2,3], Alam U.[2], Aksoy E.[2], Dalaperas S.[1], Egyir-Yawson A.[4], Malele I.[5], Ouma J.[6], Takac P.[7], Abd-Alla A.[8], Aksoy S.[2] and Bourtzis K.[1,9]

[1]Department of Environmental and Natural Resources Management, University of Western Greece, 2 Seferi St, 30100 Agrinio, Greece; [2]Yale University School of Public Health, 60 College St., 811 LEPH, New Haven, CT 06520 USA; [3]Current address: Department of Entomology, University of Kentucky, S-225 Ag. Science Center North, Lexington, KY 40546 USA; [4]Vector Genetics Laboratory, Department of Animal Science, Biotechnology & Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon, Accra, Ghana; [5]Tsetse & Trypanosomiasis Research Institute (TTRI), Majani Mapana, Off Korogwe Road, P. O. Box 1026 Tanga, Tanzania; [6]Trypanosomiasis Research Centre, Kenya Agricultural Research Institute, P.O. Box 362, Kikuyu 00902 Kenya; [7]Institute of Zoology, Department of Entomology, Slovak Academy of Science, Dubravska cesta 9, 845 06 Bratislava, Slovakia; [8]Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria; [9]Biomedical Sciences Research Center Al. Fleming, 16672 Vari, Greece - edoudoum@cc.uoi.gr

Wolbachia is a group of endosymbiotic α-Proteobacteria which infect numerous arthropod species inducing reproductive abnormalities such as cytoplasmic incompatibility (CI), parthenogenesis, feminization and male killing. Because of its unique properties, *Wolbachia* is currently being considered as a promising tool for the biological control of arthropod pests and disease vectors including *Glossina* spp. In the present study, we employed a *Wolbachia* specific 16S rRNA gene PCR assay to investigate the presence of *Wolbachia* in a total of 5339 specimens of eleven different *Glossina* species, originating from laboratory stocks and natural populations from thirteen African countries. The genotyping of the *Glossina Wolbachia* strains was based on the Multi Locus System Typing (MLST), the wsp and 16S *rRNA* genes. During this analysis, it was shown that fragments of at least six *Wolbachia* genes (*16S rRNA, fbpA, ftsZ, wsp, gatB* and *coxA*) have been transferred into tsetse fly chromosomes and have been pseudogenized by extensive deletion events. The complete characterization of the multiple *Wolbachia* genomes, cytoplasmic and nuclear, present in tsetse flies will shed light into the host-*Wolbachia* symbiotic associations and will contribute to the development and use of effective *Wolbachia*-based strategies for the control of tsetse flies and trypanosomosis.

Keywords: Wolbachia, Glossina, horizontal gene transfer

Detection of spiders microbial communities in Madeira Island

Moreira M.[1], Santos D.[2], Latorre A.[2] and Khadem M.[1]

[1]ISOPlexis Banco de Germoplasma, Universidade da Madeira, Funchal 9000-390 Portugal; [2]Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de Valencia, P.O. Box 22085, Valencia, Spain - marta_amoreira@hotmail.com

Spiders harbour heritable bacteria that are known to be reproductive manipulators in arthropods. However, only few studies have focused on spiders' microbial communities and the role that they may play in their biology. We have investigated the endoymbionts of spiders from oceanic island of Madeira, home to many endemic species of spiders. A total of six samples from four different species, *Pholcus phalangioides*, *Cyrtophora citricola* and Segestria florentina and the endemic one *Hogna insularum*, were screened for the presence of endosymbionts using universal 16S rDNA primers. Three samples were positive: two from *P. phalangioides* and one from H. insularum. The positive samples were amplified and sequenced for three gene fragments, 16S rDNA, ATP synthase F1 alpha subunit (*atpA*) and subunit I of cytochrome C oxidase (*coxA*). Multiple infections by *Rickettsia* and *Wolbachia* were found in both samples of P. phalangioides, while only one type of bacteria was present in H. insularun. Phylogenetic analysis of 16S rDNA gene sequences clustered with the *Rickettsia* from basal ancestral group of Limoniae, together with those from water beetles (*Deronectes*) and predatory bugs (*Macrolophus*). *Wolbachia* sequences of the16S rDNA gene were clustered with those reported in supergroup A. *H. insularum* was infected by *Sphingomonas* bacteria presumably due to other contamination. The quality of sequences for other genes was not high enough for phylogenetic analysis.

Keywords: Spiders, Endosymbionts, Rickettsia, Wolbachia, Madeira Island

Effect of high temperature on distribution of pathogenic *Wolbachia* strain wMelPop in *Drosophila* melanogaster brain cells

Strunov A.[1], Kiseleva E.[1] and Gottlieb Y.[2]

[1]The Institute of Cytology and Genetics, Prospekt Lavrentyeva 10, Novosibirsk, 630090, Russian Federation; [2]The Koret School of Veterinary Medicine, Faculty of Agriculture, POB 12, Rehovot, 76100, Israel - strunov.anton@gmail.com

Symbiotic bacteria together with their host are exposed to various environmental stresses. High temperature is one of the stressful factors that have a strong impact on organism biology. In *Wolbachia-Drosophila* association elevated temperature has diverse effects depending on the host species and bacterial strain. *Wolbachia* strain wMelPop actively proliferates in D. melanogaster brain and muscle tissues at 29°C and strongly reduces the longevity of its host (Min&Benzer, 1997). Due to the potential use of this pathogenic strain as a bio-control agent the purpose of my STSM project was to investigate the effect of high temperature (29°C) on wMelPop distribution in *D. melanogaster* larva and adult brain using FISH and confocal microscopy.

The data obtained has shown a strong heterogeneity of bacterial distribution in larva as well as in adult brain. There were cells free from *Wolbachia* and cells fully packed with them. Surprisingly, 29°C did not affect the bacterial distribution in 3rd-instar larva brain. *Wolbachia* titer and localization were similar to 25°C. However, the bacterial titer significantly increased already at the first day of imago stage at 29°C and then continued to grow. Bacteria dramatically over-replicated and filled almost each area of adult fly brain by 13th day of imago stage at high temperature. In contrast with that the bacterial titer at 25°C didn't changed a lot by this day. What has happened with *Wolbachia* during the pupa stage? The question will be discussed.

Keywords: Wolbachia, Drosophila melanogaster, brain, high temperature, FISH

Manipulation of mating behavior

Schneider D.I.[1], Chao T.[2], Ehrman L.[2], Däuble W.[1], Kaltenpoth M.[3] and Miller W.J.[1]

[1]Lab of Genome Dynamics, Dep. Cell and Developmental Biology, Medical University Vienna, Austria; [2]State University of New York, Division of Natural and Social Sciences, Purchase, USA ; [3]Research Group Insect Symbiosis, Max Planck Institute for Chemical Ecology, Jena, Germany. - daniela.schneider@meduniwien.ac.at

Recent research focuses on biosynthesis, expression, and biological importance of sex pheromones in insects. From these studies, we have gained insight into how crucial these complex chemical compounds are for inter-individual communication, including recognizing mating partners. Mate recognition in turn, is highly significant for sexual isolation, a key parameter of speciation. Like many other insects, *Drosophila* use a cocktail of long-chain hydrocarbons (CHCs) as sex pheromones. The composition of cuticular hydrocarbons, i.e., the pheromone pattern of an individual is characteristic, and recent studies have demonstrated that quantitative alterations in these profiles contribute significantly to sexual isolation in insects.

Extraction, detection, and precise quantification of CHCs, some occurring at quite low concentrations, are often impeded by availability and accessibility of the tested specimen, as well as the detection method itself. Hence, highly sensitive and reliable methods are needed for such analyses. We have taken advantage of the excellent equipment and expertise provided by the Research Group Insect Symbiosis at the Max Planck Institute for Chemical Ecology in Jena, Germany. This group has recently reported on a technically improved method for unambiguous and quick determination of CHCs in insects (Kroiss et al. 2011). By applying this GC-MS technique to the *D. paulistorum-Wolbachia* model system, we were able to detect and compare CHC patterns between wildtype and *Wolbachia*-knockdown lines. The improved method allowed for significant reduction of sample size than the one used before. Further, a more exact analysis of the major compounds could be performed.

Keywords: Drosophila paulistorum, cuticular hydrocarbons, gas chromatography-mass spectrometry

Role of bacterial endosymbionts for the use of predatory bugs in biological pest control

Machtelinckx T., Van Leeuwen T., Gheysen G. and De Clercq P.

Laboratory of Agrozoology, Department of Crop Protection, Coupure Links 653, B-9000 Gent, Belgium. Thijs.machtelinckx@ugent.be

Symbiotic micro-organisms are widespread in arthropods in which they play an important evolutionary and ecological role. Although such endosymbionts are well-studied, their possible use in biological control programmes is still underestimated, and generally focuses on their potential effect in pest insects. However, as these endosymbionts are able to manipulate the reproduction of their arthropod host, their presence in beneficial insects may also have a substantial impact on the use of such insects in biological control. Whereas in the past the focus of endosymbiont research in beneficial insects was most often on parasitoids, this paper will illustrate the importance of endosymbionts in predatory bugs.

The presence and role of endosymbionts in predators has been best studied in ladybugs, which are known to be infected with male killing inducing endosymbionts. Besides coccinellids, heteropteran predators have proven to be excellent biological control agents, and their zoophytophagous feeding habits make them an interesting case study to explore the importance of symbiosis in insects possessing this feeding habit. Research on the economically important predator *Macrolophus pygmaeus* indicated that endosymbionts induce a strong cytoplasmic incompatibility effect in its host. Furthermore, the wide variance in infection frequencies between different *M. pygmaeus* populations may have a substantial impact on the predator's population growth, and as such negatively influence biological control programmes employing this predatory mirid either at the production level or in the field.

These findings demonstrate that it would be warranted to expand research on endosymbiotic bacteria to other heteropteran predators in order to elucidate their potential role on the development, reproduction and feeding ecology of this group of economically important natural enemies.

Keywords: Predatory bugs, Heteroptera, Coccinellidae, endosymbionts

Posters

Genomic basis and possible evolutionary consequences of ectosymbiotic lifestyle in a sulfide-rich environment

Cerveau N., Bauermeister J. and Dattagupta S.

Georg-August-Universität Göttingen, Courant Research Center Geobiology, Goldmischmidstraße 3, 37077 Göttingen, Germany - nicolas.cerveau@geo.uni-goettingen.de

A broad continuum exists in the level of integration between hosts and symbionts. Ectosymbioses have been suggested to be the first step towards the evolution of more integrated extra- and intracellular symbioses, leading eventually to the development of obligate endosymbionts or organelles. From this perspective, ectosymbioses offer the best opportunity to study the early stages of the establishment of symbioses.

Ectosymbioses are common in sulfur-rich marine environments such as deep-sea hydrothermal vents, cold seeps, and organic-rich coastal sediments. Invertebrates living in these habitats are exposed to diverse free-living bacterial communities and are susceptible to colonization by many opportunistic, non-specific surface-dwellers. In this kind of habitat, invertebrate have developed stable ectosymbiotic associations with some epsilon and gammaproteobacteria. It has long been documented that *Thiothrix*, a gammaproteobacteria belonging to the Thiotrichaceae family are ectosymbionts of marine amphipod. Recently, *Thiothrix* bacteria were also identified as ectosymbionts of the freshwater amphipod *Niphargus* ictus from the Frasassi caves in central Italy. These caves harbor various sulfide-oxidizing bacteria, notably belonging to the *Thiothrix* genus, which form bacterial mats. *Thriothrix* bacteria are abundant in mats but the free-living strains are distinct from the bacteria found in association with N. ictus.

Our aim is to use this atypical association between *Thriothrix* and N. ictus in a fresh water ecosystem to investigate the early phase of the symbiosis. The symbiont genome will be compared with that of free-living *Thiothrix* present in bacterial mats in the amphipod habitat to uncover the genomic basis and possible evolutionary consequences of symbiotic lifestyle.

Keywords: ectosymbiont, genomic variation, sulfide-rich environment

New Supergroups and hidden *Wolbachia* diversity in aphids

Augustinos A.A.[1,9], Santos-Garcia D.[2], Dionyssopoulou E.[1,9], Moreira M.[3], Papapanagiotou A.[4], Scarvelakis M.[1], Doudoumis V.[1,9], Ramos S.[2], Aguiar A.F.[5], Borges P.A.V.[6], Khadem M.[3], Latorre A.[2,7], Tsiamis G.[1,9] and Bourtzis K.[1,8,9]

[1]Department of Environmental and Natural Resources Management, University of Ioannina, 2 Seferi St., Agrinio 30100, Greece; [2]Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, Apartado Postal 2085, 46071 Valencia, Spain; [3]ISOPlexis Gene Bank, Universidade da Madeira, Funchal 9000 Portugal; [4]Department of Greenhouse Crops and Floriculture, Technological Educational Institute of Messolonghi, Greece; [5]Laboratório de Qualidade Agrícola, Núcleo de Fitopatologia, Camacha 9135-372, Madeira, Portugal [6]Universidade dos Açores, Departamento de Ciências Agrárias CITA-A (Azorean Biodiversity Group), 9700-042 - Angra do Heroísmo, Terceira – Azores; [7]Área de Genómica y Salud, Centro Superior de Investigación en Salud Pública (CSISP), Avenida de Cataluña 21, 46020 Valencia, Spain; [8]Biomedical Sciences Research Center Al. Fleming, 16672 Vari, Greece; [9]Present address: Department of Environmental and Natural Resources Management, University of Western Greece, 2 Seferi St., Agrinio 30100, Greece - edoudoum@cc.uoi.gr

Aphid species have a major impact on agriculture since they are significant pests and also act as vectors of numerous plant diseases. They are easily dispersed by wind and due to the parthenogenetic mode of reproduction small populations can become established and rapidly increase in number. Therefore aphids are considered as significant economically invasive pests throughout the world. The about 4000 species worldwide engage in highly interesting and complex relationships with their microbial fauna. One of the key symbionts in arthropods is *Wolbachia*, an α-Proteobacterium implicated in many important biological processes and believed to be a potential tool for biological control. Aphids were thought not to harbour *Wolbachia*; however, current data suggest that its presence in aphids has been missed. The aim of the present study is to map the *Wolbachia* infection status of natural aphid populations, along with the characterization of the detected *Wolbachia* strains. Out of 426 samples from Spain, Portugal, Greece, Israel and Iran, 37 were found to harbour *Wolbachia*. Our 16S rRNA based data indicate the presence of two new *Wolbachia* supergroups in aphids, along with some strains belonging either to supergroup B or to supergroup A. Further characterization of the new aphid supergroups could not be completed using MLST and/or sequencing data from the *groEl*, *gltA* and *wsp* genes. This could be attributed to differences in the titre of the infection, multiple infections, and/or high divergence of the newly described *Wolbachia* strains of aphids. New tools are needed to further analyse the aphids *Wolbachia* strains.

Keywords: Wolbachia, insect symbiosis, aphids, 16S rRNA

Bacterial symbionts of Melophagus ovinus (Diptera: Hippoboscidae): a comparative model to the symbiotic system in tsetse flies

Husník F., Nováková E., Chrudimský T. and Hypša V.

Faculty of Science, University of South Bohemia & Institute of Parasitology, Biology Centre of ASCR, Branišovská 31, České Budějovice 37005, Czech Republic
Filip Husník: filip@paru.cas.cz, Eva Nováková: novaeva@paru.cas.cz, Tomáš Chrudimský: tomtom@paru.cas.cz, Václav Hypša: vacatko@paru.cas.cz

Insects living exclusively on vertebrate blood, such as lice, bedbugs or tsetse flies, rely on symbiotic bacteria to provide them with nutrients missing from their blood diet (mainly B-vitamins and cofactors). The sheep ked *Melophagus ovinus* (Diptera: Hippoboscidae) is a wingless obligatory blood-sucking parasite of sheep with adenotrophic viviparity and nourishment of larvae through milk glands. Here, we present basic morphological and molecular characteristics of its symbiotic system and compare it with the best known symbiotic system in tsetse fly (Diptera: Glossinidae). Similar to the tsetse flies, the sheep keds possess a specialized section of midgut (bacteriome) which harbors highly adapted obligatory mutualists transmitted vertically through the milk glands. Unlike the tsetse symbionts of the genus *Wigglesworthia*, these bacteria belong to the large and biologically diverse genus Arsenophonus (Gammaproteobacteria: Enterobacteriales). In analogy to the tsetse inhabiting symbiotic system, this symbiont is accompanied by a facultative symbiotic bacterium infecting various tissues, which originated within *Sodalis* lineage (Gammaproteobacteria: Enterobacteriales) independently of *Sodalis glossinidius*. Along microvilii of the midgut, two additional microorganisms are commonly present: *Bartonella melophagi* (Alphaproteobacteria: Rhizobiales) and *Trypanosoma melophagium* (Excavata: Kinetoplastida). Our results show that *Melophagus ovinus* contains functionally convergent, but phylogenetically independent symbiotic system to that in tsetse fly and can provide a useful comparative model of symbiosis within blood-sucking insects.

Keywords: hippoboscid fly, Arsenophonus, tsetse, Sodalis, Bartonella

Endosymbiotic Archaea - Yes, they do exist!

Lind A.E.[1] and Ettema T.J.G.[2]

[1]*Laboratories of Genome Dynamics, Centre of Anatomy and Cell Biology, Medical University of Vienna, Austria. E-mail: anders.lind@meduniwien.ac.at.* [2]***Department of Cell and Molecular Biology; Molecular Evolution, Biomedical Center, Science for Life Laboratory, Uppsala University, Uppsala, Sweden*** *- thijs.ettema@icm.uu.se*

While there are many organisms that have adapted to an intracellular lifestyle, whether as mutualists, commensals or parasites, almost all come from the same domain of life, bacteria. Even now, some 20 years after the first discovery of intracellular Archaea, few studies are being done on these organisms.

One such Archaea can be found within the eukaryotic ciliate *Nyctotherus ovalis*, whom itself is part of the hindgut micro fauna of several cockroach species. While there are other protists that harbour these kinds of endosymbionts, N. ovalis has some additional remarkable features. The hydrogen producing hydrogenosomes of *N. ovalis* contains the unique feature of having maintained a genome, whereas hydrogenosomes from other species have lost their genetic material over time.

The symbiotic Archaea found in *N. ovalis* are methanogens, converting the hydrogen from the hydrogenosomes into methane, thus providing the host with the benefit of lowering the partial pressure of hydrogen.

Because of the endosymbiotic lifestyle of these archaeons, they cannot be readily grown in pure culture. This has resulted in a lack of sequence data from these organisms. We have taken a single cell approach to sequencing of the complete genome of these organisms. Single cells were sorted and multiple displacement amplification was used in order to obtain enough genomic material for Illumina sequencing. The data obtained has been used to get a better understanding of these organisms, and to further establish the phylogeny of these Archaea.

Keywords: Archaea, Methanobrevibacter, endosymbiont, Nyctotherus ovalis

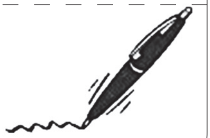
Evolutionary stasis in Blattabacterium, an ancient primary endosymbiont

Patiño-Navarrete R.[1], Ponce De León M.[2], Montero F.[2], Moya A.[1,3,4], Peretó J.[1,5] and Latorre A.[1,3,4]

[1]Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, P.O. Box 22085, E-46071 València, Spain. [2]Departamento de Bioquímica y Biología Molecular I, Facultad de Química, Universidad Complutense de Madrid, Spain. [3]Departament de Genètica, Universitat de València. [4]Centre for Public Health Research (CSISP), València. [5]Departament de Bioquímica i Biologia Molecular, Universitat de València - pereto@uv.es

Many insect species have established long-term symbiotic relationships with intracellular bacteria. Among these associations, the case of the cockroaches and *Blattabacterium cuenoti* is one of the most ancient mutualistic symbioses described up today. In the present work we present the complete genome sequence of the B. cuenoti symbiotic strain from the cockroach Blatta orientalis (BBor). The comparison of this genome with those previously published from the cockroaches *Blattella germanica* (BBge) and *Periplaneta americana* (BPam), the sub social cockroach *Cryptocercus punctulatus* (BCpu) and the termite *Mastotermes darwiniensis* (BMda), show the remarkable stasis of this symbiotic system along the 140 Myr of evolutionary history of the cockroaches. The gene content as well as the chromosome architecture is highly maintained in the five studied genomes, being the most conserved among the known endosymbionts in which more than one strain have been analysed. Evolutionary tests point that purifying selection is the major evolutionary force operating in this system but few genes are accelerated. Stoichiometric analysis of the central metabolic pathways in the five strains of *Blattabacterium* shows a strict functional conservation in terms of biosynthetic precursors production, albeit some enzymatic steps were lost in the ancestors of BBor/BPam and BCpu/BMda, affecting the citric acid and the urea cycles, respectively. Our studies suggest that the genomic and metabolic architectures in *B. cuenoti* were sculpted very early after the establishment of its symbiosis with cockroaches.

Keywords: Blattabacterium pangenome, urease, urate metabolism



The genome of *Candidatus Cardinium* hertigii, a secondary endosymbiont of the whitefly *Bemisia tabaci*

Santos-García D.[1], Beitia F.[2], Mouton L.[3], Moya A.[1], Latorre A.[1]and Silva F.J.[1]

[1]Institut Cavanilles de Biodiversitat i Biologia Evolutiva, University of Valencia, Spain; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia, Spain; [3]Laboratoire de Biométrie et Biologie Evolutive, UMR CNRS 5558, Université de Lyon, France - francisco.silva@uv.es

The whitefly *Bemisia tabaci* is a complex species composed by several biotypes, two of which, B and Q, have a wide distribution in Mediterranean countries. All individuals of this species present a gamma-proteobacterial P-endosymbiont, *Portiera aleyrodidarum*. Occasionally, the whiteflies can present also one or several S-symbionts from the genera *Rickettsia*, *Hamiltonella*, *Wolbachia*, *Arsenophonus*, *Cardinium* and *Fritschea*. While *P. aleyrodidarum* is involved in supporting insect nutrition, the function of the S-symbionts needs to be characterized, although their different distribution among biotypes suggests that they might be contributing to different ecological traits. We have performed through a metagenomic approach the sequencing of the endosymbionts of a Q biotype strain which contains, with the obligate primary endosymbiont, two secondary endosymbionts: Hamiltonella defensa and *Candidatus Cardinium* hertigii. The genome of *Candidatus Cardinium* hertigii (endosymbiont of Bemisia tabaci) has been completely sequenced (average coverage 49). It is slightly higher than 1 Mb and, together with the chromosome, includes a circular plasmid of 53 kb. As expected for most endosymbionts the average G+C content of both is low (36%). The genome contains a large fraction of repeat sequences with more than 15% of the genome formed by transposable elements. The analysis of its gene repertoire gives clues about the relation with its whitefly host.

Keywords: symbiosis, Bemisia tabaci, Candidatus Cardinium hertigii, insect endosymbiont, genomics

Distribution of *Wolbachia* strain wMelPop in *Drosophila* melanogaster brain cells analyzed with electron microscopy

Strunov A. and Kiseleva E.

Russian Federation, 630090, Novosibirsk, Prospekt Lavrentyeva 10, The Institute of Cytology and Genetics, strunov.anton@gmail.com

Wolbachia are intracellular endosymbionts that can be found in a variety of host tissues including gonads, muscles and brain. Such a vast distribution of bacteria leads to a great diversity of *Wolbachia*-host interactions range from mutualism to parasitism and much depend on bacteria strain and species of the host. The wMelPop strain of *Wolbachia* is a striking example of pathogenic endosymbionts that over-replicates in host brain and muscle cells ultimately causing tissue degeneration and premature death. Understanding the features of *Wolbachia* negative impact on host organism is the research priorities due to the potential use of wMelPop strain as an environmentally friendly, bio-control agent.

The purpose of this study was to reveal the peculiarities of wMelPop morphology and its localization in *Drosophila* melanogaster brain cells with the advantages of transmission electron microscopy (TEM).

TEM analysis has shown that wMelPop is generally found in neuron cell body (soma) as well as in glial cells and sometimes in intercellular space but not presented in neuropil. According to the bacterial titer neurons can be divided into three types with different *Wolbachia* density per soma section. The bacterial distribution throughout the brain is very uneven and varies between individuals. In addition to the typical morphology the wMelPop strain shows different ultrastructural abnormalities like electron-dense matrix, bacterial outer membrane protrusion and enlargement as well as diverse vesicle inclusions. All these findings indicate that wMelPop is in an active state and has a predetermined pattern of distribution during the early embryogenesis in D. melanogaster brain cells.

Keywords: Wolbachia, Drosophila melanogaster, brain, TEM

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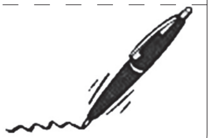


Characterization of microbial community, associated with important forest pests (Lepidoptera)

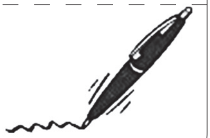
Metla Z., Halimona J., Seskena R. and Jankevica L.

Laboratory of Experimental Entomology, Institute of Biology, University of Latvia, Miera iela 3, LV-2169 Salaspils, Latvia, e-mail: zmetla@inbox.lv

Laboratory of Experimental Entomology is working on problems, that include investigation of insect and entomopathogenic microorganism interactions to obtain a theoretical basis for effective development of microbiological methods for plant protection. Investigations are carried on of midgut microflora content and structure, their interactions and the effect on their changes, caused by environmental factors. Investigation of midgut microbial community and its diversity leads us to understanding the fundamental ecology of microorganisms in insect digestive tract. As test objects served pine looper (*Bupalus piniarius* L.), nun moth (Lymantria monacha L.), pine-tree lappet moth (Dendrolimus pini L.) and gypsy moth (*Lymantria dispar* L.).

Field-collected larvae from all Lepidoptera species were monitored for pathogen occurence in population and their role in it. All insects were screened by „culture dependant" method in order to acquire information of natural gut microflora content and identified entomopathogens. Morphologically different bacteria and fungi were isolated and classified by microscopy and physiological, biochemical reactions. The richness of microorganisms associated with field-collected larvae of Lepidoptera was identified. The results demonstrated that microbial flora of larvae is complex and dominanted by gammaproteobacteria and firmicutes class bacteria. Charecterization of gut microorganisms led us to identification of more than 100 different culturable isolates. The dominant bacteria species were *Stenotrophomonas maltophilia, Serratia marcescens, Burkholderia cepacia, Corynebcaterium sp. and dominant fungi* were *Penicillium* spp. All opportunistic entomopathogenic microorganism isolates from this study will be further evaluated for their suitability as potential virulent entomopathogens.

Keywords: Lepidoptera midgut, microbial diversity, entomopathogens



A screen for host genes interacting with *Wolbachia*

Esteves S.S., Marialva M., Wilton J., Ferreira A.G. and Teixeira L.

Instituto Gulbenkian de Ciência, Oeiras, Portugal

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

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

Keywords: Wolbachia, Drosophila melanogaster

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

Badawi M., Giraud I., Grève P. and Cordaux R.

Université de Poitiers
Laboratoire Ecologie et Biologie des Interactions - UMR CNRS 7267
Equipe Ecologie Evolution Symbiose - Bat. B8 - 40 avenue du Recteur Pineau - F-86022 POITIERS Cedex FRANCE
e-mail : myriam.badawi@univ-poitiers.fr

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

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

List of participants

Alex AEBI
Agroscope Reckenholz-Tänikon
Biosafety
ZÜRICH - SWITZERLAND
alexandre.aebi@art.admin.ch

Jaime AGUILERA
European Food Safety Authority
PARMA - ITALY
jaime.aguilera@efsa.europa.eu

Serap AKSOY
Yale School of Public Health
New Haven, CT 06520-8034
serap.aksoy@yale.edu

Hisashi ANBUTSU
National Institute of Advanced Industrial Science and Technology (AIST)
TSUKUBA - JAPAN
h-anbutsu@aist.go.jp

Sandra Breum ANDERSEN
University of Copenhagen
Centre for Social Evolution
COPENHAGEN - DENMARK
SBAndersen@bio.ku.dk

Telschow ARNDT
Institute for Evolution and Biodiversity
Genome Evolution
MUENSTER - GERMANY
a.telschow@wwu.de

Myriam BADAWI
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
myriam.badawi@univ-poitiers.fr

Claudio BANDI
Universita' Degli Studi Di Milano
Dipartimento Di Patologia Animale Igiene E Sanita' Pubblica Veterinaria Sezione Di Parassitologia
MILANO - ITALY
claudio.bandi@unimi.it

Nicolas BECH
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
nicolas.bech@univ-poitiers.fr

Sophie BELTRAN
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
sophie.beltran@univ-poitiers.fr

Joanne BERTAUX
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
joanne.bertaux@univ-poitiers.fr

Karel BOLCKMANS
Koppert B.V.
BERKEL EN RODENRIJS - THE NETHERLANDS
cteunissen@koppert.nl

Jacobus J. BOOMSMA
Department of Biology, University of Copenhagen
Centre for Social Evolution
COPENHAGEN - DENMARK
JJBoomsma@bio.ku.dk

Yuval BOAZ
Hebrew University
REHOVOT-ISRAEL
yuval@agri.huji.ac.il

Didier BOUCHON
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
didier.bouchon@univ-poitiers.fr

Kostas BOURTZIS
University of Western Greece,
Department of Environmental and Natural Resources Management
AGRINIO - GREECE
kbourtz@uoi.gr

Henk R BRAIG
Bangor University
School of Biological Sciences
BANGOR - UK
h.braig@bangor.ac.uk

Christine BRAQUART-VARNIER
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
christine.braquart@univ-poitiers.fr

Hans BREEUWER
AMSTERDAM - THE NETHERLANDS
j.a.j.breeuwer@uva.nl

Marie-Christine CARPENTIER
Université Lyon 1
Laboratoire de Biométrie et Biologie Evolutive - UMR CNRS 5558
VILLEURBANNE - FRANCE
marie-christine.carpentier@univ-lyon1.fr

Bodil CASS
University of Arizona
Departement of Entomologyr
TUCSON, ARIZONA - USA
bcass@email.arizona.edu

Yves CAUBET
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
yves.caubet@univ-poitiers.fr

Ewa CHROSTEK
Instituto Gulbenkian de Ciência
Host-Microorganism Interactions
OEIRAS - PORTUGAL
echrostek@igc.gulbenkian.pt

Richard CORDAUX
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
richard.cordaux@univ-poitiers.fr

Daniele DAFFONCHIO
DISTAM, University of Milan
MILAN - ITALY
daniele.daffonchio@unimi.it

Alistair DARBY
University of Liverpool
Institute of Integrative Biology
LIVERPOOL - UK
acdarby@liv.ac.uk

Jessica DITTMER
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
jessica.dittmer@univ-poitiers.fr

Stephen DOBSON
University of Kentucky
LEXINGTON - USA
sdobson@uky.edu

Sara EPIS
Universita' Degli Studi Di Milano
Dipartimento Di Patologia Animale Igiene E Sanita' Pubblica Veterinaria Sezione Di Parassitologia
MILANO - ITALY
sara.epis@guest.unimi.it

Sara ESTEVES
Instituto Gulbenkian de Ciência
Host-Microorganism Interactions
OEIRAS - PORTUGAL
sesteves@igc.gulbenkian.pt

Guido FAVIA
University of Camerino
Molecular Entomology Lab- School of Bioscience & Biotechnology
CAMERINO - ITALY
guido.favia@unicam.it

Heike FELDHAAR
University of Bayreuth
Animal Ecology I
BAYREUTH - GERMANY
feldhaar@uni-bayreuth.de

Heather FLORES
Cornell University
Aquadro/Barbash Labs
ITHACA - USA
haf22@cornell.edu

Horacio FRYDMAN
Boston University
Frydman Lab
BOSTON - USA
hfrydman@bu.edu

Takema FUKATSU
National Institute of Advanced Industrial Science and Technology (AIST)
Bioproduction Research Institute
TSUKUBA - JAPAN
t-fukatsu@aist.go.jp

Ingrid FAYE
Department of Genetics, Microbiology and Toxicology
Stockholms universitet
STOCKHOLMS - SUEDE
ingrid.faye@gmt.su.se

Sandrine GENIEZ
New England Biolabs/ LEES
IPSWICH - USA
geniez@neb.com

Lise-Marie GENTY
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
lise.marie.genty@univ-poitiers.fr

Clément GILBERT
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
clement.gilbert@univ-poitiers.fr

Pierre GRÈVE
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
pierre.greve@univ-poitiers.fr

Tom GROOT
Koppert B.V.
BERKEL EN RODENRIJS - THE NETHERLANDS
tgroot@koppert.nl

Roy GROSS
University of Wuerzburg
Chair of Microbiology
WUERZBURG - GERMANY
roy@biozentrum.uni-wuerzburg.de

Peter HAMMERSTEIN
University Berlin
Institute for Theoretical Biology, Humboldt
BERLIN - GERMANY
p.hammerstein@biologie.hu-berlin.de

Toshiyuki HARUMOTO
National Institute of Advanced Industrial Science & Technology (AIST)
Symbiotic Evolution & Biological Functions Research Group (Fukatsu Lab)
TSUKUBA - JAPAN
t-harumoto@aist.go.jp

Louise HEATON
University of Sheffield
Siva-Jothy Lab
SHEFFIELD - UK
l.heaton@sheffield.ac.uk

Abdelaziz HEDDI
INSA de Lyon
UMR INRA/INSA de Lyon BF2I (Biologie Fonctionnelle, Insectes et Interactions)
VILLEURBANNE - FRANCE
Abdelaziz.Heddi@insa-lyon.fr

Helene HENRI
Université Lyon 1
Laboratoire de Biométrie et Biologie Evolutive - UMR CNRS 5558
VILLEURBANNE - FRANCE
helene.henri@univ-lyon1.fr

Jeremy HERREN
Ecole Polytechnique Fédérale de Lausanne
Lemaitre Lab
LAUSANNE - SWITZERLAND
jeremy.herren@epfl.ch

Matthias HORN
University of Vienna
Department of Microbial Ecology
VIENNA - AUSTRIA
horn@microbial-ecology.net

Greg HURST
University of Liverpool
Institute of Integrative Biology
LIVERPOOL - UK
g.hurst@liv.ac.uk

Filip HUSNIK
University of South Bohemia
Laboratory of molecular phylogeny and evolution of parasites
CESKE BUDEJOVICE - CZECH REPUBLIC
filip.husnik@gmail.com

Liga JANKEVICA
Institute of Biology, University of Latvia
Experimental entomology
SALASPILS - LATVIA
Jankevica.Liga@inbox.lv

Monique JOHNSON
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
monique.johnson@univ-poitiers.fr

Edouard JURKEVITCH
The Hebrew University of Jerusalem
Microbial Ecology Lab
REHOVOT - ISRAEL
jurkevi@agri.huji.ac.il

Lisa KLASSON
Uppsala University
Molecular evolution
UPPSALA - SWEDEN
lisa.klasson@icm.uu.se

Rok KOSTANJSEK
Dept. of Biology, Biotechical faculty, University of Ljubljana
LJUBLJANA - SLOVENIA
rok.kostanjsek@bf.uni-lj.si

Wieslaw KOZEK
Medical Sciences Campus, Univeristy of Puerto Rico
SAN JUAN - UNITED STATES
wjkozek@hotmail.com

Shoichiro KURATA
Graduate School of Pharmaceutical Sciences, Tohoku University
SENDAI - JAPAN
kurata@mail.pharm.tohoku.ac.jp

Amparo LATORRE
institute Cavanilles Of Biodiversity And Evolutionary Biology
Evolutionary Genetics
PATERNA, VALENCIA - SPAIN
amparo.latorre@uv.es

Antonio LAZCANO
Facultad de Ciencias, UNAM
Origen de la Vida
MEXICO D.F. - MEXICO
alar@ciencias.unam.mx

Winka LE CLEC'H
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
winka.leclech@yahoo.fr

Anders LIND
Medical University of Vienna
Lab Genome Dynamics
VIENNA - AUSTRIA
anders.lind@meduniwien.ac.at

Sandy MACDONALD
YORK-USA
sandyjmacdonald@gmail.com

Thijs MACHTELINCKX
Ghent University
Agrozoology
GHENT - BELGIUM
thijs.machtelinckx@ugent.be

Isabelle MARCADE
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
isabelle.marcade@univ-poitiers.fr

Julien MARTINEZ
Department of Genetics, University of Cambridge
Jiggins lab
CAMBRIDGE - UNITED KINGDOM
jtm35@cam.ac.uk

Humberto MARTINEZ
Texas A&M University
Mariana Mateos Lab
COLLEGE STATION - U.S.A.
lapostisima@tamu.edu

Patrick MAVINGUI
CNRS
Microbial Ecology - UMR 5557
VILLEURBANNE - FRANCE
patrick.mavingui@univ-lyon1.fr

Adrien MERVILLE
Université Lyon 1
Laboratoire de Biométrie et Biologie Evolutive - UMR CNRS 5558
VILLEURBANNE - FRANCE
adrien.merville@univ-lyon1.fr

Zane METLA
Institute of Biology, University of Latvia
Laboratory of Experimental entomology
SALASPILS - LATVIA
zmetla@inbox.lv

Wolfgang MILLER
Medical University of Vienna
Lab Genome Dynamics
VIENNA - AUSTRIA
wolfgang.miller@meduniwien.ac.at

Guillaume MINARD
CNRS-Université Lyon 1
UMR 5557 Ecologie Microbienne
VILLEURBANES - FRANCE
guillaume_minard_86@hotmail.com

Yves MONE
Université Lyon 1
Laboratoire de Biométrie et Biologie Evolutive - UMR CNRS 5558
VILLEURBANNE - FRANCE
yves.mone@univ-lyon1.fr

Marta MOREIRA
Universidade da Madeira
ISOPlexis/Banco de Germoplasma
FUNCHAL - PORTUGAL
marta_amoreira@hotmail.com

Elmina MOTTIN
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
elmina.mottin@univ-poitiers.fr

Bouziane MOUMEN
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
bouziane.moumen@univ-poitiers.fr

Laurence MOUTON
Université Lyon 1
Laboratoire de Biométrie et Biologie Evolutive - UMR CNRS 5558
VILLEURBANNE - FRANCE
laurence.mouton@univ-lyon1.fr

Andrés MOYA
Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universitat de València
Evolutionary Genetics
PATERNA, VALENCIA - SPAIN
andres.moya@uv.es

Kaare NIELSEN
University of Tromso
Dept Pharmacy
TROMSO - NORWAY
kaare.nielsen@uit.no

Evangelos NTOUNTOUMIS
University of Western Greece
AGRINIO - GREECE
edoudoum@cc.uoi.gr

Scott O'NEILL
Monash University
Scott O'Neill Lab
MELBOURNE - AUSTRALIA
scott.oneill@monash.edu

Chrystel OLIVIER
SASKATOON - CANADA
chrystel.olivier@agr.gc.ca

Juan Camilo PAREDES
Ecole Polytechnique Fédérale de Lausanne
Lemaitre Lab
LAUSANNE - SWITZERLAND
juan.paredes@epfl.ch

JULI PERETO
Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València
Evolutionary Genetics
PATERNA - SPAIN
pereto@uv.es

M. Alejandra PEROTTI
University of Reading
School of Biological Sciences
READING - UK
m.a.perotti@reading.ac.uk

Jean POPOVICI
Monash University
O'Neill Lab
MELBOURNE - AUSTRALIA
jean.popovici@monash.edu

Maryline RAIMOND
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
maryline.raimond@univ-poitiers.fr

Vincent RAQUIN
CNRS - University Lyon 1
Microbial Ecology, Lyon
VILLEURBANNE - FRANCE
vincent.raquin@univ-lyon1.fr

Carolin RATZKA
Universität Würzburg
Lehrstuhl für Mikrobiologie
WÜRZBURG - GERMANY
carolin.ratzka@biozentrum.uni-wuerzburg.de

Freddie-Jeanne RICHARD
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
freddie.jeanne.richard@univ-poitiers.fr

Markus RIEGLER
University of Western Sydney
Hawkesbury Institute for the Environment
PENRITH - AUSTRALIA
m.riegler@uws.edu.au

Panagiotis SAPOUNTZIS
CSE
COPENHAGEN - DENMARK
Sapountzis@bio.ku.dk

Davide SASSERA
Università degli Studi di Milano
DIPAV
MILANO - ITALY
davide.sassera@unimi.it

Sara SAVIC
Scientific Veterinary Institute Novi Sad
Laboratory for serology and immunology
NOVI SAD - SERBIA
sara@niv.ns.ac.rs

Daniela SCHNEIDER
Medical University of Vienna
Genome Dynamics
VIENNA - AUSTRIA
daniela.schneider@meduniwien.ac.at

Elena SHAIKEVICH
Vavilov Institute of General Genetics
Laboratory of Insect Genetics
MOSCOW - RUSSIAN FEDERATION
iaz34@mail.ru

Mathieu SICARD
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
mathieu.sicard@univ-poitiers.fr

Francisco J. SILVA
University of Valencia
Evolutionary Genetics, Institut Cavanilles de Biodiversitat i Biologia Evolutiva
PATERNA, VALENCIA - SPAIN
francisco.silva@uv.es

Barton SLATKO
New England Biolabs
Molecular Parasitology
IPSWICH MA - USA
slatko@neb.com

Catherine SOUTY-GROSSET
Université de Poitiers
Laboratoire Ecologie et Biologie des Interaction - EBI - UMR 7267
POITIERS - FRANCE
catherine.souty@univ-poitiers.f

Anton STRUNOV
Institute of Cytology and Genetics
Laboratory of morphology and function of cell structures
NOVOSIBIRSK - RUSSIAN FEDERATION
strunov.anton@gmail.com

Luis TEIXEIRA
Instituto Gulbenkian de Ciência
Host-Microorganism Interactions
OEIRAS - PORTUGAL
lteixeira@igc.gulbenkian.pt

Olle TERENIUS
Swedish University of Agricultural Sciences
Ecology
UPPSALA - SWEDEN
olle.terenius@slu.se

Gavin THOMAS
University of York
Department of Biology
YORK - UK
gavin.thomas@york.ac.uk

Amy TRUITT
Portland State University
De Rivera lab
PORTLAND - UNITED STATES OF AMERICA
amtruitt@pdx.edu

Claire VALIENTE MORO
Université Claude Bernard Lyon 1
UMR CNRS 5557 Ecologie Microbienne
VILLEURBANNE - FRANCE
claire.valiente-moro@univ-lyon1.fr

Jan VAN DEN ABBEELE
BELGIUM
jvdabbeele@itg.be

Fabrice VAVRE
Université Lyon 1
Laboratoire de Biométrie et Biologie Evolutive - UMR CNRS 5558
VILLEURBANNE - FRANCE
fabrice.vavre@univ-lyon1.fr

Christoph VORBURGER
Institute of Integrative Biology, ETH Zürich & EAWAG
DÜBENDORF - SWITZERLAND
christoph.vorburger@eawag.ch

Jennifer WHITE
University of Kentucky
LEXINGTON, KY - UNITED STATES
jenwhite.uk@gmail.com

Meg WOOLFIT
Monash University
Beth McGraw/Scott O'Neill
MELBOURNE - AUSTRALIA
meg.woolfit@monash.edu

Jason WULFF
University of Kentucky, Department of Entomology
White Lab
LEXINGTON, KY - UNITED STATES
jsnwlff@gmail.com

Ilya A. ZAKHAROV-GEZEKHUS
Institute of General Genetics
Animal Genetics
MOSCOW - RUSSIA
iaz34@mail.ru

Einat ZCHORI-FEIN
Agricultural Research Organization
Entomology
RAMAT YISHAY - ISRAEL
einat@agri.gov.il

Flore ZÉLÉ
CNRS
Maladie infectieuse et vecteurs Ecologie Génétique Evolution Controle
MONTPELLIER CEDEX 5 - FRANCE
flore.zele@ird.fr

Renate ZINDEL
Agroscope Reckenholz-Tänikon, ART
Biosafety
ZÜRICH - SWITZERLAND
renate.zindel@art.admin.ch

Roman ZUG
Institute for Theoretical Biology, Humboldt University Berlin
BERLIN - GERMANY
roman.zug@biologie.hu-berlin.de