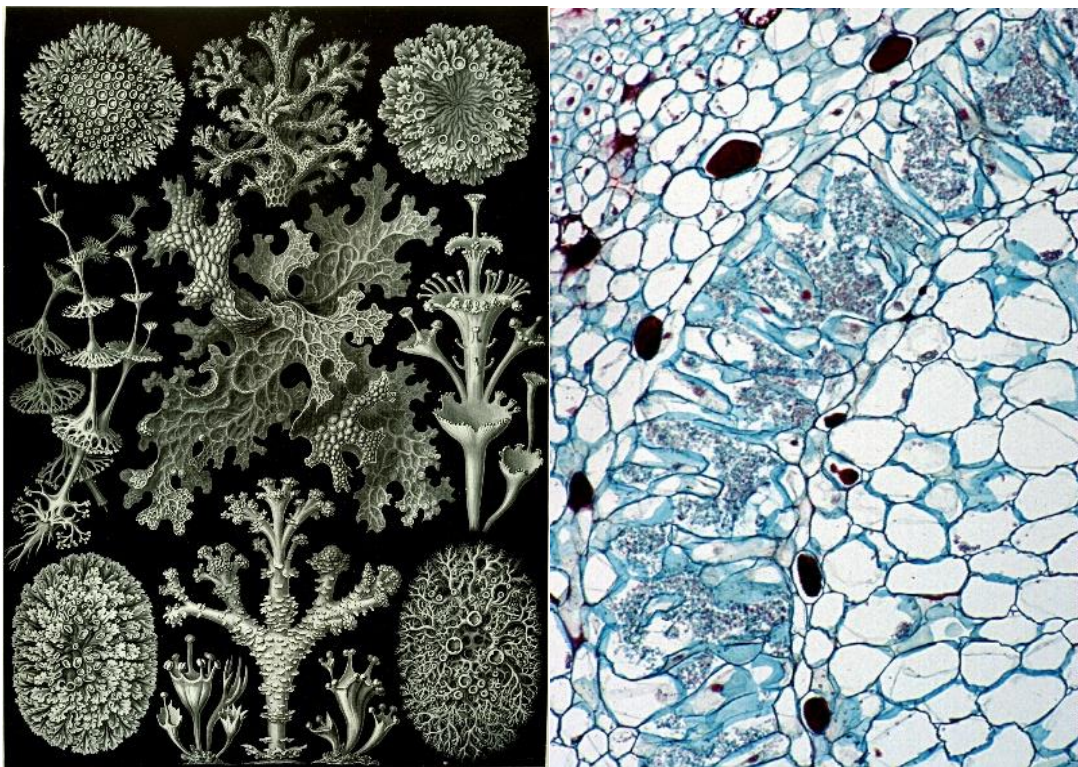




# International Conference on Holobionts

Paris April 19-21, 2017

## Conference Abstracts



# Session 1

## "Holobionts and evolution"



### Genetic variation and evolution of holobionts

#### Authors

**Eugene Rosenberg and Ilana Zilber-Rosenberg – Invited**

Tel Aviv University, Ramat Aviv, 61390 Tel Aviv, Israël

Consideration of the holobiont with its hologenome as a level of selection leads to underappreciated modes of genetic variation and evolution. The hologenome is comprised of two complimentary parts: host and microbiome genomes. Changes in either part result in variation that can be selected for or against. The host genome is highly conserved, and genetic changes within it occur slowly, whereas the microbiome genome is dynamic and changes rapidly in response to the environment by increasing or reducing particular microbes, acquisition of novel microbes, horizontal gene transfer, and mutation. These genetic variations can be transferred to offspring by a variety of mechanisms that will be discussed. As Darwin wrote, “It is not the strongest of the species that survives but the most adaptable”. We argue that rapid changes in the microbiome genome could allow holobionts to adapt and survive under changing environmental conditions, thus providing the time necessary for the host genome to evolve if required.

### Levels of selection and symbioses, with examples from insects

#### Author

**Nancy A. Moran - Invited**

The University of Texas at Austin, Dept Integrative Biology, 2506 Speedway, Austin, United States

Animals and microorganisms often live in close association, and at least for a while their fates can be closely intertwined. Thus, selection potentially can act on the combined unit of host plus symbiotic organisms. But fitness interests of these parties are generally not the same over longer time scales: microorganisms may escape their hosts to colonize others, hosts may discard their symbionts for more beneficial ones, and even when their fates are permanently fused, as in some obligate symbioses, the fitness interests can involve an element of antagonism rather than pure mutualism. Observations such as a highly consistent microbiota within a host species, and differences in microbiota between host species, don't imply anything about the nature or the evolutionary history of an association between host and microbiota. These ideas are developed with examples from insect symbioses including extreme obligate intracellular symbiosis and more labile gut symbioses.

# Theoretical and practical insight into how symbiotic microorganisms might increase acclimation and adaptation capacity of complex organisms

## Authors

**Antton Alberdi**<sup>1</sup>, M Thomas Gilbert<sup>1-3</sup>

<sup>1</sup> Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen, Denmark.

<sup>2</sup> Trace and Environmental DNA Laboratory, Department of Environment and Agriculture, Curtin University, Perth, Western Australia 6102, Australia.

<sup>3</sup> NTNU University Museum, N-7491 Trondheim, Norway.

Symbiotic microorganisms might provide complex organisms with increased capacity to acclimate (individual level process) and adapt (population or species level process) to environmental variation [1,2]. This hypothesis lies in the facts that (1) the associated microbial metagenome harbours genes that provide hosts with extended physiological capabilities [3,4], and (2) microbial assemblies and their gene load can rapidly vary [5,6]. Each organism or group of organisms can harbour an ensemble of microbial taxa or genes (potential microbial space), which limits the set of functions that microorganisms could potentially provide to a host. Potential microbial spaces are seldom fulfilled though; instead, each host at a given moment hosts a fraction of the microbial taxa that could potential harbour (realised microbial space). The breadth of the potential microbial space of a host, as well as the capacity to change its realised microbial space (metagenomic plasticity) responding to environmental variation are thus two key properties that might determine the microorganism-driven acclimation and adaptation capacity of hosts. In theory, organisms with larger potential microbial spaces and metagenomic plasticity would have larger acclimation and adaptation capacity compared to hosts with narrow potential microbial spaces and very static realised microbial spaces. In order to gain insight into microorganism-driven acclimation and adaptation and assess its biological relevance, it is necessary to (1) study the potential microbial spaces and metagenomic plasticity of wild organisms by extensive field sampling and environment manipulation experimentation combined with meta'omics approaches, as well as to (2) unveil the specific factors (environment, microbial interaction, host genome, host behaviour) that shape potential microbial spaces and metagenomic plasticity of hosts, combining the aforementioned experimental approaches with genome-wide analyses (e.g. GWAS) and genome engineering (e.g. CRISPR/Cas9).

## References

1. Alberdi A, Aizpurua O, Bohmann K, Zepeda-Mendoza ML, Gilbert MTP. Do Vertebrate Gut Metagenomes Confer Rapid Ecological Adaptation? *Trends Ecol Evol.* 2016; 31: 1–11.
2. Vannier N, Mony C, Bittebière A-K, Vandenkoornhuyse P. Epigenetic Mechanisms and Microbiota as a Toolbox for Plant Phenotypic Adjustment to Environment. *Front Plant Sci.* 2015; 6: 405–8.
3. Kohl KD, Stengel A, Dearing MD. Inoculation of tannin-degrading bacteria into novel hosts increases performance on tannin-rich diets. *Environmental Microbiology.* 2016; 18: 1720–1729.
4. Chevalier C, Stojanović O, Colin DJ, Suarez-Zamorano N, Tarallo V, Veyrat-Durebex C, et al. Gut microbiota orchestrates energy homeostasis during cold. *Cell.* 2015; 163: 1360–1374.
5. Candela M, Biagi E, Maccaferri S, Turroni S, Brigidi P. Intestinal microbiota is a plastic factor responding to environmental changes. *Trends Microbiol.* 2012; 20: 385–391.
6. Goedbloed DJ, Sanchez E, Reinhardt T, Tebbe CC, Bhuju S, Geffers R, et al. Amphibian gut microbiota shifts differentially in community structure but converges on habitat- specific predicted functions. *Nat Commun.* 2016; 7: 1–12.

## Keywords

Evolution, microbiome, microbiota, metagenome, metagenomic plasticity





## How long does *Wolbachia* remain on board?

### Authors

Marc Bailly-Bechet<sup>1\*</sup>, Patricia Martins-Simões<sup>1,2,3\*</sup>, Gergely Szöllősi<sup>4</sup>, Gladys Mialdea<sup>1</sup>, Marie-France Sagot<sup>1,2</sup> and **Sylvain Charlat<sup>1\*</sup>**

<sup>1</sup> Laboratoire de Biométrie & Biologie Evolutive - CNRS - Université Lyon 1 - Bat. Mendel, 43 boulevard du 11 novembre - 69622 Villeurbanne – France

<sup>2</sup> Erable Team, INRIA Grenoble Rhône-Alpes, France.

<sup>3</sup> Centre International de Recherche en Infectiologie (CIRI), INSERM U1111 – CNRS UMR5308, Equipe Pathogénie des Staphylocoques, Faculté de médecine Laennec, 7 rue Guillaume Paradin, Lyon, France

<sup>4</sup> ELTE-MTA “Lendület” Evolutionary Genomics Research Group, Pázmány P. stny. 1A., 1117 Budapest, Hungary

*Wolbachia* bacteria infect about half of all arthropods, with diverse and extreme consequences ranging from sex-ratio distortion and mating incompatibilities to protection against viruses. These phenotypic effects, combined with efficient vertical transmission from mothers to offspring, satisfactorily explain the invasion dynamics of *Wolbachia* within species. However, beyond the species level, the lack of congruence between the host and symbiont phylogenetic trees indicates that *Wolbachia* horizontal transfers and extinctions do happen and underlie its global distribution. But how often do they occur? And has the *Wolbachia* pandemic reached its equilibrium? We addressed these questions by inferring recent acquisition / loss events from the distribution of *Wolbachia* lineages across the mitochondrial DNA tree of 3,600 arthropod specimens, spanning 1,100 species from Tahiti and surrounding islands. We show that most events occurred within the last million years, but are likely attributable to individual level variation (e.g. imperfect maternal transmission) rather than population level variation (e.g. *Wolbachia* extinction). At the population level, we estimate that mitochondria typically accumulate 4.7% substitutions per site during an infected episode, and 7.1% substitutions per site during the uninfected phase. Using a Bayesian time calibration of the mitochondrial tree, these numbers translate into infected and uninfected phases of approximately 7 and 9 million years. Infected species thus lose *Wolbachia* slightly more often than uninfected species acquire it, supporting the view that its present incidence, estimated slightly below 0.5, represents an epidemiological equilibrium.

### Keywords

*Wolbachia*, Evolution, Dynamics

# Divergent microbial functional isoforms drive niche specialisation and hologenomic homeostasis in the rumen microbiome

## Authors

Francesco Rubino<sup>1,2</sup>, Ciara Carberry<sup>2,3</sup>, Sinéad M Waters<sup>2</sup>, David Kenny<sup>2</sup>, Matthew S McCabe<sup>2</sup>,

**Christopher J Creevey<sup>1</sup>**.

<sup>1</sup> Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Aberystwyth, UK;

<sup>2</sup> Animal and Bioscience Research Department, Teagasc, Grange, Dunsany, Co., Meath, Ireland

<sup>3</sup> School of Agriculture, University College Dublin, Dublin, Ireland.

One of the mechanisms that may govern hologenomic homeostasis is niche specialisation and competitive exclusion of microbes in roles important for the holobiont (Zilber-Rosenberg et al 2008; Bordenstein & Theis 2015). This seems to be maintained even in the presence of horizontal gene transfer which would otherwise erode the competitive advantage of the niche specialists and cause instability in the microbiome. One explanation may be that the horizontal acquisition of a single isoform of a novel gene is not enough to maintain a competitive advantage in fluctuating environmental conditions where a range of isoforms may be required to, for instance, maintain enzymatic activity (Stewart, 1976; Nevo, 2001). The rumen microbiome of sheep and cattle is a case in point; temperature and pH fluctuate daily in response to ambient conditions and the biomass consumed by the host (Mishra et al., 1970). A symbiotic relationship exists between ruminants and their microbiome which allows access to the host of otherwise inaccessible nutrients in plant material. To address this we investigated the genomic signatures of niche specialisation in the rumen microbiome. We generated individual metagenomic libraries from 14 cows fed an ad libitum diet of grass silage and calculated functional isoform diversity for each microbial gene identified. The animal replicates were used to calculate confidence intervals to test for differences in diversity of functional isoforms between microbes that may drive niche specialisation. We identified 153 genes with significant differences in functional isoform diversity between the two most abundant bacterial genera in the rumen (*Prevotella* and *Clostridium*). We found *Prevotella* possesses a more diverse range of isoforms capable of degrading hemicellulose, whereas *Clostridium* for cellulose. Furthermore, significant differences were observed in key metabolic processes indicating that isoform diversity plays an important role in maintaining their niche specialisation (Rubino et al 2016). Our results support the notion that microbial isoform diversity of the holobiont maintains hologenomic homeostasis and the methods developed represent a novel approach for untangling complex interactions between microorganisms and for identifying signatures of hologenomic selection.

## References

Bordenstein, R., Kevin R. Theis. (2015) "Host biology in light of the microbiome: ten principles of holobionts and hologenomes." *PLoS Biol* 13.8 : e1002226. Mishra M, M, Stanley RW, Johnson HD. (1970). Effect of diet and ambient temperature-humidity on ruminal pH, oxidation reduction potential, ammonia and lactic acid in lactating cows. *J Anim Sci* 30: 1023– 1028. Nevo E. (2001). Evolution of genome-phenome diversity under environmental stress. *Proc Natl Acad Sci USA* 98: 6233–6240. Rubino F, Carberry C, Waters SM., Kenny D., McCabe M. & Creevey CJ. In Press. Divergent functional isoforms drive niche specialisation for nutrient acquisition and use in rumen microbiome. *ISMEJ*. Stewart SS. (1976). Factors affecting the cellulolytic activity of rumen contents. *Appl Environ Microbiol* 33: 497–502. Zilber-Rosenberg, I and E Rosenberg. (2008) "Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution." *FEMS microbiology reviews* 32.5 : 723-735.

## Keywords

Homeostasis, Hologenome, Niche Specialisation, Microbiome, Rumen.



# Life history and eco-evolutionary dynamics in light of the gut microbiota

## Authors

Emilie Macke<sup>1</sup>, Aurélie Tasiemski<sup>2</sup>, François Massol<sup>2</sup>, Martijn Callens<sup>1</sup>, **Ellen Decaestecker**<sup>1</sup>

<sup>1</sup> Laboratory Aquatic Biology, KU Leuven (Kulak), Department of Biology, E. Sabbelaan 53, B-8500, Kortrijk, Belgium

<sup>2</sup> CNRS, Université de Lille - Sciences et Technologies, UMR 8198 Evo-Eco-Paleo, SPICI group, F-59655 Villeneuve d'Ascq, France

The recent emergence of powerful genomic tools, such as high-throughput genomics, transcriptomics and metabolomics, combined with the study of gnotobiotic animals, have revealed overwhelming impacts of gut microbiota on the host phenotype. In addition to provide their host with metabolic functions that are not encoded in its own genome, evidence is accumulating that gut symbionts affect host traits previously thought to be solely under host genetic control, such as development and behavior. Metagenomics and metatranscriptomics studies further revealed that gut microbial communities can rapidly respond to changes in host diet or environmental conditions through changes in their structural and functional profiles, thus representing an important source of metabolic flexibility and phenotypic plasticity for the host. Hence, gut microbes appear to be an important factor affecting host ecology and evolution which is, however, not accounted for in life-history theory, or in classic population genetics, ecological and eco-evolutionary models. In this forum, we shed new light on life history and eco-evolutionary dynamics by viewing these processes through the lens of host-microbiota interactions. We follow a three-level approach. First, current knowledge on the role of gut microbiota in host physiology and behavior points out that gut symbionts can be a crucial medium of life history strategies. Second, the particularity of the microbiota is based on its multilayered structure, composed of both a core microbiota, under host genetic and immune control, and a flexible pool of microbes modulated by the environment, which differ in constraints on their maintenance and in their contribution to host adaptation. Finally, gut symbionts can drive the ecological and evolutionary dynamics of their host through effects on individual, population, community and ecosystem levels. In conclusion, we highlight some future perspectives for integrative studies to test hypotheses on life history and eco-evolutionary dynamics in light of the gut microbiota.

## References

Macke, E., Tasiemski, A., Massol, F., Callens, M., Decaestecker, E. (2016). Life history and eco-evolutionary dynamics in light of the gut microbiota. *Oikos* DOI 10.1111/oik.03900.

## Keywords

Eco-evolutionary dynamics, life-history, gut microbiome

# Evolution of the gut microbiome in wild versus captive mammals

## Authors

**Frédéric Delsuc**<sup>1</sup>, Se Jin Song<sup>2</sup>, Jessica L. Metcalf<sup>2</sup>, Katherine R. Amato<sup>2</sup>, John G. Sanders<sup>3</sup>, Nico L. Avenant<sup>4</sup>, Duncan N. MacFadyen<sup>5</sup>, Rob Knight<sup>4</sup> and Valerie J. McKenzie<sup>3</sup>

<sup>1</sup> CNRS, Université de Montpellier, Montpellier, France.

<sup>2</sup> University of Colorado, Boulder, CO, USA.

<sup>3</sup> University of California, San Diego, CA, USA.

<sup>4</sup> National Museum, Bloemfontein, South Africa.

<sup>5</sup> University of Pretoria, E Oppenheimer & Son, Pretoria, South Africa.

Understanding how the gut microbiome has evolved in mammals is a question currently attracting a lot of interest in the context of human microbiome research. One current limitation of mammalian microbiome studies is that they are mostly based on zoo-kept animals in which captivity might have influenced the evolution of their gut microbes. Indeed, captive animals experience a range of changes that may influence the microbiome, such as diet changes, medical treatments, and reduced contact with natural sources of bacterial diversity. Previous studies on the subject have revealed a decrease in bacterial diversity in captive animals. However, these studies have been relatively limited in the range of species investigated. Here we assemble a data set built from field trips specifically aimed at the sample of fecal material from an extended diversity of mammalian species. This new data set allow us to compare using 16S rRNA metabarcoding the composition of the gut microbiome between wild and captive individuals in 25 species of placental mammals. We show that the previously reported pattern of decreased bacterial diversity in captivity is not observed universally, with heterogeneous responses being observed in different orders. We also report that host traits, such as taxonomic order, diet type, and gut physiology all influence the effect of captivity on the microbiome. Finally, we discuss the broad functions that are involved with the bacterial groups responding to captivity. Overall, our work highlights the importance of sampling wild populations for understanding gut microbiome evolution and function.

## Keywords

Evolution, Gut microbiome, 16S RNA metabarcoding, Mammals, Captivity

# Deep-sea mussels and their symbionts: recent findings and open questions

## Authors

**Sébastien Duperron**<sup>1,2</sup>, Bérénice Piquet<sup>1,3,4</sup>, François H. Lallier<sup>3,4</sup>, Ann C. Andersen<sup>3,4</sup>, Bruce Shillito<sup>1</sup>

<sup>1</sup> Sorbonne Universités, Université Pierre et Marie Curie, UMR 7208 MNHN CNRS UPMC IRD, Adaptation aux Milieux Extrêmes –Paris, France

<sup>2</sup> Institut Universitaire de France, Paris, France

<sup>3</sup> Sorbonne Universités, Université Pierre et Marie Curie, UMR 7144, ABICE, Station Biologique de Roscoff, France

<sup>4</sup> Centre National de la Recherche Scientifique (CNRS), UMR 7144, Adaptation et Diversité en Milieu Marin, Station Biologique, F-29680 Roscoff, France

Bathymodioline mussels rank among the most abundant metazoans at deep-sea reducing habitats such as hydrothermal vents, cold seeps, wood and whale falls worldwide. They owe their success to efficient symbiotic associations with one-to-several lineages of bacteria that fulfill most of the mussel's nutritional requirements by exploiting the oxic-anoxic interface, and have become one of the major example of deep-sea symbiosis. Recent years have seen advances in our understanding of these symbioses, which are presented here. Studying the diversity of symbionts in different mussel species, we discovered that symbioses were much more diverse than previously thought, including bacteria with unusual lifestyles (such as living inside nuclei) and metabolisms. The composition of symbiont communities can vary over the geographical distribution area of a single *Idas* species, a variability most likely made possible by the environmental acquisition of local bacterial lineages. The advent of in vivo experimentation under pressure allowed to demonstrate that mussel symbioses are flexible and react quickly to changes in their environment. Over time, symbiosis in mussels has evolved in a complex way, and may even have disappeared over short evolutionary timescales at some stages. Compared with other metazoan groups displaying chemosynthetic symbioses, Bathymodioline mussels are able to associate in a flexible way with a broader phylogenetic and metabolic diversity of bacteria, and how this is achieved remains to be explained. Altogether, recent findings question some assumptions about chemosynthetic symbioses, and these original features may be key to the success of deep-sea mussels in the various habitats they colonize.

## Keywords

Mussels, chemosynthesis, deep-sea, hydrothermal vents, cold seeps



# “Specialist” vs “Generalist” gut microbiomes are linked to host range in frugivorous fruit flies

**Author**

**Edouard Jurkevitch**

Boaz Yuval, Department of Entomology, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, POB12, Rehovot, Israel

Fruit flies (Tephritidae, subfamilies Dacinae and Trypetinae) form a large and diverse group of phytophagous species, several of which are frugivores that negatively affect fruit production worldwide. In the highly polyphagous Mediterranean fruit fly (*Ceratitis capitata*), a complex composed of numerous Enterobacteriaceae is inoculated into the fruit by ovipositing females. This microbiome fixes atmospheric nitrogen and degrades pectin, contributing to larval and adult nutrition<sup>1,2</sup>. While these core functions are conserved, a high microdiversity in metabolic capabilities is apparent at the strain level<sup>3</sup>. Furthermore, individual strains are differentially sensitive to egg antimicrobial compounds and contribute differentially to larval development. In contrast, the olive fly (*Bactrocera oleae*) almost exclusively associates with a non-cultivable, maternally transmitted bacterium (*Candidatus Erwinia dacicola*). The fly's symbionts compensate for the inability of adult flies to synthesize essential nutrients and to metabolize intractable sources of nitrogen<sup>4,5</sup>. While the symbionts are not required for larval development in ripe (black) olives, they are essential for development in unripe (green) olives where they may inactivate oleuropein – a toxic component of green but not black olives<sup>6</sup>. We propose that a shift from saprophagy to phytophagy occurred in Tephritid flies mediated by the ability of females to inoculate environmental, rot-inducing bacteria into living plant tissues. As this can be brought about by rather generic functions, a generalist population forms the bulk of the microbiota enabling colonization of a wide range of host fruits with low local selection. By requiring the overcoming of strong barriers preventing larval development, monophagy imposes strong selection on specialist functions, thereby restricting the taxonomic and possibly the genetic diversity of the microbiome.

## References

1. Behar, A., Yuval, B., and Jurkevitch, E. 2005. Enterobacteria-mediated nitrogen fixation in natural populations of the fruit fly, *Ceratitis capitata*. *Molecular Ecology*. 14:2637-2643.
2. Behar, A., Jurkevitch, E., Yuval, B. 2008. Bringing back the fruit into fruit fly-bacteria interactions. *Molecular Ecology*. 17:1375–1386.
3. Aharon, Y., Pasternak, Z., Ben Yosef, M., Behar, A., Lauzon, C., Yuval, B., and E. Jurkevitch. 2013. Phylogenetic, metabolic and taxonomic diversity shape the Mediterranean fruit fly microbiota across ontogeny. *Applied and Environmental Microbiology*. 79: 303-513.
4. Ben Yosef, M., Aharon, Y., Jurkevitch, E., and Yuval, B. 2010. Give us the tools and we will do the job: Symbiotic bacteria affect olive fly fitness in a diet dependent fashion. *Proceedings of the Royal Society B: Biological Sciences*. 277:1545-1552.
5. Ben-Yosef, M., Pasternak, Z., Jurkevitch, E., and B. Yuval. 2014. Symbiotic bacteria enable olive flies (*Bactrocera oleae*) to exploit intractable sources of nitrogen. *Journal of Evolutionary Biology* 27.12: 2695-2705.
6. Ben-Yosef, M., Pasternak, Z., Jurkevitch, E., Yuval, B. 2015. Symbiotic bacteria enable olive fly larvae to overcome host defences. *Royal Society Open Science* 2:7.

## Keywords

Fruit fly symbionts, *Ceratitis capitata*, *Bactrocera olea*. symbiont diversity



# Symbiotic algae enslave the cnidarian biological clock to their own rhythm

## Authors

Michal Sorek<sup>1</sup>, Yisrael Schnytzer<sup>1</sup>, Hiba Waldman Ben-Asher<sup>1</sup>, Vered Chalifa Caspi<sup>2</sup>, Chii-Shiarn Chen<sup>3</sup>, David J. Miller<sup>4</sup>, **Oren Levy<sup>1</sup>**

<sup>1</sup> The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel

<sup>2</sup> National Institute of Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel

<sup>3</sup> National Museum of Marine Biology and Aquarium, Checheng, Pingtung, Taiwan, ROC

<sup>4</sup> ARC Centre of Excellence for Coral Reef Studies and Department of Molecular and Cell Biology, James Cook University, Townsville, 4811 Australia

Circadian clocks are self-sustained endogenous timers that enable organisms to anticipate daily environmental rhythms and adjust their physiology and behavior accordingly. Whereas the molecular mechanisms underlying the circadian clock are largely conserved across the Metazoa, the integration of molecular clocks in symbiotic and holobiont systems has received limited attention. In corals and many other cnidarians (basal metazoans), rhythmic behaviours are complicated by oscillations in tissue oxygen tension over light/dark cycles that are driven by photosynthetic and respiratory activities of the endosymbionts. Moreover, coastal ecosystems such as coral reefs are exposed to tidal changes that occur on cycles of approximately 12.4h. Whereas the circadian clock mechanisms of metazoans are relatively well established, circatidal clocks are much less well understood. To better understand the integration of biological clocks in cnidarian hosts of the photosynthetic dinoflagellate *Symbiodinium*, daily rhythms of biological activity and gene expression were studied in symbiotic and aposymbiotic morphs of the sea anemone *Aiptasia diaphana*. As expected, circadian cycles of activity and gene expression dominated in symbiotic morphs whereas, in aposymbiotic morphs, circatidal patterns were more common. Counter-intuitively, expression of a significant number of genes ( $n = 227$ ) shifted from 12h to 24h rhythms in symbiotic morphs. The presence of photosymbionts extensively modifies behavioural, physical and transcriptional rhythms of the host, thus the dinoflagellates are essentially the “masters”, and the host the “slave”, in the association.

## References

- 1 Levy, O., Kaniewska, et al., (2011). Complex diel cycles of gene expression in the coral-algal symbiosis. *Science* 331: 175.
- 2 Levy, O., et al., (2007). Light-responsive cryptochromes from the simplest marine eumetazoan animals. *Science* 318: 467-470.
- 3 Kaniewska P, Alon S, et al., (2015) Signaling cascades and the importance of moonlight in coral broadcast mass spawning. *eLife* <http://dx.doi.org/10.7554/eLife.09991>
- 4 Sorek M, et al., (2014) Circadian clocks in symbiotic corals: the duet between *Symbiodinium* algae and their coral-host MarineGenomics. <http://dx.doi.org/10.1016/j.margen.2014.01.003>
- 5 Reitzel AM, et al., (2013) Circadian clocks in the Cnidaria: environmental entrainment, molecular regulation, and organismal outputs *Integr. Comp. Biol.* 53: 118-130. doi:10.1093/icb/ict024
- 6 Ganot P, et al., (2011). Adaptations to endosymbiosis in a cnidarian-dinoflagellate association: differential gene expression and specific gene duplications. *PLoS Genet*, 7(7), e1002187.
- 7 Miwa, I. (2009). Regulation of circadian rhythms of *Paramecium bursaria* by symbiotic *Chlorella* species. In *Endosymbionts in Paramecium* (pp. 83-110). Springer Berlin Heidelberg.
- 8 Heath-Heckman, et al., (2013). Bacterial bioluminescence regulates expression of a host cryptochrome gene in the squid-vibrio symbiosis. *MBio*, 4(2), e00167-13.

## Keywords

Biological clock, cnidarians circadian and circatidal rhythm, symbiosis, host, sea anemone, symbiodinium, holobiont



# Good fences make good neighbors: Immune compartmentalization strategy in insect symbiosis

## Authors

**Justin Maire**<sup>1</sup>, Florent Masson<sup>1,\$</sup>, Agnès Vallier<sup>1</sup>, Séverine Balmand<sup>1</sup>, Carole Vincent-Monégat<sup>1</sup>, Nicolas Parisot<sup>1</sup>, Anna Zaidman-Rémy<sup>1</sup>, Abdelaziz Heddi<sup>1</sup>

<sup>1</sup> Univ Lyon, INSA-Lyon, INRA, BF2I, UMR0203, F-69621, Villeurbanne, France.

<sup>\$</sup> Global Health Institute, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), Station 19, Lausanne 1015, Switzerland.

Many insects thriving on nutritionally unbalanced diets have evolved symbiotic associations with intracellular bacteria (endosymbionts), which provide them with metabolic supplements, thereby increasing their adaptive abilities. While host-endosymbiont coevolution is known to pair with massive genomic changes in the bacterial partner, host's genome evolution remains elusive, especially how the host immune system is shaped in response to a chronic bacterial presence. Many insects have evolved a compartmentalization strategy consisting in maintaining endosymbionts in specialized cells, the bacteriocytes, where the symbiont is preserved and controlled. In the cereal weevil *Sitophilus* spp., which houses the Gram-negative bacterium *Sodalis pierantonius*, bacteriocytes form an organ, the bacteriome. We previously reported that this organ displays a specific internal immune program, adapted to both symbiosis maintenance and control. Despite the massive presence of endosymbionts, antimicrobial peptides (AMP) show a very low basal expression in the bacteriome. Only one AMP, the Coleopteracin A (CoLA), is highly and constantly produced in this symbiotic organ, and was shown to prevent bacterial cytokinesis and endosymbiotic escape from the bacteriome. To understand the regulation of this bacteriome specific genetic program, we first addressed the bacteriome immunocompetency, by testing its capacity to induce AMP encoding genes in response to exogenous bacterial infections. We show that all tested AMP encoding genes, including *colA*, are highly up-regulated in the bacteriome upon bacterial challenge, albeit in a weaker manner as compared to the systemic larval immune response. Intriguingly, combined genomic, transcriptomic and functional analyses indicate that an IMD-like pathway is responsible for AMP expression in both standard (i.e. *colA* expression in the presence of symbionts) and infected conditions (i.e. AMP cocktail expression upon infection). The molecular regulations allowing the bacteriome to exhibit two distinct, yet IMD-dependent immune programs, depending on the symbiotic and infectious status, thus remain to be unraveled. We are currently investigating the hypothesis of a differential recruitment of transcription factors downstream of IMD. This study highlights how insect immune system is shaped in response to symbiosis, ensuring endosymbiont seclusion and global symbiotic homeostasis while maintaining immune responsiveness to exogenous, pathogenic bacteria.

## References

Login FH, Balmand S, Vallier A, Vincent-Monégat C, Vigneron A, et al. Antimicrobial peptides keep insect endosymbionts under control. *Science*. 2011, 334(6054):362–5. Masson F, Vallier A, Vigneron A, Balmand S, Vincent-Monégat C, et al. Systemic Infection Generates a Local-Like Immune Response of the Bacteriome Organ in Insect Symbiosis. *J Innate Immun*. 2015, 7(3):290–301.

## Keywords

Innate immunity, antimicrobial peptide, bacteriocyte, evolution, Coleoptera, *Sitophilus*



# Unorthodox transmission modes of endosymbionts in hybrids and the symbiotic origin of speciation

## Authors

**Wolfgang Miller**<sup>1</sup>, Daniela I. Schneider<sup>2</sup>, Lisa Klasson<sup>3</sup>, Lee Ehrman<sup>4</sup>

<sup>1</sup> Medical University of Vienna, Schwarzschanerstr. 17, A-1090 Vienna, Austria

<sup>2</sup> Lab Genome Dynamics, Center of Cell and Developmental Biology, Medical Univ Vienna, Vienna, Austria;

<sup>3</sup> Molecular Evolution, Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden;

<sup>4</sup> Natural Sciences, State University of New York, Purchase College, Purchase, New York, USA.

Although not a new idea, recent studies suggest that differences in the composition of symbiotic microbes between hosts can lead to reproductive isolation, and as a consequence also to speciation. Despite the fact that microbes are universally present in eukaryotes, they are rarely considered as a driving force of speciation, and chances are their contribution to speciation is overlooked. Symbiotic bacteria of the genus *Wolbachia* are known to affect their hosts' reproduction in adaptive manners to improve the propagation of the maternally transmitted endosymbiont throughout populations. These reproductive alterations that can result in postmating isolation via cytoplasmic incompatibilities, have recently been shown to foster also premating isolation in some host-symbiont associations such as the neotropical *Drosophila paulistorum* species complex, giving even more reason to assume that symbionts can play a significant role in host speciation. Here we will present most recent data on the involvement of *Wolbachia* in host speciation using two different insect systems, i.e., Neotropical *Drosophila* and African tsetse flies, both presently under incipient speciation in nature, carrying closely related but incompatible *Wolbachia* strains. We will also show that naturally incompatible and sterile interspecies hybrids of both systems can be rescued by means of mild paternal *Wolbachia*-knockdown before forced mating, giving rise to fertile progeny and thereby stable hybrid lines via sib mating. Even more surprisingly, such rescued hybrid lines show complete sexual isolation to their parental lines plus unambiguous signatures of paternal inheritance of both their cytoplasmic endosymbionts, i.e. of mitochondria and *Wolbachia*.

## Keywords

*Wolbachia*, mitochondria, mutualism, hybridization, introgression, reproductive isolation, speciation



# **Towards population-level microbiome monitoring: the Flemish gut flora project**

**Author**

**Jeroen Raes – Invited**

KU Leuven, Herestraat 49 - box 1028, 3000 Leuven, Belgium

Alterations in the gut microbiota have been linked to various pathologies, ranging from inflammatory bowel disease and diabetes to cancer. Although large numbers of clinical studies aiming at microbiome-based disease markers are currently being performed, our basic knowledge about the normal variability of the human intestinal microbiota and the factors that determine this still remain limited. Here, I will present a large-scale study of the gut microbiome variation in a geographically confined region (Flanders, Belgium). A cohort of >5000 individuals from the normal population is sampled for microbiome analysis and extensive metadata covering demographic, health- and lifestyle-related parameters is collected. Based on this cohort, a large-scale cross-sectional study of microbiome variability in relation to health as well as parameters associated to microbiome composition is being performed. In this presentation, I will discuss our experiences in large-scale microbiome monitoring, show how the development of dedicated computational approaches can assist in microbiome analysis and interpretation, and first results coming out of this effort.

# Metagenomic investigation of deep-sea vent tubeworm endosymbionts from Mid-Cayman Rise reveals new insights into metabolism and diversity

## Authors

**Julie Reveillaud**<sup>1,2</sup>, Colleen Cavanaugh<sup>2,3</sup>, Julie Huber<sup>2</sup>

<sup>1</sup>INRA/CIRAD, UMR 117 ASTRE, Montpellier, France

<sup>2</sup>Josephine Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA, USA

<sup>3</sup>Harvard University, Department of Organismic and Evolutionary Biology, Cambridge, MA 02138, USA

Two species of vestimentiferan tubeworms (*Escarpia* sp. and *Lamellibrachia* sp.2) were reported at an area of low-temperature hydrothermal diffuse vent flow at the Mid-Cayman Rise (MCR) in the Caribbean Sea. Here, we used 16S rRNA and ITS gene cloning, together with electron microscopy and a cultivation-independent metagenomic approach to characterize chemosynthetic symbionts in the trophosome tissue of *Escarpia* and *Lamellibrachia* at the MCR. 16S rRNA and ITS gene phylogenetic analysis indicated all MCR individuals harbored endosymbionts that were >99% identical, with ITS sequences forming a separate well-supported clade that diverged from those of symbionts of seep and vent vestimentiferans from the Pacific, Gulf of Mexico, and Mediterranean Sea. The metagenomes of the symbionts of two specimens of each tubeworm species were sequenced and two distinct Gammaproteobacteria genomes of more than 4 Mbp assembled. An Average Nucleotide Identity of 86.5% between these genomes, together with distinct 16S sequences, suggest the presence of several MCR endosymbiont phylotypes, with one draft genome shared between one *Escarpia* and two *Lamellibrachia* individuals. Genes for sulfur and hydrogen oxidation, nitrate (assimilatory and dissimilatory) reduction, glycolysis and the Krebs cycle, peptide, sugar, and lipid transporters, and both rTCA and CBB carbon fixation cycles were detected in the genomes, highlighting key and shared functions with symbiont metagenomes of *Riftia*, *Tevnia*, and *Ridgeia* from the Pacific. The potential for a second hydrogen oxidation pathway (via a bidirectional hydrogenase), formate dehydrogenation, a catalase, and several additional peptide transporters were found exclusively in the MCR symbionts. Marked gene content and sequence dissimilarity at the rRNA operon and whole genome level between vent and seep endosymbionts studied herein suggest symbionts from the MCR belong to novel tubeworm endosymbiont strains, possibly adapted to H<sub>2</sub>-rich (19 mM) systems. The present study further adds new evidence that tubeworm endosymbionts can potentially switch from autotrophic to heterotrophic metabolism and suggest their versatile metabolic potential may enable the host to exploit a wide range of environmental conditions.

## Keywords

Tubeworm, endosymbionts, metagenomics, Mid-Cayman Rise

# The evolution of interdependency by neutral evolution in holobionts

## Author

**Marc-André Selosse**<sup>1,2</sup>

<sup>1</sup> Institut de Systématique, Évolution, Biodiversité (ISYEB - UMR 7205 – CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, CP50, 75005, Paris, France

<sup>2</sup> Department of Plant Taxonomy and Nature Conservation, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland

In holobionts the evolution of each partner is partly driven by the other partners (co-evolution). The evolution of holobionts is often viewed as a progressive evolution, based on the emergence of new adaptive properties that enhance the fitness, or diversify the niche. In many cases, such evolution enhances the interdependence between partners, because new functions are only achieved in symbiosis. The present paper focuses on the emergence of interdependence, and emphasizes that it can arise even without emergence of any new property, nor any progressive evolution. I propose two examples where such a neutral evolution may have acted. First, this may explain why microbiotas are acting both in plants and animals as developmental signals for immunity maturation (and also for maturation of nervous system), as revealed by observation of germ-free animals and non-mycorrhizal plants. Since no evidence supports that microbial signals are more relevant than endogenous ones, a neutral evolution can account for such dependencies: any hypothetical endogenous signal can be lost because microbial colonization, reliably occurring at germination or birth, can substitute for it. Second, neutral evolution may explain the extreme genetic reduction in some endosymbiotic mitochondria and plastids, that fully lost their genome in some eukaryotic lineages (respectively, anaerobic or achlorophyllous lineages). Yet, they persisting as bodies surrounded by two membranes dividing in host cytoplasm. This extreme evolution of dependence to the host is facilitated by the genetic redundancy between the host and the endosymbiotic bacterium. Generally, when two initially independent partners permanently interact, redundant properties become unstable: a mutation in one of the partner can be complemented by the presence of the other, or even by a complementing mutation in the other. Independency is then lost without any gain of function, nor any positive selection, in a neutral evolution. Moreover, the accumulation of such ratchet steps over times progressively forbids reversion to independency. Neutral evolution is a major driver of reciprocal dependencies within holobionts.

## References

M.A. Selosse, A. Bessis, M.J. Pozo, 2014. Microbial priming of plant and animal immunity: symbionts as developmental signals. *Trends in Microbiology* 22: 607-613.

## Keywords

Coevolution, dependency, drift, mutualism

# Darwinian selection induces lamarckian adaptation in a holobiont model

## Authors

**Yoav Soen**<sup>1</sup>, Osmanovic D<sup>2</sup>, Kessler DA<sup>2</sup>, Rabin Y<sup>2,3</sup>

<sup>1</sup> Weizmann Institute of Science, Herzl, Rehovot 76100, Israel

<sup>2</sup> Department of Physics, Bar-Ilan University, Ramat Gan 52900, Israel

<sup>3</sup> NYU-ECNU Institute of Physics at NYU Shanghai 200062, China

Current models of animal evolution focus on selection of individuals, ignoring the much faster selection of symbiotic bacteria. To investigate the evolutionary implications of this population structure, we introduced a Population Genetics model of holobionts exposed to toxic stress. The stress can be alleviated by selection of resistant individuals (host and bacteria) and by secretion of a detoxification agent (“detox”). By defining a new measure we show that selection of resistant bacteria over one generation of hosts leads to stress-dependent increase in the tolerance of the hosts’ offspring. This benefit is mediated by co-alleviation of toxic and physiologic stress and typically persists over multiple generations of host. Prolonged exposure leads to independent adaptation by ‘group selection’ of bacterial communities with higher detox per bacterium. The latter is accompanied by stress-induced variability and by a previously unrealized type of assimilation of host phenotypes, which we term ‘bacterial assimilation’ (a bacterial equivalent of genetic assimilation by host-intrinsic alleles). Overall, these findings show that Lamarckian adaptation, group selection and stress-induced variability can arise via interactions between two levels of Darwinian selection within a holobiont system. The conclusions and modelling framework are applicable to diverse types of holobiont systems.

## Keywords

Holobiont, Darwinian selection, Lamarckian adaptation, evolution, stress, host-microbe interactions, symbiosis, group selection, stress-induced variability, bacterial assimilation, population genetics



## Session 2

# "Emerging approaches to holobiont research"



## From simple to complex: phytobiomes and the vision for agriculture in 2050

### Author

**Kellye Eversole**

International Alliance for Phytobiomes Research, 2481 NE Marywood Court, Lee's Summit, Missouri, USA 64086

To meet the demands of a global human population expected to exceed 9.6 billion by 2055, crop productivity in sustainable agricultural systems must improve considerably in the face of a steadily changing climate and increased biotic and abiotic stressors. Traditional agricultural sciences have relied mostly on research within individual disciplines and linear, reductionist approaches for crop improvement as well as for production methods and practices. While significant advancements have been made in developing and characterizing genetic and genomic resources for crops, we still have a very limited understanding of genotype by environment by management (GxExM) interactions that determine productivity, sustainability, quality, and resistance to biotic and abiotic stressors. Embracing complexity and the non-linear organization and regulation of biological systems would enable a paradigm shift in breeding and crop production by allowing us to move towards a holistic, systems level approach that integrates a wide range of disciplines (e.g., geophysics, biology, agronomy, physiology, genomics, genetics, breeding, physics, pattern recognition, feedback loops, modeling, and engineering) and knowledge about phytobiomes (i.e., plants, their associated macro- and micro-organisms, and the geophysical environment of distinct geographical sites). By focusing on the phytobiome, by 2050, we will be able to elucidate, quantify, model, predict, and ultimately prescribe the cropping systems, methods, and management practices most suited for sustainable production on a particular farm, grassland, or forest. To ensure progress towards this vision, the new International Alliance for Phytobiomes Research, an industry-academic consortium, has been created.

### Keywords

Phytobiomes, precision agriculture, weather models, predictive analytics, plant microbiome, soil microbiome, multi-disciplinary science

# Multi-scale characterization of symbiont diversity in the pea aphid complex through metagenomic approaches

## Authors

**Cervin Guyomar**<sup>1</sup>, Fabrice Legeai<sup>1,2</sup>, Christophe Mougél<sup>1</sup>, Claire Lemaitre<sup>2</sup>, Jean-Christophe Simon<sup>1</sup>

<sup>1</sup> INRA, UMR 1349 INRA/Agrocampus Ouest/Université Rennes 1, Institut de Génétique, Environnement et Protection des Plantes (IGEPP), Le Rheu, France

<sup>2</sup> INRIA/IRISA/GenScale, Campus de Beaulieu, Rennes, France

Most metazoans are involved in durable symbiotic relationships with microbes which can take several forms, from mutualism to parasitism. The advances of NGS technologies and bioinformatics tools have opened new opportunities to shed light on this hidden but very influential diversity. The pea aphid is a model insect system for symbiont studies. It harbors both an obligatory symbiont supplying key nutrients and several facultative symbionts bringing some novel functions to the host, such as protection against natural enemies and thermal stress. The pea aphid is organized in a complex of biotypes, each adapted to a specific host plant of the legume family and having its own symbiont composition. Yet, the metagenomic diversity of the biotype-associated symbiotic community is still largely unknown. In particular, little is known on how the symbiotic genomic diversity is structured at different scales: across host biotypes, amongst individuals of the same biotype, or within individual aphids. We used high throughput whole genome metagenomic sequencing to characterize with a fine resolution the metagenomic diversity of both individual resequenced aphids and biotype specific pooled aphids. By a reference genome mapping approach, we first assessed the taxonomic diversity of the samples and built symbiont specific read sets. We then performed a genome-wide SNP-calling, to examine the differences in bacterial strains between samples. Our results revealed different diversity patterns at the three considered scales for the pea aphid symbionts. At the inter-biotype and intra-biotype scales, the primary symbiont *Buchnera* and some secondary symbionts such as *Serratia* showed a biotype specific diversity. We showed evidence for horizontal transfer of a *Hamiltonella* strain between biotypes, and found two distinct strains of *Regiella* symbionts within some biotypes. At the finest intra-host diversity scale, we also showed that these two strains of *Regiella* may coexist inside the same aphid host. This study highlights the huge potential of bioinformatics analyses of metagenomic dataset in exploring microbiote diversity in relation with host variation.

## Keywords

Metagenomics, aphids, diversity, symbionts, SNP-calling

# A transcriptomic approach to study marine plankton holobionts

## Authors

**Arnaud Meng**<sup>1,†</sup>, Erwan Corre<sup>2</sup>, Pierre Peterlongo<sup>4</sup>, Camille Marchet<sup>4</sup>, Adriana Alberti<sup>5</sup>, Corinne Da Silva<sup>5</sup>, Patrick Wincker<sup>5</sup>, Ian Probert<sup>6,7</sup>, Noritoshi Suzuki<sup>8</sup>, Stéphane Le Crom<sup>1</sup>, Lucie Bittner<sup>1\*</sup>, Fabrice Not<sup>6,7\*</sup>

<sup>1</sup> Sorbonne Universités, UPMC Univ Paris 06, Univ Antilles Guyane, Univ Nice Sophia Antipolis, CNRS, Evolution Paris Seine - Institut de Biologie Paris Seine (EPS - IBPS), 75005 Paris, France.

<sup>2</sup> CNRS, UPMC, FR2424, ABiMS, Station Biologique, Roscoff 29680, France.

<sup>4</sup> INRIA Rennes - Bretagne Atlantique/IRISA, EPI GenScale, Rennes 35042, France.

<sup>5</sup> CEA - Institut de Génomique, GENOSCOPE, 2 rue Gaston Crémieux, 91057 Evry, France.

<sup>6</sup> CNRS, UMR 7144, Station Biologique de Roscoff, Place Georges Teissier, Roscoff, France.

<sup>7</sup> Sorbonne Universités, Université Pierre et Marie Curie (UPMC) Paris 06, UMR 7144, Station Biologique de Roscoff, Place Georges Teissier, Roscoff, France.

<sup>8</sup> Institute of Geology and Paleontology, Graduate School of Science, Tohoku University, Sendai City, 980-8578, Japan.

\* These authors contributed equally to this work.

† Corresponding authors. ([arnaud.meng@etu.upmc.fr](mailto:arnaud.meng@etu.upmc.fr))

Symbiosis is a widespread phenomenon in the biosphere. In the water column, planktonic organisms are key component of pelagic ecosystems and many species form mutualistic association with microalgae forming a photosymbiosis [1]. Here we intended to investigate the genetic basis of photosymbiosis, through a transcriptomic approach on marine plankton organisms. We focused on associations occurring between radiolarian host (protist, zooplankton) and dinoflagellates symbiont (protist, phytoplankton) living inside the host cell. These holobionts are widespread in oligotrophic open oceans and have fundamental implications in biogeochemical carbon, silica and strontium cycles [2, 3]. The study of such non-model holobionts RNA-seq datasets requires *de novo* assembly, which implies considerable computational resources and the potential production of chimeric sequences [4]. We therefore developed an original strategy aiming at accelerating and improving *de novo* assembly. We used SRC\_Linker to compare our holobionts transcriptomes to publicly available data generating two datasets, one composed of reads from the symbionts and another with reads from the host. Independent assemblies were performed in parallel, accelerating the study process, and minimizing the proportion of chimeric sequences. Our strategy produced a comprehensive genomic dataset for Radiolaria [5], and offers a large-scale comparison strategy to study holobionts [6]. These new sequences obtained will be used for phylogenomics investigation, as reference for environmental metagenomic studies and to understand and characterize the molecular basis of symbiotic relationships in the plankton.

## References

[1] Janoušková, J. *et al.* “Major transitions in dinoflagellate evolution unveiled by phylotranscriptomics”. in: PNAS 201614842 (2016). doi:10.1073/pnas.1614842114 [2] Tristan Biard *et al.* “*In situ* imaging reveals the biomass of giant protists in the global ocean”. In: *Nature* advance online publication (Apr. 20, 2016). issn: 0028-0836. doi: 10.1038/nature17652. [3] Lionel Guidi *et al.* “Plankton networks driving carbon export in the oligotrophic ocean”. In: *Nature* 532.7600 (Apr. 28, 2016), pp. 465–470. issn: 0028-0836. doi: 10.1038/nature16942. [4] Sergio Balzano *et al.* “Transcriptome analyses to investigate symbiotic relationships between marine protists”. In: *Frontiers in Microbiology* 6 (Mar. 17, 2015). issn: 1664-302X. doi: 10.3389/fmicb.2015.00098. [5] Burki, F. *et al.* “Evolution of Rhizaria: new insights from phylogenomic analysis of uncultured protists”. In *BMC Evolutionary Biology* 10, 377 (2010). [6] Sangwan, N., Xia, F. & Gilbert, J. A. “Recovering complete and draft population genomes from metagenome datasets”. In: *Microbiome* 4, 8 (2016).

## Keywords

Transcriptomics, symbiosis, plankton



# Genomic analysis of the main bacterial symbiont of the polycyclic guanine alkaloid-producing sponge *Crambe crambe*

## Authors

**Marcelino T Suzuki**<sup>1</sup>, Victor le Layec<sup>1</sup>, Sapna Chitlapilly-Dass<sup>1</sup>, Olivier Thomas<sup>2</sup>, Gregory Genta-Jouve<sup>3</sup>, Thierry Perez<sup>4</sup>, Raphael Lami<sup>1</sup> and Didier Stien<sup>1</sup>

<sup>1</sup> Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes (LBBM), Observatoire Océanologique, F-66650 Banyuls/Mer, France

<sup>2</sup> National University of Ireland Galway Marine Biodiscovery School of Chemistry, College of Science Galway, Ireland

<sup>3</sup> Laboratoire de Chimie-Toxicologie Analytique et Cellulaire (C-TAC) UMR-CNRS 8638 COMETE Faculté de Pharmacie, Université Paris Descartes 75270 Paris, France

<sup>4</sup> Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale IMBE UMR CNRS7263 / IRD 237 / Aix Marseille Université, Station Marine d'Endoume, 13007 Marseille, France

Marine sponges are known sources of complex molecules, many of which are thought to be produced by associated symbiotic microorganisms. Due to their cytotoxic or antimicrobial activities, many hypotheses exist for the role of these compounds to the holobiont, such predator deterrence, antifouling microbiome control. As in most cases, the concentrations of these compounds are low in the sponges, these putative roles remain unproven. A notable exception, polycyclic guanidine alkaloids (PGA) from crambeidae sponges are present at high concentrations and have established ecological roles such as antifouling agents. Paradoxically, neither their organismal origin nor their biosynthesis is known. Recently we have discovered that a single betaproteobacterium species is present and dominates the microbiota of the crambeidae sponge *Crambe crambe* (Croue et al. 2013) and novel polycyclic guanidine alkaloids structures that led to the proposal of a new biosynthetic hypothesis for PGA biosynthesis (Genta-Jouve et al. 2014). We have assembled a partial genome from the major symbiont of *C. crambe* from single cell and shotgun metagenomics sequences and will present results of an ongoing analysis of these sequences in the context of the symbiosis at large, and the implication on this bacterium in the synthesis or degradation of PGAs.

## References

Croue, J., West, N.J., Escande, M.L., Intertaglia, L., Lebaron, P., Suzuki, M.T.\* 2013 A single betaproteobacterium dominates the microbial community of the crambescidine-containing sponge *Crambe crambe*. *Sci Rep* 3:2583 <http://dx.doi.org/10.1038/srep02583> Genta-Jouve, G., Croué, J., Weinberg, L., Cocandeau, V., Holderith, S., Bontemps, N., Suzuki, M and O.P. Thomas. 2014 Two-Dimensional Ultra High Pressure Liquid Chromatography Quadrupole/Time-of-Flight Mass Spectrometry for Semi-Targeted Natural Compounds Identification. *Phytochemistry Letters* 10: 318-323 <http://dx.doi.org/10.1016/j.phytol.2014.10.029>

## Keywords

Marine sponge, *Crambe crambe*, single cell genome, polycyclic guanidine alkaloids



# Understanding and manipulating the plant holobiont

## Author

**Etienne Yergeau**

Centre INRS-Institut Armand-Frappie, 531 boul. des Prairies, H7V1B7 Laval, Canada

Plants and their microbiota form an inseparable entity known as a holobiont. The concept of the hologenome (all the genomes of the holobiont) as an evolutionary unit suggests mechanisms that could be harnessed to rapidly evolve/adapt holobionts. For this presentation, I will discuss recent studies that aimed at understanding and manipulating the microbial part of the plant holobiont. More specifically, I will: 1) compare the capacity of various plants to recruit beneficial microorganisms under stressful conditions, 2) contrast the effects of the plant genotype and the environment on the stress response and 3) show that manipulating the microbiota can modify the phenotype of the host plant. Globally, my research shows that it is possible to modify the plant holobiont through microbiota manipulation, highlighting the central role of microbes in plant phenotypic plasticity. More research will be however necessary to completely understand the mechanisms involved and apply them for e.g. increase crop yields.

## Keywords

Microbiota manipulation, metatranscriptomics, agriculture, phytoremediation

# Session 3

## "Microbiota and host health"



### ***Homo sapiens symbioticus* : an endangered species ?**

#### **Author**

**Joël Doré – Invited**

INRA, MetagenoPolis, 78350 Jouy en Josas, France

During the first months of life, the newborn develops as an intimate symbiosis between human cells and complex microbial communities that colonize the skin, intestinal contents and all mucosal surfaces of the body. As any cell, our microbial symbionts are recognized as ‘self’ by our immune system and homeostasis of symbiosis is essential for the maintenance of health and wellbeing. With a rich diversity of species and on average 25 times more genes than the human genome, the intestinal microbiota contributes major functions that pertain to host nutrition, physiology and ensure protection from colonization by environmental microbes.

An alteration of the intestinal microbiota - called dysbiosis - has been documented for a broad range of immune-mediated, chronic disorders. The latter have all increased in incidence over the past 60 years, to a point where one in four persons of the world population is affected today. After 25 years of exploration of dysbiosis in chronic conditions, we have come to realize that it corresponds to an alteration of man-microbes symbiosis.

Imposed over 2 to 3 generations, nutritional transition, exposure to environmental xenobiotics including therapeutics, as well as major changes in perinatal management and environment all combined drastic effects on man-microbes symbiosis. On top of this, it would only take a few feedback loops in host-microbes relationships to challenge the comforting concept of a continuum between health and disease. We propose and will provide preliminary evidence that alternative stable states derived from critical transition are a basic feature of altered man-microbes symbiosis and could characterize major diseases of modern societies.

Specific nutrients (fibers, polyphenols) and live microbes (probiotics, microbiotherapy preparations) would be bioactives of choice to restore symbiosis or prevent the onset or aggravation of chronic diseases. The current context hence calls for an urgent change in paradigm; towards preventive nutrition and clinical management addressed to man as a true man-microbes symbiosis.

## Harnessing insect symbiosis for the population control of agricultural pest and disease vector species

### Author

**Kostas Bourtzis**

Insect Pest Control Laboratory IAEA - Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Wagramer Strasse 5, A-1400 Vienna, Austria

Insect species have been masters in establishing sophisticated symbiotic associations with diverse microorganisms during evolution, particularly with bacterial species. These symbiotic bacteria have been shown to influence the biology, physiology, ecology and the evolution of their insect hosts and they play a catalytic role in nutrition, reproduction, mating behaviour, fitness, immunity, as well as in determining their pest status. Due to their unique biological properties, several insect symbionts and particularly the ones manipulating the reproductive properties of their insect hosts and providing protection against pathogens and parasites, such as *Wolbachia*, have been exploited for applications. A number of studies have shown that *Wolbachia* can be used as a tool in integrated pest / vector management strategies either for population suppression or for population replacement. In my presentation, I will focus on how we can exploit insect symbiosis for pest and disease control with an emphasis on the population suppression of major mosquito vector species via the integration of the sterile insect technique and *Wolbachia* symbiosis. Data from laboratory, semi-field and open field pilot trials against *Aedes* mosquito populations will be presented.

### Keywords

*Wolbachia*, sterile insect technique, pest and disease control

# Integrative analysis of the coral holobiont thermotolerance under contrasted thermal environment

## Authors

**Kelly Brener-Raffalli**<sup>1</sup>, Marine Pratlong<sup>2,3</sup>, Jeremie Vidal-Dupiol<sup>4</sup>, Mehdi Adjeroūd<sup>5</sup>, Pascal Romans<sup>6</sup>, Rémi Pillot<sup>6</sup>, Lionel Feuillassier<sup>6</sup>, Didier Aurelle<sup>2</sup>, Pierre Pontarotti<sup>3</sup>, Anne Haguenauer<sup>2</sup>, Guillaume Mitta<sup>1</sup> and Eve Toulza<sup>1</sup>

<sup>1</sup> Université de Perpignan Via Domitia, CNRS UMR 5244, Interactions Hôtes-Pathogènes-Environnements (IHPE), 66860 Perpignan France

<sup>2</sup> Aix-Marseille Université, CNRS, IRD, Avignon Université, IMBE UMR 7263, 13007, Marseille, France

<sup>3</sup> Aix-Marseille Université, CNRS UMR 7353, Équipe Évolution Biologique et Modélisation, Campus Saint-Charles, 3 place Victor Hugo, 13331 Marseille France

<sup>4</sup> UMR 241 Écosystèmes Insulaires Océaniques, IFREMER / Centre du Pacifique, Département Ressources Biologiques Environnement, Unité Ressources Marines en Polynésie Française, BP 7004, 98719 Taravao, Polynésie Française

<sup>5</sup> Institut de Recherche pour le Développement, UMR 9220 ENTROPIE, Laboratoire d'Excellence "CORAIL", UPVD 52 Avenue Paul Alduy, 66860 Perpignan, France

<sup>6</sup> Sorbonne Universités, UPMC Univ Paris 06, UMS 2348, Centre de Ressources Biologiques Marines, Observatoire Océanologique, F-66650 Banyuls/Mer, France

In the context of global change, corals are being submitted to an increase in sea surface temperatures that are already reaching values they never experienced in their lifetime. In order to better understand the mechanisms of thermal stress resistance of the coral, we developed an integrative approach on a coral holobiont from the *Pocillopora* genus. We performed an ecologically realistic thermotolerance experiment on *Pocillopora damicornis*, and we compared the response for two populations that naturally experience contrasted thermal regimes. One population from Oman is submitted to high thermal variations along the year and the other one from New-Caledonia is submitted to low annual thermal variations. We analyzed three genotypes for each population. We considered the whole coral holobiont, i.e. the cnidarian itself, its associated microbial community and the symbiotic dinoflagellate. We followed the transcriptomic response of the cnidarian using RNAseq, the dynamics of the microbiota (using 16S metabarcoding) as well as the Symbiodinium assemblages (using ITS2 metabarcoding) during the stress for the different populations and genotypes. Our results revealed that the holobiont response is strongly influenced by the host genome and highly dependent on the thermal regime they are experiencing in the field.

## Keywords

Coral, holobiont, symbiodinium, pocillopora, thermal stress, microbiota, RNAseq, metabarcoding

# Using nanoscale imaging technologies to understand differential susceptibility to coral bacterial infection

## Authors

**Emma Gibbin**<sup>1</sup>, A. Gavish<sup>2</sup>, I. Domart-Coulon<sup>3</sup>, L. Søgaard Jensen<sup>1</sup>, E. Kramarsky-Winter<sup>2</sup>, O. Shapiro<sup>2,4</sup>, A. Meibom<sup>1</sup>, A. Vardi<sup>2</sup>

<sup>1</sup> École Polytechnique Fédérale de Lausanne, Switzerland.

<sup>2</sup> Weizmann Institute of Science, Rehovot, Israel.

<sup>3</sup> Museum National d'Histoire Naturelle, MCAM UMR7245CNRS-MNHN, Paris, France

<sup>4</sup> Volcani Center for Agricultural Research, Rishon LeZion, Israel

Reef-building corals form complex relationships with photosynthesizing dinoflagellates (*Symbiodinium* sp.) and a consortia of microbial partners. Collectively known as the coral holobiont, the complex signaling and metabolic interactions that occur between the different partners enables corals to thrive in the oligotrophic waters of the tropics. Exposure to above-average seawater temperatures disrupts these relationships and thus, the metabolic interactions that sustain the holobiont, resulting in the expulsion of *Symbiodinium* and/or the manifestation of disease. Over the past decade, there has been an increase in the prevalence and severity of both episodic bleaching and disease outbreaks. It is perhaps unsurprising that thermally-stressed corals are more susceptible to disease, but it is not clear whether this is due to increased virulence of microbial pathogens or a reduction in the immune response of the coral host. We use the association between *Pocillopora damicornis* and *Vibrio coralliilyticus* as a model to address this knowledge gap. This relationship is particularly interesting in the context of climate change as *V. coralliilyticus* only becomes virulent in conditions 2 to 5 °C above ambient temperatures. A paired experimental design was implemented whereby infected (INF) *P. damicornis* fragments inoculated with <sup>15</sup>N-labeled, DsRed-tagged *V. coralliilyticus* for two hours, were paired with non-infected, control (CON) fragments. Both INF and CON fragments were exposed to isotopically H<sup>13</sup>CO<sub>3</sub><sup>-</sup>-labeled seawater and photosynthesis-saturating light levels during the experiment. The progression of the infection was monitored in real time using the “Coral-on-a-Chip” microfluidic system. Once clear symptoms of disease (the development of lesions, biofilms and/or tissue necrosis) were observed, the experiment was quenched and the samples fixed. The onset of symptoms differed temporally between fragments, ranging from 3.5 to 9 hours post-infection (HPI), while some fragments failed to develop disease symptoms over the duration of the light period (13.5 HPI). Following fixation, samples were embedded in resin, sectioned and prepared for immunolocalisation and Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS) imaging. Immunolocalisation, achieved via the application of a primary anti-RFP antibody detected with Alexafluor647 conjugated secondary IgG, permitted the localization of labelled *Vibrio* pathogens within coral tissues, while NanoSIMS analysis enabled us to track simultaneously the photosynthetically fixed H<sup>13</sup>CO<sub>3</sub><sup>-</sup> in the symbiont dinoflagellates and the presence of <sup>15</sup>N-labeled pathogens in the coral tissue. Here, we present high-resolution images of the infection process, from the initial inoculation of the coral to the colonization of tissue and the development of disease symptoms, while simultaneously quantifying the reduction in photosynthetic performance of the symbiont dinoflagellates as infection progresses. Our approach and results add a unique mechanistic perspective to the complexities of coral disease, which is a crucial step towards understand how corals will fare under global climate change.

## Keywords

Coral disease, infection, vibrio, NanoSIMS, immunolocalisation





# Multi-kingdom microbial consortia maintain microbial homeostasis in roots and promote plant health

## Authors

Paloma Durán<sup>1</sup>, Thorsten Thiergart<sup>1</sup>, Eric Kemen<sup>1</sup>, Carlos Alonso-Blanco<sup>2</sup>, Fabrice Roux<sup>3</sup>, Jon Ågren<sup>4</sup>, Paul Schulze-Lefert<sup>1</sup>, **Stéphane Hacquard**<sup>1</sup>,

<sup>1</sup> Department of Plant-Microbe Interactions, MIPZ, Cologne, Germany

<sup>2</sup> Centro Nacional de Biotecnología, CSIC, Madrid, Spain

<sup>3</sup> Laboratoire des Interactions Plantes-Microorganismes, INRA, Castanet-Tolosan, France

<sup>4</sup> Department of Ecology and Genetics, EBC, Uppsala University, Uppsala, Sweden.

In nature, healthy plants cohabit with a plethora of microbes, such as bacteria, fungi, and oomycetes, forming complex microbial consortia that impact plant growth and health. Here we aimed at investigating the complex microbial communities that are established on plant roots, where bacteria, fungi and oomycetes are not considered as separated entities but rather as internal drivers of overall community structure. We profiled the microbial communities associated with *Arabidopsis* roots from a large-scale European survey (16 natural populations). Using amplicon sequencing of the bacterial 16s rRNA gene and fungal/oomycete ITS, we identified structural convergence for root-associated bacterial communities, whereas root-associated fungal and oomycetal communities were more similar to soil's, hosting more site-specific members. Moreover, we showed that soil characteristics are good predictors of bacterial community structure in soil but not in roots. Using comprehensive *Arabidopsis* root-associated bacterial, fungal and oomycete culture collections with gnotobiotic plant systems, we investigated how multi-kingdom microbial communities interact with each other and affect plant host fitness without any external cues. We showed that re-colonization of *Arabidopsis* by oomycetal and fungal communities have a strong deleterious impact on plant growth, whereas in combination with bacteria, this phenotype is recovered. This bacteria growth rescue is associated with a fungal community shift, demonstrating that inter-kingdom interactions are key for microbial community establishment and plant health.

## Keywords

Plant microbiota, fungi, bacteria, oomycetes, microbial interactions

# NOD2 influences intestinal resilience and fungal signatures after antibiotic perturbation

## Authors

**Jacqueline Moltzau-Anderson**<sup>1,2</sup>, Ateequr Rehman<sup>1</sup>, Maren Paulsen<sup>1</sup>, Simone Lipinski<sup>1</sup>, Wei-Hung Pan<sup>1</sup>, Robert Häsler<sup>1</sup>, Richa Bharti<sup>1</sup>, Christian Kautz<sup>1</sup> and Philip Rosenstiel<sup>1</sup>

<sup>1</sup> Institute for Clinical Molecular Biology (IKMB), Christian-Albrechts-University (CAU) Kiel, Germany

<sup>2</sup> Max Planck Institute (MPI) for Evolutionary Biology, Plön, Germany

Microbial communities are important for physiological homeostasis in the mammalian gut. In Crohn's disease, risk alleles on the NOD2 locus disrupt and destabilize the NOD2 protein, which senses bacterial cell wall products and is highly expressed in specialized intestinal epithelial cells. A disturbed resilience is hypothesized to be an environmental factor precipitating disease manifestation through this genetic variation. Thus, understanding how the microbiota interacts with the host's genotype, to respond to antibiotics as a selective pressure, is crucial to determine how stability of community composition is maintained. Using a mouse model deficient in the Crohn's Disease risk gene, NOD2, we aimed to investigate the role of this innate immune receptor for microbial resilience after a perturbation. Wild-type C57BL6(WT) and knock-out NOD2(KO) mice were treated for two weeks with broad-spectrum antibiotics and the fecal microbial composition was followed for 10 weeks. Using 16S and ITS1 sequencing, we determined the bacterial and fungal community composition, respectively. Additionally, we assessed the occurrence of selected known resistance genes using qPCR. Antibiotics significantly altered the composition of the microbial gut community in both genotypes, with *Escherichia/Shigella* becoming the dominant bacterial genera during the two-week administration. However, while bacterial diversity decreased in both genotypes, fungal diversity increased, and numerous significant correlations between the bacterial and fungal microbiota were identified as co-occurrence or co-exclusion interactions. Interestingly, our results demonstrated a phenotypic variation, where the NOD2 genotype impairs resilience of the bacterial gut microbiota leading to a delayed recovery. Furthermore, antibiotic use caused a significant increase in antibiotic resistance genes, and was independent of genotype. Despite its relevance in disease, the fungal microbiota have been largely ignored. Here we demonstrate a complex relationship between gut bacteria and fungi, where antibiotic perturbation creates niche availability and the expansion of potentially opportunistic genera. Additionally, the delayed recovery in the bacteriome of the KO creates a 'window of opportunity', which may promote pathogen proliferation and the development of gastrointestinal inflammation.

## Keywords

NOD2, IBD, resilience, antibiotic perturbation, gut microbiome

## Medicine through a hologenomic lens

### Author

**Kevin R. Theis - Invited**

Wayne State University, 540 East Canfield, Room 7130 Scott Hall, 48201 Detroit, USA

Biologists and philosophers of biology are increasingly focusing on the ecological and evolutionary dynamics of host-microbial organization, interaction, and function. They are pursuing questions about organismality, individuality, levels of selection, fidelity of microbiome transmission and/or transgenerational association among hosts, the scope of host-microbiota cooperation and conflict, and the scales at which ecology and evolution feed into each other in the context of host-microbial symbioses. In addition to providing a vocabulary and framework for contemporary dialogue on hosts in light of their ubiquitous and complex microbiomes, foundational theses such as the hologenome concept of evolution have theoretical and practical ramifications for the study of animal biology, including medicine. In this presentation, I will explain how viewing a syndromic medical problem through a hologenomic lens can translate to enhanced research and clinical care in medicine. I will use evolutionary and precision medicine as a theoretical framework for discussion and perinatal medicine as a developing practical proof-of-concept.

# Helminth burden and ecological factors associated with wild host gastrointestinal microbiota

## Authors

**Lindsay K Newbold**<sup>1</sup>, Sarah J Burthe<sup>2</sup>, Anna E Oliver<sup>1</sup>, Hyun S Gweon<sup>1</sup>, Christopher J Barnes<sup>1,3</sup>, Francis Daunt<sup>2</sup> and Christopher J van der Gast<sup>1,4</sup>

<sup>1</sup> NERC Centre for Ecology & Hydrology, Wallingford, UK

<sup>2</sup> NERC Centre for Ecology & Hydrology, Penicuik, UK

<sup>3</sup> National History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

<sup>4</sup> Manchester Metropolitan University, School of Healthcare Science, Manchester, UK

Infection by gastrointestinal helminths of humans, livestock and wild animals is common, but the impact of such endoparasites on wild hosts and their gut microbiota represents an important overlooked component of population dynamics. Wild host gut microbiota and endoparasites occupy the same physical niche spaces with both affecting host nutrition and health. However, associations between the two are poorly understood. This work utilised a non-destructive sampling method (Endoscopy) on the commonly parasitized European shag (*Phalacrocorax aristotelis*) a 2kg seabird, as a model for wild host-parasite-microbiota interactions. Forty live adults from the same colony were sampled. Endoscopy was employed to quantify helminth infection in situ. Microbiota from the proventriculus (site of infection), cloacal and faecal gastrointestinal tract microbiomes were characterised using 16S rRNA gene-targeted high-throughput sequencing. Increasingly strong associations between helminth infection and microbiota composition were found, progressing away from the site of infection. This work within live wild animals, forms a foundation to better understand the ecological mechanisms that underpin the three-way relationship between helminths, microbiota and hosts.

## References

Newbold, L.K., Burthe, S.J., Oliver, A.E., Gweon, H.S., Barnes, C.J., Daunt, F., and van der Gast, C.J. (2016) Helminth burden and ecological factors associated with alterations in wild host gastrointestinal microbiota. ISME J advance online publication. doi:10.1038/ismej.2016.153

## Keywords

European shags, helminth infection, microbiome, parasites

# Biodiversity of the human gut microbiome: influence of diet and parasitism

## Authors

**Laure Segurel**<sup>4, 5</sup>, E. Morton<sup>1</sup>, J. Lynch<sup>1</sup>, A. Froment<sup>2</sup>, S. Lafosse<sup>3</sup>, E. Heyer<sup>4</sup>, M. Przeworski<sup>5</sup>, R. Blekhman<sup>1</sup>

<sup>1</sup> University of Minnesota, Minneapolis, United States of America

<sup>2</sup> IRD, Paris, France

<sup>3</sup> CNRS, Paris, France

<sup>4</sup> MNHN, Paris, France

<sup>5</sup> Columbia University, New York, United States of America

Humans live in close proximity with multiple microbial communities living in/on them, of which the gut microbiome. This intimate relationship has been challenged many times during human evolutionary history. It has notably been shown that there is a loss of biodiversity in urban industrialized populations when compared to rural populations. However, we know little about the relative contribution of diet, hygiene or host genetics to these patterns. Here, we focus on fine-scale comparisons of African rural populations in order to contrast the gut microbiomes of populations that inhabit similar environments but have different diets (hunter-gatherers, farmers) and to evaluate the effect of parasitism on microbiome composition. To this end, we profiled the gut microbiota and intestinal parasites in 64 individuals from Cameroon. We found that the presence of an intestinal protozoa, *Entamoeba*, is strongly correlated with microbial composition and diversity. We also found gut communities to vary significantly with subsistence mode, with some taxa previously shown to be enriched in other hunter-gatherers groups. Our study thus highlights the substantial variability in gut microbiome composition among closely related populations and suggests an important role for eukaryotic gut inhabitants, one that has been largely overlooked in studies of the microbiome to date.

## Keywords

Gut microbiome, *Entamoeba*, subsistence, lifestyle, diet



# Session 4

## "Mechanisms for holobiont assembly"



### Structure and function(s) of the plant root microbiota

#### Author

**Paul Schulze-Lefert - Invited**

Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany.

E-mail: [schlef@mpipz.mpg.de](mailto:schlef@mpipz.mpg.de)

We have previously shown that healthy roots of *Arabidopsis thaliana*, grown in natural soils, are colonized by a bacterial consortium with well-defined taxonomic structure. Members of this root microbiota belong mainly to the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Bulgarelli *et al.*, *Nature* 2012). A comparison of the bacterial root microbiota of *A. thaliana* with *A. thaliana* relatives, grown under controlled environmental conditions or collected from natural habitats, demonstrated a largely conserved microbiota structure with quantitative, rather than qualitative, species-specific footprints (Schl ppi *et al.*, *PNAS* 2014). Unlike this, the root microbiota of monocotyledonous barley and dicotyledonous *A. thaliana*, grown in the same soil type, revealed a similar overall structure, but with several bacterial taxa uniquely enriched in the Brassicaceae (Bulgarelli *et al.*, *Cell Host&Microbe*, 2015). This suggests bacterial root microbiota structure is an ancient plant trait that was already present in the last common ancestor of monocotyledonous and dicotyledonous plants. We have isolated more than 8000 *A. thaliana* root- and leaf-associated microbiota members as pure bacterial cultures, representing the majority of *A. thaliana* microbiota members that are detectable by culture-independent community profiling methods, and generated whole-genome sequence drafts for a core collection of 400 isolates (Bai *et al.*, *Nature* 2015). I will discuss how we utilize synthetic communities and gnotobiotic plant systems to define principles underlying root microbiota establishment. I will also present our reductionist approaches to examine proposed microbiota functions in plant health.

# A core microbiota of plant and earthworm interaction? Phylogenetic and functional aspects

## Authors

**Manuel Blouin**<sup>1</sup>, Samuel Jacquioud<sup>1</sup>, Arnaud Mounier<sup>1</sup>, Aymé Spor<sup>1</sup>, Ruben Puga-Freitas<sup>2</sup>, Christophe Mougel<sup>3</sup>, Cécile Monard<sup>4</sup>

<sup>1</sup> UMR Agroécologie (AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté), France

<sup>2</sup> UMR 7618 IEES-Paris (CNRS, INRA, UPMC, IRD, UPEC), France

<sup>3</sup> UMR 1349 IGEPP (INRA - Agrocampus Ouest – Université Rennes 1), France

<sup>4</sup> UMR 6553 ECOBIO (CNRS, Université de Rennes 1), France

The core microbiota concept has been proposed to describe the subset of a microbiota (e.g. the rhizosphere microbial community) associated with a given host (e.g. a plant) going beyond macroenvironment differences (e.g. soil type), and characterized by taxonomic markers (e.g. 16S rRNA gene sequences). Its existence has been questioned by geographical studies, showing the overruling soil type effect in shaping microbial communities. As far as biotic determinants are concerned, several “hosts” or macroorganisms are impacting a given habitat and its specific microbial community. In soils, there is an overlap between the so-called rhizosphere and the drilosphere, defined as the functional domains under the influence of plant roots and earthworms respectively. Plants and earthworms have been sharing the same soils over geological times, thus microbial communities living at the congruence of rhizosphere and drilosphere could be specific of plant-earthworm interaction, as a consequence of their coevolution. Here we tested the hypothesis of a specific core microbiota at the interface of two interacting macroorganisms, in three different soils. We grew barley in microcosms in the presence/absence of the endogeic earthworm *Aporrectodea caliginosa*. We investigated the structure of microbial communities and quantified their abundance. Because interactions between earthworms and plants have been proposed to be mediated through the mineralization of organic matter and the release of nutrients, we also studied the abundance of genes involved in nitrogen cycling in different micro environments (bulk soil, rhizosphere and earthworm casts). 16S rRNA gene amplicon sequencing revealed the presence of microsites core microbiota over the three soil types. Despite an expected strong influence of soil type on microbial community composition (59% of explained variance), significant effects of both microsites (13%) and macro-organisms presence (7%) were observed. Many genes of the nitrogen cycle were influenced by soil type, whereas earthworms were only responsible for an increase in the abundance of the *ureC* gene. The interaction between plant and soil type was modifying the abundance of *nosZ*, *nifH* and *narG*, in the rhizosphere suggesting a strong control of the nitrogen cycle by the plant dependent on soil fertility. A refined analysis on each soil separately revealed a significant interaction between plant and earthworm presence on the structure of the rhizosphere and drilosphere microbiota. This suggests that assembly of microbial community in plant rhizospheres and earthworm casts was dependent on the mutual presence of earthworm and plant respectively. Several bacterial species were significantly enriched at the interface of plant rhizosphere and earthworm casts, suggesting that direct/indirect relationships between macroorganisms associated microbiota resulted in recruiting and promoting specific members.

## Keywords

Archaea, bacteria, fungi, core microbiota, nitrogen cycle, plant – earthworm interaction, soil fertility



# The woodlice holobiont: an all in one toolbox for animal-microbe interactions of ecological relevance

## Authors

**Didier Bouchon**<sup>1</sup>, Martin Zimmer<sup>2</sup>, Jessica Dittmer<sup>3</sup>

<sup>1</sup> UMR CNRS 7267, Université de Poitiers, Poitiers, France

<sup>2</sup> Leibniz Center for Tropical Marine Ecology, Bremen, Germany

<sup>3</sup> Rowland Institute at Harvard, Harvard University, Cambridge, MA, USA

Bacterial symbionts represent essential drivers of arthropod ecology and evolution, influencing host traits such as nutrition, reproduction, immunity, and speciation. However, the majority of work on arthropod microbiota has been conducted in insects and more studies in non-model species across different ecological niches will be needed to complete our understanding of host–microbiota interactions. In this context, terrestrial isopod crustaceans constitute an emerging model to investigate symbiotic associations with potential relevance to ecosystem functioning. Terrestrial isopods comprise a group of crustaceans that have evolved a terrestrial lifestyle and represent keystone species in terrestrial ecosystems, contributing to the decomposition of organic matter and regulating the microbial food web. As for many symbioses, the association between isopods and microorganisms had initially been viewed as highly specific binary interactions between one host and one symbiont. We present the current knowledge of terrestrial isopods as holobionts representing a large scope of host–symbiont associations, including reproductive parasites, bacterial pathogens and several symbionts specifically associated with digestive tissues. The high diversity of the woodlice holobiont may constitute an all-in-one tool box for the study of host-microbe interactions. We will discuss several particular associations that offer opportunities for the study of symbiosis: *Wolbachia* as a model to study genetic conflicts, *Rickettsiella* as a model to study transitions between pathogenicity and mutualism, *Rhabdochlamydia porcellionis* as a model to study complex pathogen life cycles, *Hepaticola* and *Hepatoplasma* as models to study nutritional symbiosis and symbiont-symbiont interactions, *Bacilloplasma* as a model to study ancient host–symbiont relationships and viruses and Acanthocephalan parasites as models to study host manipulations. We will identify future directions to (i) fully understand the functional roles of particular bacteria (both intracellular or intestinal symbionts and environmental gut passengers), and (ii) whether and how the host-associated microbiota could influence the performance of terrestrial isopods as keystone species in soil ecosystems.

## References

Bouchon, D., Zimmer, M., Dittmer, J. (2016) The terrestrial isopod microbiome: An all-in-one toolbox for animal-microbe interactions of ecological relevance. *Frontiers in Microbiology*, (7):1472. Dittmer, J., Lesobre, J., Moumen, B., Bouchon, D. (2016) Host origin and tissue microhabitat shaping the microbiota of the terrestrial isopod *Armadillidium vulgare*. *FEMS Microbiology Ecology*, 92(5):fiw063. Dittmer, J., Beltran-Bech, S., Lesobre, J., Raimond, M., Johnson, M., Bouchon, D. (2014) Host tissues as microhabitats for *Wolbachia* and quantitative insights into the bacterial community in terrestrial isopods. *Molecular Ecology*, 23 (10):2619-2635.

## Keywords

*Wolbachia*, microbiota, terrestrial isopods



# The inner help: bleached *Exaiptasia pallida* (Anthozoa, Actiniaria) farms and allocates remaining endosymbionts ensuring the inheritance of *Symbiodinium* spp. to offspring

## Authors

**Isabel Casillas-Barragán**<sup>1</sup>, Marcelo Visentini Kitahara<sup>2</sup>, Márcio Reis Custódio<sup>1</sup>

<sup>1</sup> Departamento de Fisiologia Geral, Instituto de Biociências, Universidade de São Paulo

<sup>2</sup> Departamento de Ciências do Mar, Universidade Federal de São Paulo

Loss of the associated microalgae in symbiotic cnidarians, commonly known as bleaching, impairs energy budget and several physiological processes of the host, eventually leading to its death. However, in some cases, host animals reestablish the association, using mechanisms that are still poorly understood. Understanding the recovery time span might accurate predictions about consequences of eventual successive bleaching events. In this study, we investigated bleaching recovery and pigmentation patterns on different functional body parts of bleached *Exaiptasia pallida*, an actiniarian that lives in symbiosis with the dinoflagellate *Symbiodinium* spp.. We measured time of holobiont's pigmentation at each body part in an oligotrophic environment during 46 days at three ontogenetic stages through a six levels color scale. Also, we analyzed histochemical composition of the symbioma and its ultrastructure through scanning and transmission electron microscopy. We found a sequence of pigmentation recovery after bleaching notwithstanding its ontogeny but delayed on earlier stages. Independently on holobiont's size, tentacles pigmented first. After 10 and 24 days, all medium and small sized individuals pigmented the pedal disc respectively, and after 14 and 28 days, the oral disc. A prolific pedal laceration was observed after 16 and 26 days in adult and smaller individuals respectively. Also, we observed *E. pallida* regurgitating viable endosymbiont clumps embeded on a proteoglycan matrix before pigmentation process was accomplished. Based on our observations, we propose a mechanism of assembling of the holobiont system regarding proliferation of endogenous symbionts after bleaching (tentacles; pedal disc; oral disc; column). Also, we propose a mechanism of disassembling of the holobiont system, production and expulsion of symbiont clump (flagella interaction; host membrane cell dissipation; endosymbiont membrane interaction, endosymbiont detachment, clumping and clump expulsion). We discuss if these mechanisms are driven by host or endosymbiont. The pigmentation sequence apparently favors light capture and vertical transmission of the endosymbiont to the host's offspring while endosymbiont clump expulsion during pigmentation may favor dinoflagellate dispersion. Nevertheless, time span between bleaching events might have different effects on the success of the pigmentation recovery. If the time span is shorter than 40 days, young individuals of the population might not have time to recover their maximal pigmentation levels. If it is shorter than 10 days, hosts might not transfer endosymbionts offspring. Shorter than a week, the photosynthetic energy supply of the host may be reduced.

## Keywords

Bleaching recovery strategy, pigmentation pattern, symbiont clumping, zooxanthellae regulation, recovery time span, disassembling mechanism, dispersal strategy

# Seasonal and diet-driven assembly of a core microbiota associated to macroalgal digestion in the marine herbivore holobiont

## Authors

**Angélique Gobet**<sup>1</sup>, Laëtitia Mest<sup>1</sup>, Morgan Perennou<sup>2</sup>, Simon Dittami<sup>1</sup>, Claire Caralp<sup>3</sup>, Céline Coulombet<sup>4</sup>, Sylvain Huchette<sup>4</sup>, Sabine Roussel<sup>3</sup>, Gurvan Michel<sup>1</sup> and Catherine Leblanc<sup>1</sup>

<sup>1</sup> UMR 8227 CNRS UPMC, LBI2M, Station Biologique de Roscoff (SBR), France

<sup>2</sup> Genomer & ABiMS platforms, FR 2424 CNRS UPMC, SBR, France

<sup>3</sup> LEMAR, UMR 6539, IUEM, Plouzané, France

<sup>4</sup> France Haloties, Plouguerneau, France

The importance of host digestive microbiota is increasingly coming into the focus of ecological and clinical research due to their major role in host digestion, host health and host development. There are numerous studies on the digestive microbiota of mammals and terrestrial herbivores but little is known about marine herbivores. Among them, generalists consume red, green, and brown algae, each presenting specific composition in complex polysaccharides. Subsequently, each macroalga presents a specific epiphytic microbiota and the digestive microbiota of marine herbivore is expected to vary with a monospecific algal diet. Therefore, we investigated the effect of 4 monospecific diets (*Palmaria palmata*, *Ulva lactuca*, *Saccharina latissima*, *Laminaria digitata*) on the digestive microbiota of a generalist marine herbivore in its natural environment over one year. Our model is a generalist herbivore able to digest the 3 algal types; the abalone, a gastropod of primary interest due to its economic importance in several countries in Asia, South Africa, and South America. The microbiota from abalone digestive gland, sampled every 2 months, was explored using metabarcoding. Despite differences in algal diet composition, we unexpectedly found three bacterial genera that constantly dominated the community: *Psychrilyobacter*, *Mycoplasma*, and *Vibrio*. Phylogenetically close genomes indicated their ability to ferment pyruvate and only *Vibrio* seemed able to degrade alginate from brown macroalgae. Key aerobic primary degraders of algal polysaccharides were found in a less abundant core microbiota associated with each algal diet. Despite finding few diet-specific OTUs, diversity analyses showed diet-specific patterns of the bacterial community and this was further confirmed by correlations between individual bacterial groups and algal characteristics. Abalone digestive gland may represent a particular niche where abalone-specific bacteria cohabit with transient bacteria able to degrade algal polysaccharides.

## Keywords

Holobiont, digestive microbiota, algal digestion, *Psychrilyobacter*, *mycoplasma*, *vibrio*



# Bacteriophages in the freshwater polyp Hydra and their role in holobiont maintenance

## Authors

**Tim Lachnit**, Janina Lange, Thomas Bosch

Zoological Institute, Kiel Universität, Am Botanischen Garten 1-9, 24118 Kiel, Germany

All multicellular organisms are associated with a host specific bacterial community. This close association between bacteria and its host is beneficial for both partners and forms a complex unit termed “holobiont”. Controlling the composition of associated bacteria is essential for the stability and function of the holobiont, but the factors contributing to this host specific bacterial colonization are poorly understood. While research has focused on the composition and control of host associated bacteria as key components of holobionts, viruses have been totally disregarded. Viruses are compared to bacteria the most abundant and diverse entity in the world and are responsible to cause high mortalities in bacteria. Recently we have demonstrated that the freshwater polyp Hydra is not only associated with a host specific bacterial community but carries also a divers viral community, composed of both eukaryotic and prokaryotic viruses (phages). Moreover, we found first evidence that bacteriophages play an important role in the colonization control of Hydra by simple bacteria-bacteria interaction experiments. Genome sequencing of Hydra associated bacteria revealed that more than 50% feature an intact prophage sequence in their genome. The possession of a prophage not only protects Hydra associated bacteria from superinfection, we could also demonstrate that phages of Hydra associated bacteria can be reactivated by different environmental factors and are able to cross-infect other bacteria. Moreover, we could show that the prophage of the most dominant bacterial colonizer of Hydra can be induced by different bacterial strains. For this reason we expect Hydra associated phages to play an important role in holobiont maintenance.

## Keywords

Phage, prophage, virus, interaction

## New insights into the *Rimicaris exoculata* holobiont model

### Authors

**Simon Le Bloa**<sup>1,2,3</sup>, Lucile Durand<sup>1</sup>, Céline Boidin-Wichlaz<sup>4</sup>, Valérie Cueff- Gauchard<sup>1,2,3</sup>, Josiane Le Bars<sup>1,2,3</sup>, Laure Taupin<sup>5</sup>, Charlotte Marteau<sup>5</sup>, Alexis Bazire<sup>5</sup>, Aurélie Tasiemski<sup>4</sup>, Marie-Anne Cambon-Bonavita<sup>1,2,3</sup>.

<sup>1</sup> Ifremer, Centre Bretagne, Laboratoire de Microbiologie des Environnements Extrêmes, DEEP/LM2E, UMR 6197 Ifremer-CNRS-UBO, ZI Pointe du Diable, CS 10070, 29280 Plouzané, France

<sup>2</sup> Université de Brest, Laboratoire de Microbiologie des Environnements Extrêmes, UMR 6197 Ifremer-CNRS-UBO, Technopôle Iroise, 4 place Nicolas Copernic, 29280 Plouzané, France

<sup>3</sup> CNRS, Laboratoire de Microbiologie des Environnements Extrêmes, UMR 6197 Ifremer-CNRS-UBO, Technopôle Iroise, 4 place Nicolas Copernic, 29280 Plouzané, France

<sup>4</sup> Université de Lille-CNRS UMR8198, Evo, Eco and Paleo unit (EEP), SPICI group, Villeneuve d'Ascq, France

<sup>5</sup> Université de Bretagne-Sud, EA 3884, LBCM, Rue de Saint Maudé, 56321 Lorient, France

Deprived of light, the deep-sea hydrothermal vents are extremes ecosystems sustained by microbial chemosynthesis. These environments are colonized by animal species living in close relationships with these chemoautotrophic micro-organisms, eating them or establishing long term interactions with them, may they be trophic or not only. The shrimp *Rimicaris exoculata* is an endemic hydrothermal species of the Mid-Atlantic Ridge (MAR) sites. This crustacean represents the predominant macrofauna of some sites of the MAR. It lives in symbiotic association with two distinct microbial communities qualified as ectosymbiosis. One is located in its gill chamber and one in its gut. First, our work focused on the study of bacterial communication (Quorum Sensing or QS) within the ectosymbiotic communities during the molting and life cycles of *R. exoculata*. Then, we focused on an antimicrobial peptide (AMP) identification and search for its function in *R. exoculata* immunity and in controlling symbionts. Two QS genes (*luxS* and *luxR*) were identified in the *R. exoculata* ectosymbiotic community at different shrimp molt stages and life stages at the Rainbow, TAG, Snake Pit and Logatchev vent sites. RT-PCR experiments highlighted *lux* gene expression at TAG, Snake Pit and Rainbow vent sites, suggesting a potential QS activity and its possible roles in epibiont colonisation processes. Phylogenetic analysis has shown the presence of three haplotypes for *luxS* (Epsilonproteobacteria) and four haplotypes for *luxR* (Gammaproteobacteria) genes distributed separately along the four sites. As these genes are more divergent than that of 16S rRNA, they could be then used as biogeographical genetic markers tools to trace back the origin of individuals to a location or between locations along its life cycle. This work reports also the first description of an AMP in an extremophile arthropod (namely Re-crustin). The study shows a constitutive expression of Re-crustin in all tissues in contact with the environment with a higher expression level in the gill chambers area on which the ectosymbiotic community develops. Looking at the temporal dynamic of the Re-crustin production during acquisition events, loss and recolonization of symbionts post molting suggests a participation of this AMP in the control of the ectosymbiosis in *Rimicaris exoculata*. Altogether these data support the role of the immune system in the adaptation to extreme environment through the establishment of vital ectosymbiosis.

### Keywords

Hydrothermal vent, *Rimicaris exoculata*, symbiose, quorum sensing, antimicrobial peptide, genetic marker



# Stability and functionality of the microbial communities associated with temperate gorgonians and the precious red coral *Corallium rubrum*

## Authors

**Jeroen van de Water**<sup>1</sup>, Christian R. Voolstra<sup>2</sup>, Denis Allemand<sup>1</sup>, Christine Ferrier-Pages<sup>1</sup>

<sup>1</sup> Centre Scientifique de Monaco

<sup>2</sup> Red Sea Research Center, King Abdullah University of Science and Technology

Gorgonians are key habitat-forming species of temperate benthic communities. However, gorgonian populations have dramatically declined due to local human impacts and mass mortality events caused by elevated seawater temperatures and microbial disease outbreaks in recent decades. Despite these threats, relatively little is known about the symbioses between gorgonian hosts and their microbial associates. Using 16S rRNA gene amplicon sequencing, we revealed that 90 - 95% of the bacterial communities associated with six gorgonian species from the Mediterranean Sea are represented by bacteria that are consistently present on both spatial and temporal scales (core microbiome). While the core microbiomes of soft gorgonians, such as *Eunicella* spp., *Leptogorgia sarmentosa* and *Paramuricea clavata*, are comparatively simple and composed of up to 5 bacterial species belonging to the genus *Endozoicomonas* (Order Oceanospirillales) and clade BD1-7 (Order Cellvibrionales), the precious red coral *Corallium rubrum* possessed a core microbiome which is unique within the Anthozoans and is dominated by 12 bacteria from the phylum Spirochaetes (70%) and the Oceanospirillales family ME2 (25%). Interestingly, we also found significant overlap in the microbiome composition of gorgonian species belonging to the same taxonomic family, but not across families. Overall, this shows that the membership of the gorgonian microbiome is very consistent, likely under tight regulation by the host, and suggests that these few bacteria are important for holobiont health. We therefore also investigated the localization of these microbial associates within the host tissues using catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) and generated the metagenomes of three species (*C. rubrum*, *P. clavata* and *E. cavolini*) representing taxonomic families with distinct microbiomes to elucidate the function of their core microbiome members. As we did observe some shifts in the bacterial assemblages of gorgonians at locations affected by local human impacts, we also investigated the effects eutrophication and metal pollution may have on the functionality of the host-associated bacteria using a metatranscriptomics approach. We will present results from these studies showing novel microbial associations within Anthozoans, their potential symbiotic interactions, and how stress may impact the health of the coral holobiont through the disruptions of host-microbial symbioses.

## Keywords

Gorgonian, holobiont, bacteria, *Endozoicomonas*, Oceanospirillales, Spirochaetes, core microbiome

# The microbiota heritability in a clonal plant: toward the concept of meta-holobiont

## Authors

**Nathan Vannier**<sup>1</sup>, Cendrine Mony<sup>1</sup>, Anne-Kristel Bittebiere<sup>2</sup>, Philippe Vandenkoornhuyse<sup>1</sup>

<sup>1</sup> Université de Rennes 1, CNRS, UMR 6553 EcoBio, Campus Beaulieu, Avenue du Général Leclerc, 35042 Rennes Cedex (France)

<sup>2</sup> Université de Lyon 1, CNRS, UMR 5023 LEHNA 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex (France)

Plants are colonized by a high diversity of symbiotic microorganisms forming a microbiota and providing additional functions to their host<sup>1,2</sup>. The plant microbiota can be considered as a toolbox allowing plants to buffer local environmental changes<sup>2</sup>. It represents then a major compartment influencing the plant fitness. Under the frame of the plant holobiont and hologenome hypothesis<sup>3</sup>, the transmission of symbiotic partners to progeny is investigated in our study. Most of the plants in nature are clonal. We thus tested under controlled conditions the hypothesis of an active transmission of the plant microbiota between ramets (i.e. from mother to clonal daughters) using *Glechoma hederacea*<sup>4</sup>. For each plant individual, we analyzed microbial assemblages using high throughput sequencing of amplicons of Archaea, Bacteria and Fungi using DNA extracts from root samples of the mother and daughter ramets. We demonstrated the vertical transmission of a significant proportion of the mother Bacteria and Fungi assemblages to the daughters but not of Archaea. This reveals the heritability of a specific core microbiota from mother plants to their descendants. More broadly, these results support the understanding of the plant as a holobiont rather than as a standalone entity. In addition, our results raised the holobiont concept to another layer of complexity because clonal plants are organized in networks in which microorganisms appear to be transmitted between holobionts. From this perspective, this clonal network can be seen as a meta-holobiont and may be a qualitative model for studying holobionts assembly and evolution.

## References

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A. & Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytologist* 206, 1196-1206. Vannier, N., Mony, C., Bittebiere, A. K. & Vandenkoornhuyse, P. (2015). Epigenetic mechanisms and microbiota as a toolbox for plant phenotypic adjustment to environment. *Frontiers in Plant Science* 6, 1159. Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews* 32, 723–735. Vannier, N., Bittebiere, A. K., Vandenkoornhuyse, P. & Mony, C. (2016). AM fungi patchiness and the clonal growth of *Glechoma hederacea* in heterogeneous environments. *Scientific Reports* 6, 37852.

## Keywords

Vertical transmission, Bacteria, Fungi, Archae, amplicon sequencing

# Session 5

## "Metabolic interactions between host and microbiota"



### Marine sponges: life in a sea of microbes

#### Authors

**Lucía Pita Galán**<sup>1</sup>, Ute Hentschel Humeida<sup>2</sup> - **Invited**

<sup>1</sup> GEOMAR Helmholtz Centre for Ocean Research, Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

<sup>2</sup> Christian-Albrechts-University Kiel, Christian-Albrechts-Platz 4, 24118 Kiel

lpita@geomar.de

Throughout evolution, marine sponges (phylum Porifera) have evolved in a literal sea of microbes. In spite of filtering thousands of liters of seawater per day, they harbor specific symbiotic communities within their mesohyl tissue. A sponge individual can harbor up to thousands of symbiont lineages that are located extracellularly within the mesohyl matrix. The sponge holobiont is thus a prominent example of a complex host-microbe association. The natural variability of sponge-microbe associations across species and environments provides a meaningful ecological framework to investigate animal-microbe-environment interactions through experimentation in the field and also in aquaria. For instance, by monitoring the microbiome of sympatric closely-related species over time and space, we observed that the symbiotic microbiota is species-specific and highly persistent despite changes in the bacterioplankton of the surrounding seawater. Furthermore, the sponge species (host taxonomy) has an affect on the composition of the symbiotic microbiome, but the precise mechanisms are still unknown. Next-generation sequencing technologies have revealed genomic features of their microbiota that may allow for a symbiotic lifestyle. In the sponge host, recent analysis of genomes and transcriptomes revealed immune receptors that are potentially involved in microbial recognition. Our overall aim is to provide insights into the mechanistic underpinnings that mediate host-microbiota-environment interaction in this ancient animal phylum.



# Plant nutrient resource strategies control the rhizosphere microbiota structure and functions

## Authors

Julien Guyonnet<sup>1</sup>, Amélie Cantarel<sup>1</sup>, Laurent Simon<sup>2</sup>, Audrey Dubost, Philippe Ortet<sup>3</sup>, Mohamed Barakat<sup>3</sup>, Wafa Achouak<sup>3</sup>, **Feth-el-Zahar Haichar**<sup>1</sup>.

<sup>1</sup> Université de Lyon, UMR 5557 Ecologie microbienne, Université Lyon 1, CNRS, INRA USC 1364, F-69622 Villeurbanne Cedex, France

<sup>2</sup> Université de Lyon, UMR5023 LEHNA, Université Lyon 1, CNRS, ENTPE, F-69622 Villeurbanne Cedex, France

<sup>3</sup> CNRS, BVME UMR 7265, ECCOREV FR 3098, 13108 Saint Paul – Lez - Durance, France, Aix Marseille Université, 13284 Marseille Cedex 07, France.

The rhizosphere is active and dynamic in which newly generated carbon, derived from root exudates, and ancient carbon of soil organic matter (SOM), are available for microbial growth. Root exudation impacts microbial community functions including nitrogen cycling process (Haichar et al, 2012). Root exudation is related to plant physiology, which can be measured via functional traits. These functional traits are used to classify plant species according to their performances. Indeed, fast-growing plant species with higher photosynthetic capacity and rapid rates of N acquisition are called competitive species in contrast to slower-growing conservative species with lower biomass N concentrations but a longer lifespan (Aerts & Chapin, 2000). The aim of this study was to determine the impact of plant strategy (from conservative to competitive), on denitrifying activity and bacterial diversity through root exudation. To do this, we have grown in the same soil, six species of Poaceae grassland (2 conservatives, 2 intermediates and 2 conservatives) for 10 weeks, that we further incubated, under <sup>13</sup>CO<sub>2</sub> during one week. This labelling allowed to estimate the rate of root exudation for each plant species and to perform DNA-SIP (stable isotope probing) approach to identify active microbiota involved in root exudates assimilation in the root compartment, in the root adhering-soil and those involved in SOM degradation. In addition, we measured denitrifying activity to determine interactions between plants and denitrifiers influenced by root exudates. We have demonstrated that root exudation was linked to plant nutritional strategy. In fact, the rate of exudation is more important for competitive and intermediates plants than for conservatives ones, owing to the root system architecture. In addition, microbial denitrification activity is correlated with exudation rate. The diversity of microbiota involved in root exudates assimilation and/or in SOM degradation determined by 16S rDNA metabarcoding, revealed differences for each plant species microbiota. To our knowledge, this is the first work demonstrating the impact of plant nutritional strategy on denitrifiers through root exudation.

## References

Aerts R. & Chapin F.S. 2000, Adv. Ecol. Res., 30: 1-67. Haichar FeZ *et al* 2012. FEMS Microbiol. Ecol. 81(2) : 291-302

## Keywords

Microbiota, root exudation, plant nutrient strategy, Stable isotope probing, active bacteria, denitrification



# Influence of insect microbial symbionts on plant nutritional status, immunity and morphology: consequences for plant exploitation

## Authors

**David Giron**<sup>1</sup>, Mélanie Body<sup>1</sup>, Franck Dedeine<sup>1</sup>, Géraldine Dubreuil<sup>1</sup>, Nicolas Faivre<sup>1</sup>, Elisabeth Huguet<sup>1</sup>, Wilfried Kaiser<sup>1</sup>, Hui Zhang<sup>1</sup>, Carlos Lopez-Vaamonde<sup>1,2</sup>

<sup>1</sup> Institut de Recherche sur la Biologie de l'Insecte (IRBI), UMR 7261, Centre National de la Recherche Scientifique/Université François-Rabelais de Tours, Tours, France

<sup>2</sup> INRA, UR0633 Zoologie Forestière, F-45075 Orléans, France

Insect symbionts can directly affect insect–plant interactions by providing insects with key nutrients or by interfering with the plant to modulate food provisioning to insects and plant defences. Due to their regulatory role, phytohormones have been a privileged target of arthropods over the course of the evolutionary arms race between plants and their biotic partners. The ability to perceive, interpret and manipulate plant signals is likely to provide insect herbivores with novel adaptive capacities enabling them to invade new ecological niches. Our extensive quantification of phytohormones in *Malus domestica* infested by the apple-tree leaf-miner *Phyllonorycter blancardella* (Lepidoptera: Gracillariidae) show that leaf-mining insects produce and deliver cytokinins (CKs) to the plant, thereby enabling insects to overtake the plant senescing programme and to control their nutritional supply under fluctuating environmental conditions. Analyses also reveal that strategies underlying the plant manipulation may be shared between herbivorous insects with distinct life histories. Our study further suggests that bacterial symbionts may contribute to the production of CKs through the synthesis of specific forms of CKs. A strong correlation was also found between the level of an endosymbiotic bacterium, *Wolbachia* and the amount of CKs in the mine. Insects treated with antibiotics created mines that contained significantly lower concentrations of CKs. Using a combination of molecular phylogenies and ecological data, we also show that the acquisitions of the altered phenotype and *Wolbachia* infections have been associated through the evolutionary diversification of Gracillariidae.

Our study provides key findings towards the understanding of molecular mechanisms underlying this intricate plant-insect-microbe interaction and converging experimental evidences strengthen the idea that insect-associated microbes are active players in plant manipulation to the benefit of the insect host.

## References

Giron D *et al.* (2017). Influence of Microbial Symbionts on Plant-Insect Interactions. In N. Sauvion, P-A. Calatayud, D. Thiéry (Volume Eds), *Advances in Botanical Research series: Vol 81. Insect-Plant Interactions in a Crop Protection Perspective* (pp. 225-247). Elsevier, GBR. Giron D *et al.* (2016). Insect-induced effects on plants and possible effectors used by galling and leaf-mining insects to manipulate their host-plant. *Journal of Insect Physiology*, 84, 70-89. Zhang H *et al.* (2016). Leaf-mining by *Phyllonorycter blancardella* reprograms the host-leaf transcriptome to modulate phytohormones associated with nutrient mobilization and plant defense. *Journal of Insect Physiology*, 84, 114-127. Giron D *et al.* (2013). Cytokinins as key regulators in plant–microbe–insect interactions: connecting plant growth and defense. *Functional Ecology*, 27, 599-609. Gutzwiller F *et al.* (2015). Correlation between the green-island phenotype and *Wolbachia* infections during the evolutionary diversification of Gracillariidae leaf-mining moths. *Ecology and Evolution*, 5, 4049-4062. Kaiser *et al.* (2010). Plant green-island phenotype induced by leaf-miners is mediated by bacterial symbionts. *Proceedings of the Royal Society B: Biological Sciences*, 277, 2311–2319.

## Keywords

Herbivores, insect symbiosis, plant manipulation, food provisioning, *Wolbachia*, Cytokinins, Leaf-miner

# The newcomers: distantly related bacteria have settled as co-obligate symbionts in aphids

## Authors

**Alejandro Manzano-Marín**, Armelle Coeur D'Acier, Anne-Laure Clamens, Emmanuelle Jousset

Centre de Biologie pour la Gestion des Populations (CBGP - UMR1062) - INRA, 755 avenue du campus Agropolis, CS 30016, 34988 Montferrier-sur-Lez, France

Typically, aphids house the obligate nutritional bacterial symbiont *Buchnera* inside specialised cells called bacteriocytes. *Buchnera* supplies the aphid with essential amino acids and vitamins thus insuring the correct development of its host 1. However, some *Buchnera* lineages have lost the ability to fulfil this role, either triggered or rescued by new and younger endosymbionts. One such case are the aphid species from the Lachninae subfamily, where an ancient loss of the riboflavin biosynthetic genes in the genome of *Buchnera* was accompanied by the acquisition of a co-obligate partner, putatively *Serratia symbiotica* 2. However, co-obligate symbioses are not restricted to this subfamily, and examples of these have been previously reported mainly by microscopic studies 3,4,5. Through whole genome sequencing, we have reconstructed the genomes of the co-obligate endosymbionts from several aphid species belonging to different subfamilies, mainly the Lachninae. We have corroborated that these co-obligate symbionts indeed supplement essential metabolic auxotrophies found in *Buchnera*, mainly that of riboflavin. Not surprisingly, they have evolved genomes with similar core metabolic capabilities, with some even bringing new ones to the symbiotic consortium. Also, we have determined that these co-symbionts have evolved from diverse facultative symbiotic taxa associated to aphids (e.g. *S. symbiotica*, *Candidatus Fukatsuia symbiotica*, *Sodalis*) as well as free-living bacterial strains (e.g. *Erwinia*). These findings show that co-obligate symbiosis in aphids is more widespread than previously thought. This suggests a fragile mono-symbiotic association between the aphid host and its *Buchnera* symbiont, whose highly degenerated genome could undergo simple metabolic losses which could lead to a “lucky” secondary symbiont establishing as a co-obligate one.

## References

1 Douglas A.E. (1989) Mycetocyte Symbiosis in Insects. *Biol. Rev.* 64: 409–434. 2 Manzano-Marín A., Simon J.-C., and Latorre A. (2016) Reinventing the Wheel and Making It Round Again: Evolutionary Convergence in *Buchnera* – *Serratia* Symbiotic Consortia between the Distantly Related Lachninae Aphids *Tuberolachnus salignus* and *Cinara cedri*. *Genome Biol. Evol.* 8: 1440–1458. 3 Fukatsu T. and Ishikawa H. (1998) Differential immunohistochemical visualization of the primary and secondary intracellular symbiotic bacteria of aphids. *Appl. Entomol. Zool.* 33: 321–326. 4 Michalik A., Szklarzewicz T., Janakowska W., and Wiczorek K. (2014) Endosymbiotic microorganisms of aphids (Hemiptera: Sternorrhyncha: Aphidoidea): Ultrastructure, distribution and transovarial transmission. *Eur. J. Entomol.* 111: 91–104. 5 Buchner P. (1953) Endosymbiose der Tiere mit Pflanzlichen Mikroorganismen Birkhäuser Basel, Basel.

## Keywords

Secondary symbiont, co-obligate symbiont, metabolic complementation, aphid endosymbiont, multipartite interactions

# Are antimicrobial peptides enabling metabolic integration of endosymbionts?

## Authors

**Peter Mergaert**<sup>1\*</sup>, Yoshitomo Kikuchi<sup>2,3</sup>, Shuji Shigenobu<sup>4</sup>, Eva C. M. Nowack<sup>5</sup>

<sup>1</sup> Institute for Integrative Biology of the Cell, UMR9198, CNRS, Université Paris-Sud, CEA, Gif-sur-Yvette, France

<sup>2</sup> Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Hokkaido Center, Sapporo, Japan

<sup>3</sup> Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

<sup>4</sup> National Institute for Basic Biology (NIBB), Okazaki, Japan

<sup>5</sup> Department of Biology, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

\* Correspondence: peter.mergaert@i2bc.paris-saclay.fr

Antimicrobial peptides (AMPs) are effectors of innate immunity that attack potentially harmful microbes<sup>1</sup>. However, recent studies found that AMPs are also massively produced by eukaryotic hosts during symbiotic (i.e. mutualistic) interactions with bacteria. Only in a few systems, specific functions of symbiotic AMPs have been studied experimentally revealing, among other roles, their capacity to permeabilize symbiont membranes and to facilitate metabolite-flow across the host-symbiont interface<sup>2-4</sup>. We propose that an ancestral role of these peptides is the facilitation of metabolic exchange between the symbiotic partners through membrane permeabilization. This function may be particularly critical for the integration of the endosymbiont and host metabolism in interactions involving bacteria with strongly reduced genomes lacking most small metabolite transporters. We also hypothesize that AMPs have acted in a similar way at the onset of plastid and mitochondrion evolution, after a host cell took up a bacterium and needed to extract nutrients from it in the absence of dedicated transporters. Transient symbiotic AMP-induced membrane permeabilizations as well as regulation of the activity of these peptides can reconcile metabolite flow across endosymbiont membranes and the maintenance of the proton motive force.

## References

1. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature* 415, 389-395.
2. Van de Velde, W., Zehirov, G., Szatmari, A., Debreczeny, M., Ishihara, H., Kevei, Z., Farkas, A., Mikulass, K., Nagy, A., Tiricz, H., Satiat-Jeunemaitre, B., Alunni, B., Bourge, M., Kucho, K., Abe, M., Kereszt, A., Maorti, G., Uchiumi, T., Kondorosi, E., and Mergaert, P. (2010). Nodule specific peptides govern terminal differentiation of bacteria in symbiosis. *Science* 327, 1122-1126.
3. Salzman, N.H., Hung, K., Haribhai, D., Chu, H., Karlsson-Sjöberg, J., Amir, E., Tegatz, P., Barman, M., Hayward, M., Eastwood, D., Stoel, M., Zhou, Y., Sodergren, E., Weinstock, G.M., Bevins, C.L., Williams, C.B., and Bos, N.A. (2010). Enteric defensins are essential regulators of intestinal microbial ecology. *Nat. Immunol.* 11, 76-83.
4. Carro, L., Pujic, P., Alloisio, N., Fournier, P., Boubakri, H., Hay, E., Poly, F., François, P., Hoher V., Mergaert, P., Balmand, S., Rey, M., Heddi, A., and Normand, P. (2015). *Alnus* peptides modify membrane porosity and induce the release of nitrogen-rich metabolites from nitrogen-fixing *Frankia*. *ISME J.* 9, 1723–1733.

## Keywords

Symbiosis, antimicrobial peptides, metabolic collaboration, metabolite transport, organellogenesis

## Understanding the role of *Archaea* for plant holobionts

### Authors

**Julian Taffner**<sup>1</sup>, Armin Erlacher<sup>1</sup>, Anastasia Bragina<sup>1</sup>, Henry Müller<sup>1</sup>, Christin Zachow<sup>2</sup>, Christine Moissl-Eichinger<sup>3</sup> and Gabriele Berg<sup>1</sup>

<sup>1</sup> Institute of Environmental Biotechnology, Graz University of Technology, 8010 Graz, Austria

<sup>2</sup> Austrian Centre of Industrial Biotechnology (ACIB GmbH), 8010 Graz, Austria

<sup>3</sup> Department of Internal Medicine, Medical University of Graz, 8010 Graz, Austria

Plants are known to harbour a wide diversity of microorganisms, such as bacteria and fungi, influencing plant nutrition, resistance to stress and fitness. Thereby they collectively form a holobiont. Furthermore recent studies showed that actually Archaea also shape the microbiome of plants, but their ecological roles and interactions with their hosts remained mostly unclear. To get a broader insight into the structure and function of plant-associated Archaea, we compared 37 different plants of the families Pinaceae, Ericaceae, Cyperaceae, Poaceae, Hylocomiaceae, Polytrichaceae, Aulacomniaceae, Sphagnaceae, Typhaceae, Brassicaceae, Oleaceae and Amaranthaceae, in a combined approach using 16S rRNA amplicon sequencing, whole metagenome shotgun sequencing and fluorescence in situ hybridization confocal laser scanning microscopy (FISH-CLSM). First insights into the community structure, habitat preferences and functions of plant-associated Archaea were provided. The highest relative abundances of Archaea were detected in the endosphere of olive trees (*Olea europaea* L.), with up to 67.3% of total reads, in dwarf shrubs (*Vaccinium myrtilloides* and *V. oxycoccus*, with 33.0% and 31.7% respectively), and in the rhizosphere of sugar beets (*Beta vulgaris* L.) with 33.0%. Across all habitats the archaeal community structure was clearly dominated by Euryarchaeota, followed by the less abundant phylum of Crenarchaeota and Thaumarchaeota, except in *O. europaea*, where Thaumarchaeota were predominant. Moreover, we observed signatures for putative adaptation mechanisms of Archaea on plant holobionts, including those for higher chemotaxis, nutrient cycling like CO<sub>2</sub> fixation, stress response, especially against oxidative stress, and archaeon stability. Complementary FISH-CLSM analyses showed specific colonization pattern prevail over randomized root colonization.

### Keywords

*Archaea*, metagenomics, bog vegetation, plant holobiont



# Transtegumental absorption of ectosymbiotic bacterial by-products in the hydrothermal shrimp *Rimicaris exoculata*: An unusual way of eating

## Authors

**Magali Zbinden**<sup>1</sup>, J. Ponsard<sup>2</sup>, P. Compère<sup>2</sup>, L. Corbari<sup>3</sup>, B. Shillito<sup>1</sup>, V. Cuff-Gauchard<sup>4</sup>, L. Durand<sup>4</sup>, G. Lepoint<sup>2</sup>, A. Joassin<sup>2</sup> and MA. Cambon-Bonavita<sup>4</sup>

<sup>1</sup> Sorbonne Universités, Univ Paris 06, UMR CNRS MNHN 7208 Biologie des Organismes Aquatiques et Ecosystèmes (BOREA), Equipe Adaptation aux Milieux Extrêmes, 7 Quai St Bernard, 75005 Paris, France

<sup>2</sup> Laboratoire de Morphologie fonctionnelle et évolutive, Département de Biologie, Ecologie et Evolution, Université de Liège, Liège, Belgium

<sup>3</sup> ISYEB - UMR 7205 – CNRS, MNHN, UPMC, EPHE, Muséum National d'Histoire Naturelle, Département Systématique et Evolution, Sorbonne Universités, CP26, 57 rue Cuvier, 75231 Paris Cedex 05, France

<sup>4</sup> Ifremer, DEEP/Laboratoire de Microbiologie des Environnements Extrêmes, UMR6197, Technopôle Brest Iroise, BP70, Plouzané

*R. exoculata*, the most extensively studied hydrothermal shrimp species to date, occurs on the active chimney walls, in amazingly dense swarms of 1500 to 3000 individuals per square meter (Segonzac 1992), where it lives within steep chemical and thermal gradients, resulting from the mixing of hot, reduced hydrothermal fluid and oxygenated seawater. This species harbours a luxuriant bacterial community in its enlarged gill chamber. The highly specialized morphological features of *Rimicaris* (hypertrophied branchial chamber and mouthparts, bacteriophage setae) suggest a real bacterial culture in the shrimp pre-branchial chamber and argue for a trophic symbiosis (Gebruk *et al.* 1993). Strong evidences, based on stable isotope signatures and essential fatty acid composition, showed that the shrimp gets organic matter mainly from its epibionts (Rieley *et al.* 1999). Yet this evidence of symbiont–host nutritional relationships was indirect, and how carbon is transferred from bacteria to shrimp long remained an open question. It has long been thought that shrimp fed on the epibiotic bacteria living within its carapace, by scraping and grazing on them (Gebruk *et al.* 1993). Recently, we carried out *in vivo* experiments in a pressurised aquarium with isotope-labelled inorganic carbon (Ponsard *et al.* 2013) to test an alternative hypothesis of direct transfer of dissolved molecules across the shrimp integument (Casanova *et al.* 1993; Zbinden *et al.* 2004). In doing so, we provided the first *in-vivo* experimental evidence of nutritional transfer of bacteria-fixed carbon to shrimps: Bacterial epibionts assimilate inorganic carbon by chemoautotrophy, and a direct nutritional transfer of soluble bacterial by-products to the host occurs by transtegumental absorption across the gill chamber integument, rather than via the digestive tract. Usually rejected for crustaceans and especially decapods, uptake of nutrients across the integument can be regarded here as a peculiar adaptation for this symbiotic shrimp and constitute an unusual way of eating.

## References

- Casanova B, Brunet M, Segonzac M. 1993. L'impact d'une épibiose bactérienne sur la morphologie fonctionnelle de crevettes associées à l'hydrothermalisme médio-Atlantique. *Cahiers de Biologie Marine* 34: 573-588.
- Gebruk A, Pimenov N, Savvichev A. 1993. Feeding specialization of bresiliid shrimps in the TAG site hydrothermal community. *Marine Ecology Progress Series* 98: 247-253.
- Ponsard J, Cambon-Bonavita M-A, Zbinden M, Lepoint G, Joassin A, Corbari L, Shillito B, Durand L, Cuff-Gauchard V, Compère P. 2013. Inorganic carbon fixation by chemosynthetic ectosymbionts and nutritional transfers to the hydrothermal vent host-shrimp, *Rimicaris exoculata*. *ISME Journal* 7: 96-109.
- Segonzac M. 1992. Les peuplements associés à l'hydrothermalisme océanique du Snake Pit (dorsale médio-Atlantique, 23°N, 3480m): composition et microdistribution de la mégafaune. *Compte-Rendus de l'Académie des Sciences*. 314, série III: 593-600.
- Zbinden M, Le Bris N, Gaill F, Compère P. 2004. Distribution of bacteria and associated minerals in the gill chamber of the vent shrimp *Rimicaris exoculata* and related biogeochemical processes. *Mar. Ecol. Prog. Ser.* 284: 237-251.

## Keywords

Chemosynthesis, hydrothermal vent, ectosymbiose, *Rimicaris exoculata*



## From genetics to hologenetics

### Authors

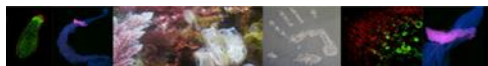
**Jacques Beckmann<sup>1</sup>, Thierry Heulin<sup>2</sup>** – Invited

<sup>1</sup> Faculté de Biologie et Médecine, Université de Lausanne, 1015 Lausanne, Suisse

<sup>2</sup> CNRS-CEA, LEMIRE BIAM Bât 177 CEA Cadarache, 13108 Saint-Paul lez Durance, France

Precision medicine, as currently advocated, remains essentially a highly genome-centric initiative. While the germline genome is an essential component, we contend that there will be no effective delivery of precision medicine without diligent consideration of the complex multicomponent holobiome, i.e. inclusion also of the virome and microbiome. The latter are a sensor for the extrinsic and intrinsic ecosystem in which we dwell and evolve, ensuring the robustness needed for survival in rapidly changing, and potentially adverse, environments. It endows the host with the capacity to rapidly adapt and adjust to changing environments, or to face adverse or stressful conditions. It was also causally implicated in numerous diseases as well as in our well-being. Thus, consideration of the hologenome might result in improved understanding and treatment of genetic diseases. Examples will be proposed.

## Session Posters



# Denitrifying process in the rhizosphere under plant species control

## Authors

Feth el Zahar Haichar<sup>1</sup>, Julien Guyonnet<sup>1</sup>, Laurent Simon<sup>2</sup>, Mohamed Barakat<sup>3</sup>, Philippe Ortet<sup>3</sup>, Thierry Heulin<sup>3</sup>, **Wafa Achouak<sup>3</sup>**

<sup>1</sup> Université Lyon1, CNRS, UMR5557, INRA, USC1364, Ecologie Microbienne, Groupes Fonctionnels Microbiens et Cycle de l'Azote, Villeurbanne F-69622, France

<sup>2</sup> Université de Lyon, UMR5023 LEHNA, Université Lyon 1, CNRS, ENTPE, F-69622 Villeurbanne Cedex, France

<sup>3</sup> UMR 7265 CNRS-SEA-AMU, ECCOREV FR 3098, 13108 Saint Paul-Lez-Durance, France

Denitrification is a major process affecting soil greenhouse gases emissions and soil nitrogen availability. It is well known that the major factors favouring denitrification are proceeding at a higher rate in the rhizosphere: nitrate concentration (via the N assimilation by plants) and oxygen partial pressure (via root respiration) are decreased, whereas C availability (via rhizodeposition) is generally increased (Mounier et al., 2004), and where the impact on denitrifying activity has been previously reported (Henry et al., 2008, Haichar et al., 2012). However, little is known about how denitrification is regulated in the rhizosphere. Hence, understanding the regulation of denitrification and the role of organic carbon, released through plant root exudation, in the production of N<sub>2</sub>O and in shaping denitrifying bacterial diversity is of global importance and offers the opportunity for managing soils to lower net N<sub>2</sub>O emission. In the present study, we question whether there is a relationship between the rate of denitrification and i) the source of root exudates by using four plant species (*Triticum aestivum*, *Brassica napus*, *Medicago truncatula*, and *Arabidopsis thaliana*) cultivated in the same soil; and ii) the structure of microbiota selected by each plant species in the rhizosphere. For that purpose, denitrification activity was measured in the root tissue and in the root adhering-soil (RAS), and the structure of microbiota was determined by metabarcoding of *rrs* gene. In addition, to understand how each plant species modulates denitrification activity mainly in the root compartment, we determined the rate of nitrogen uptake from nitrate and from ammonia by each plant species. We provide evidence that each plant species shaped specifically bacterial community, and favoured certain bacterial functions in the root compartment, as revealed by PICRUST tool (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, Langille et al. 2013) based on sequenced genomes of most closely related bacteria. The N<sub>2</sub>O production rates measured by adding nitrate as sole nitrogen source, revealed a higher denitrifying activity on plant roots where root exudation is more important than in the RAS for all studied plants. Denitrifying activity was higher on *T. aestivum* and *A. thaliana* roots, which showed a lesser preferential uptake of NO<sub>3</sub><sup>-</sup>.

## References

Mounier E, Hallet S, Chèneby D, Benizri E, Gruet Y, *et al.* (2004) Influence of maize mucilage on the diversity and activity of the denitrifying community. *Environ Microbiol* 6: 301–312. Henry S., Texier S., Hallet S., Bru D., Dambreville C., Chèneby D., *et al.* (2008) Disentangling the rhizosphere effect on nitrate reducers and denitrifiers: insight into the role of root exudates. *Environ. Microbiol.* 10 3082–3092. Haichar FZ, Roncato MA, Achouak W (2012) Stable isotope probing of bacterial community structure and gene expression in the rhizosphere of *Arabidopsis thaliana* FEMS Microbiol Ecol 81:291-302. Langille, M. G.I.\*; Zaneveld, J.\*; Caporaso, J. G.; McDonald, D.; Knights, D.; Reyes, J.; Clemente, J. C.; Burkepile, D. E.; Vega Thurber, R. L.; Knight, R.; Beiko, R. G.; and Huttenhower (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *C. Nature Biotechnology*, 1-10. 8.

## Keywords

Rhizosphere, denitrification, root exudates, microbiota

# Rice varieties loss within a centennial Chinese agro-system: Impact on rice microbiota and disease emergence

## Authors

**Pascal Alonso**<sup>1</sup>, Denis Filloux<sup>1</sup>, Romain Ferdinand<sup>1</sup>, Laurence Blondin<sup>1</sup>, Jin Bai Hui<sup>2</sup>, Xiahong He<sup>2</sup>, Christian Verniere<sup>1</sup> and Philippe Roumagnac<sup>1</sup>

<sup>1</sup> UMR BGPI, Campus International de Montferrier-Baillarguet, 34398 Montpellier

<sup>2</sup> The National Center for Agricultural Biodiversity, Yunnan Agricultural University, Kunming 650201, China

Recent studies have discovered that there are likely many plant virus species that have mutualistic relationships with their hosts. Whereas some viruses are essential for the survival of their hosts, others simply give their hosts a fighting edge in the highly competitive natural world. It is noteworthy that several studies have shown that either cryptic harmless plant viruses or acute plant viruses can reduce the impact of abiotic stress on their host plant. In addition, viruses, such as bacteriophage can play a key-role in the control of plant bacterial outbreaks. Hence, it is now widely accepted that the composition, the population structure and the activity of the host microbiome is a crucial parameter of the host health. We plan to test during my PhD study the hypothesis that modification of the plant microbiome (bacteriome and virome) may significantly have an impact on the plant pathogen dynamics, and further on the pathogen emergence or non-emergence. Therefore, we here aim at characterizing the endophyte microbiome of pilot rice agrosystems from the Yunnan Province of China. Areas with different rice cultural practices (traditional agriculture vs conventional agriculture) will be surveyed for 2-3 years. In addition, the effect of the very recent increasing use of modern rice varieties in the Yuanyang terraces where mix of traditional rice varieties have been used for centuries will also be studied. Metagenomics-based analyses of both the microbial and the virus communities inhabiting the plant endosphere of three compartments (roots, stems and leaves) will be carried out. Culturomics, 16S rRNA barcoding and virion-associated nucleic acid metagenomics-based approaches will be used in this study.

## Keywords

Metagenomics, culturomics, microbiota, diversity, agrosystem

# Collateral damages of the corallivorous gastropod *Drupella cornus* on coral microbial associates

## Authors

**Jean-Christophe Auguet**<sup>1</sup>, Y. Bettarel<sup>1</sup>, S. Bouchard<sup>2</sup>, T. Bouvier<sup>1</sup>, C. Bouvier<sup>3</sup>, V.N. Bui<sup>4</sup>, C. Desnues<sup>2</sup>, T.Y. Hoang<sup>4</sup>, T.C. Mai<sup>4</sup>, P. Got<sup>3</sup>, T.T. Pham<sup>5</sup>

<sup>1</sup> IRD, Université Montpellier 2- UMR MARBEC, France

<sup>2</sup> CNRS, UMR URMITE, France

<sup>3</sup> CNRS, Université Montpellier 2- UMR MARBEC, France

<sup>4</sup> IBT, VAST, Hanoi, Viet Nam

<sup>5</sup> IMER, Haiphong, Viet Nam

The grazing activity by specific marine organisms represents a growing threat to the survival of many scleractinian species. For example, the recent proliferation of the corallivorous gastropod *Drupella cornus* now constitutes a critical case in all South East Asian waters. If the damaging effects caused by this marine snail on coral polyps are relatively well known, the incidence of grazing on coral microbial associates might also potentially impair the defense mechanisms and nutritive properties of their bacterial and viral epibionts. In this study, we compared the main ecological traits of coral-associated bacterial and viral communities living in the mucus layer of *Acropora formosa* and *Acropora palmata*, between healthy and predated individuals (i.e., colonized by *D. cornus*), in the Bay of Nha Trang (Vietnam). Preliminary results show a substantial impact of the presence of the gastropod on a variety of ecological markers (abundance, diversity, respiration, metabolic capacity, life strategy, etc.) for both viral and bacterial communities, whatever the species considered. Particularly, predated corals presented a profound bacterial dysbiosis mainly related to the development of bacteria belonging to known coral pathogen orders (i.e, Vibrionales, Alteromonadales, Campylobacterales and Oceanospirillales). Our results suggest that predation, beyond its direct deleterious mechanical effects to corals, might be also able to cause significant alterations in their symbiotic microbiota, which may subsequently promote the emergence of opportunistic pathogenic infections.

## Keywords

*Drupella cornus*, *Acropora*, bacterial and viral epibionts, dysbiosis, pathogens

# Oxylipins at the core of a phytopathogen-endophyte interaction

## Authors

**Margot Bärenstrauch**<sup>1</sup>, Bastien Nay<sup>1</sup>, Soizic Prado<sup>1</sup>, Stéphane Mann<sup>1</sup> and Caroline Kunz<sup>1,2</sup>

<sup>1</sup> Sorbonne Universités, Muséum National d'Histoire Naturelle, CNRS, Laboratoire Molécules de Communication et Adaptation des Microorganismes (MCAM), UMR 7245 CNRS-MNHN, CP54, 57 rue Cuvier, 75005 Paris

<sup>2</sup> Sorbonne Universités, UPMC Université Paris 06, UFR 927, Paris, France

Most plants are harbouring a wide diversity of microorganisms, called endophytes. The study of the cultivable microbial community of the Asian conifer *Cephalotaxus harringtonia*, led our team to the discovery of the Ascomycete *Paraconiothyrium variable* (Dothideomycetes, Pleosporales). Endophytic fungi, involved in mutualistic associations with plants, can have profound impact on plant ecology as they provide fitness benefits to their host, such as protection against phytopathogens (Rodriguez et al, 2009). Indeed, they produce many metabolites displaying fungicidal or antibacterial activity (Kusari et al, 2012). The endophyte *P. variable*, inhabiting the conifer needles, exhibited a strong antagonistic activity towards the ubiquitous soil-borne phytopathogen *Fusarium oxysporum*. Interestingly, this pathogen is responsible for seedling-blight in some conifers. During the metabolic interaction between both microorganisms, two oxylipins identified as 13-keto-9,11-octadecadienoic acid (13-KODE) and 13-hydroperoxy-9,11-octadecadienoic acid (13-HpODE) are overproduced. Such oxidized fatty acids are widely studied as signalling molecules in the context of host-pathogen interactions (Tsitsigiannis, 2007). However, few is known about their role in the chemical communication within the microbiote itself. Besides, this oxylipin production is accompanied by a decrease in beauvericin secretion, one of the most potent mycotoxins of *Fusarium* species and a virulence factor on infected plants (López-Berges, 2013). Previous results showed that exogenous application of 13-KODE lowers beauvericin production, synthesized by a nonribosomal peptide synthetase encoded by the *beas* gene. This points out a probable role of endophytic oxylipins in beauvericin downregulation (Combès, A. et al. 2012). The goal of our work is to elucidate the origin, endophytic or pathogenic, of the two oxylipins 13-KODE and 13-HpODE during the antagonistic interaction. In this perspective, we started to construct *P. variable* endophyte mutants lacking these oxylipins. We identified and cloned two lipoxygenase genes (*Pvlo*x1 and *Pvlo*x2) of *P. variable* and started establishing a genetic transformation system to obtain *Pvlo*x1 and *Pvlo*x2 mutants. A q-PCR approach will give insight about the expression patterns of the two endophyte *Pvlo*x genes as well as the two *lox* genes and the *beas* gene of *F. oxysporum* during the antagonistic interaction. Furthermore, we currently express the two *Pvlo*x genes in *Escherichia coli*, to elucidate their biochemical activity and to test their implication in 13-KODE and 13-HpODE biosynthesis.

## Keywords

Endophytic fungi, microbial community, phytopathogen, metabolic interaction, oxylipins, mycotoxin



## Prokaryote-nematode interactions in marine extreme environments

### Authors

**Laure Bellec**<sup>2</sup>, M.A. Cambon-Bonavita<sup>2</sup>, V. Cueff-Gauchard<sup>2</sup>, S. Fuchs<sup>1</sup>, D. Zeppilli<sup>1</sup>

<sup>1</sup> IFREMER, Centre Brest, REM/EEP/LEP, ZI de la pointe du diable, CS10070, 29280 Plouzané, France

<sup>2</sup> IFREMER, Centre Brest, REM/EEP/LMEE, UMR6197, ZI de la pointe du diable, CS10070, 29280 Plouzané, France

Nematodes are the most abundant and diversified metazoans in terrestrial and marine ecosystems. The discovery of abundant and well-adapted nematode species in extreme environments has opened new frontiers in the ecology and biology of meiofauna from marine extreme environment. In their natural environment, nematodes are exposed to bacteria, archaea, viruses and fungal pathogens but little is known about nematode/prokaryote interactions in marine environments, and even less in marine extreme environments. In the framework of the research-project PIONEER (Prokaryote-nematode Interaction in marine extreme environments: a uNiquE source for ExploRation of innovative biomedical applications), we aim to identify the relationships between bacteria and nematodes in several extreme environments. We sampled deep-sea and shallow hydrothermal vents and anoxic sediments. We amplified the 16S rDNA gene by Sanger-sequences clone libraries to determine the prokaryote diversity and additionally to characterize epibiotic or endobiotic microbial community, we used Scanning Electron Microscopy (SEM) and Fluorescence In Situ Hybridization (FISH). First results from the Lucky Strike vent field (Mid-Atlantic Ridge) showed that an epibiotic microbial community, highly represented by Gammaproteobacteria lineages, associated with a new species of nematode (*Oncholaimus dyvae* sp. nov.). This study opens to unexplored prokaryote–eukaryote interactions in deep-sea hydrothermal vents. The overall impact of the PIONEER project is the discovery of the microbial diversity associated to marine nematode and a greater understanding of evolutionary mechanisms from this holobiont.

### Keywords

Nematode, deep sea, eukaryote-prokaryote interactions

## The viral imprint in coral holobiont

### Authors

**Yvan Bettarel**<sup>1</sup>, Hanh Kim-Nguyen<sup>2</sup>, Thierry Bouvier<sup>1</sup>

<sup>1</sup> UMR MARBEC (Marine Biodiversity, Exploitation and Conservation), Université de Montpellier2, France

<sup>2</sup> Institute of Oceanography of Nha Trang, VAST, Vietnam

There is increasing suspicion that viral communities play a pivotal role in maintaining coral health, yet their main ecological traits still remain poorly characterized. In this study, we examined the seasonal distribution and reproduction pathways of viruses inhabiting the mucus of the scleractinians *Fungia repanda* and *Acropora formosa* collected in the Nha Trang Bay (Viet Nam), during a 11-month survey. The strong coupling between epibiotic viral and bacterial abundance suggested that phages are dominant among coral-associated viral communities. Mucosal viruses also exhibited significant differences in their main features between the two coral species and were also remarkably contrasted with their planktonic counterparts. For example, their abundance (inferred from epifluorescence counts), lytic production rates (KCN incubations), and the proportion of lysogenic cells (Mitomycin-C inductions) were respectively 2.6, 9.5, and 2.2 fold higher in mucus than in the surrounding water. Both lytic and lysogenic indicators were tightly coupled with temperature and salinity, suggesting that the life strategy of viral epibionts is strongly dependent upon environmental circumstances. Finally, our results suggest that coral mucus may represent a highly favorable habitat for viral proliferation, promoting the development of both temperate and virulent phages. Herein, we discuss on how such an optimized viral arsenal could be crucial for coral viability by presumably forging complex links with both symbiotic and adjacent non-symbiotic microorganisms.

### References

- Bettarel Y, Bouvier T, Nguyen-Kim H, Pham TT (2015) The versatile nature of coral-associated viruses. *Environmental Microbiology* 17: 3433-3439
- Nguyen-Kim H, Bettarel Y, Bouvier T, Bouvier C, Doan-Nhu H, Nguyen-Ngoc L, Nguyen-Thanh T, Tran-Quang H, Brune J (2015) Coral mucus is a hot spot for viral infections. *Applied and Environmental Microbiology* 81: 5773-5783

### Keywords

Viruses, coral, holobiont, bacteria, lysogeny, Vietnam, ecology

# Bacterial metabarcoding and MS metabolomics on insect cuticle: the case of parabiosis in French Guiana “Ant gardens”

## Authors

**Caroline Birer**<sup>1</sup>, Lucie Zinger<sup>2,3</sup>, Gregory Genta-Jouve<sup>4</sup>, Yannick Estevez<sup>1</sup>, Alexandre Maciuk<sup>5</sup>, Bruno Figadère<sup>5</sup>, Niklas Tysklind<sup>1</sup>, Christophe Duplais<sup>1\*</sup>

<sup>1</sup> Ecology of French Guianan Forests (EcoFoG), UMR8172 AgroParisTech, CNRS, Cirad, INRA, Université des Antilles, Université de Guyane, Kourou, French Guiana, France

<sup>2</sup> Université Toulouse 3 Paul Sabatier, CNRS, ENFA, UMR 5174 EDB, F-31062 Toulouse, France

<sup>3</sup> Present affiliation : Institut de Biologie de l'École Normale Supérieure, École Normale Supérieure, Paris Sciences et Lettres Research University, CNRS UMR 8197, INSERM U1024, F-75005 Paris, France

<sup>4</sup> Organic, Medicinal, Extractive Chemistry and Experimental Toxicologie (COMETE) UMR8638, CNRS-Université Paris Descartes, Paris, France

<sup>5</sup> Biomolecules: Conception, Isolation and Synthesis (BioCIS), UMR 8076 Université Paris Sud - CNRS, Châtenay-Malabry, France

Symbiotic interactions with microorganisms have played a major role in the evolutionary history of host species (1). In tropical forests, ants (Formicinae) have high species diversity, ecological success, social behavior, versatility in nesting modes, and they represent a relevant model in host-microbe interactions to understand what host factors shape the bacterial community composition and what functions do bacteria provide to hosts. In this context, it has been demonstrated that gutbacteria (Rhizobiales) increase nitrogen assimilation in the diet of herbivorous ants enabling them to colonize and persist in arboreal habitats (2). Whereas cuticular actinobacteria associated with fungus-growing ants are known to protect the fungus cultivar from various pathogens by biosynthesizing antimicrobial metabolites (3), in general the diversity, host-specificity, transmission and functional role of cuticular bacteria in ants remain unknown. To reveal which bacterial communities produce a specific molecules' cocktail and how this mixture might improve host fitness, it is necessary to correlate cuticular microbiota and cuticular metabolome analysis. We have recently optimized a DNA extraction protocol for the bacterial metabarcoding of ant cuticle (4) and we have combined this approach with a Mass Spectrometry (MS) metabolomics analysis. Herein by studying parabiosis in ant gardens in French Guiana, we accessed in our preliminary results the cuticular bacterial composition of *Camponotus femoratus* and *Crematogaster levior* to reveal high environmental acquisition whereas on the contrary MS metabolomics show a greater metabolome specificity with ant species. Future directions on statistical analysis strategies for combining metabarcoding and metabolomics data are discussed.

## References

1. M. McFall-Ngaia, et al. Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl. Acad. Sci. USA*, 2013, 110, 3229-3236.
2. J. A. Russell, et al. Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. *Proc. Natl. Acad. Sci. USA*, 2009, 106, 21236-21241.
3. C. S. Sit, et al. Variable genetic architectures produce virtually identical molecules in bacterial symbionts of fungus-growing ants. *Proc. Natl. Acad. Sci. USA*, 2015, 112, 13150-13154.
4. C. Birer, et al. Comparative analysis of DNA extraction methods to study the body surface microbiota of insects: a case study with ant cuticular bacteria. 2016, submitted.

## Keywords

Ants, cuticle, microbiota, metabarcoding, metabolomics

# Natural products from symbiotic bacteria are essential for nematode development and growth

**Author**

**Helge B. Bode**

Goethe Universität Frankfurt, Germany

Bacteria of the genera *Xenorhabdus* and *Photorhabdus* live in symbiosis with nematodes of the genera *Steinernema* and *Heterorhabditis*, respectively.<sup>1,2</sup> The bacteria/nematode pair infects and kills insect larvae living in the soil, an efficient principle used in organic farming. Work during the last 10 years has shown that the bacteria are prolific producers of several natural products (often called secondary or special metabolites), several of them being toxic to insects or food competitors like soil living protozoa, fungi or bacteria.<sup>3-5</sup> We have recently revealed that some of these natural products are also essential for nematode development and/or maintaining bacteria-nematode specificity. Moreover, we have developed molecular tools that allowed the specific production of single natural product classes in the bacteria that usually produce several natural product classes in parallel. Thus we can now address the role of these natural products during symbiosis.

## References

1. Goodrich-Blair, H. & Clarke, D. J. Mutualism and pathogenesis in *Xenorhabdus* and *Photorhabdus*: two roads to the same destination. *Mol. Microbial.*, 64, 260–268 (2007).
2. Waterfield, N. R., Ciche, T. & Clarke, D. *Photorhabdus* and a host of hosts. *Annu. Rev. Microbial.*, 63, 557–574 (2009).
3. Bode, H. B. Entomopathogenic bacteria as a source of secondary metabolites. *Current Opinion in Chemical Biology*, 13, 224–230 (2009).
4. Challinor, V. L. & Bode, H. B. Bioactive natural products from novel microbial sources. *Annals of the New York Academy of Sciences*, 1354, 82–97 (2015).
5. Vizcaino, M. I., Guo, X. & Crawford, J. M. Merging chemical ecology with bacterial genome mining for secondary metabolite discovery. *J Ind Microbial Biotechnol*, 41, 285–299 (2013).

## Keywords

Entomopathogenic bacteria, *Photorhabdus*, *Xenorhabdus*, nematodes, *Heterorhabditis*, *Steinernema*, secondary metabolites

# Insights into the lignocellulose degradation from the woodlice holobiont

## Authors

**Marius Bredon**<sup>1</sup>, Cyril Noël<sup>2</sup>, Jessica Dittmer<sup>3</sup>, Benjamin Herran<sup>1</sup>, Joanne Bertaux<sup>1</sup>, Pierre Grève<sup>1</sup>, Maryline Raimond<sup>1</sup>, Bouziane Moumen<sup>1</sup>, Didier Bouchon<sup>1</sup>

<sup>1</sup> UMR CNRS 7267, Ecologie et Biologie des Interactions, Université de Poitiers, Poitiers, France

<sup>2</sup> UMR CNRS 5654, Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Matériaux, Université de Pau et des Pays de l'Adour, France

<sup>3</sup> The Rowland Institute at Harvard, Cambridge, USA

Lignocellulose is the main component of plants and is composed of cellulose, lignin and hemicellulose. Its digestion requires the collective action of multiple Carbohydrate-Active enZymes (called CAZymes), typically acting together as a cocktail with complementary, synergistic activities and modes of action. Many invertebrates express some lignocellulose-degrading enzymes, but in most of them efficient degradation of lignocellulose is only possible thanks to mutualistic associations with endosymbionts. Due to their important role in the decomposition of organic matter, terrestrial isopods are recognised as keystone species in terrestrial ecosystems. Past studies have shown that they can digest cellulose, and thus are able to produce some cellulases themselves (Kostanjsek et al. 2010). Although marine isopods like *Limnoria quadripunctata* secrete all the enzymes necessary for cellulose digestion in the absence of gut microbes (King et al. 2010), terrestrial isopods would not be able to digest cellulose without the help of their microbiota (Bouchon et al. 2016). Similar to termites, it has been suspected that several hepatopancreatic symbionts may be involved in the lignocellulose degradation in terrestrial isopods completing the CAZyme repertoire of their hosts (Zimmer et al. 2002). To test this hypothesis, transcriptomic and metagenomic approaches in several woodlice holobionts have been used. We compared CAZymes identified from the microbiome with CAZymes present in their isopod hosts. Based on metagenomic analyses of several digestive tissues from the pill bug *Armadillidium vulgare*, we identified many genes coding for CAZymes. Some of them may be involved in the degradation of cellulose, hemicellulose and lignin. In parallel, we identified other different CAZymes in the terrestrial isopod transcriptomes. In-depth analysis of interactions from both the microbiome and the host enabled us to identify complete metabolic pathways for the lignocellulose digestion. Our primary results provide an insight into the role of the microbiome in the evolution of terrestrial isopods and their adaptive radiation in terrestrial habitat.

## References

Bouchon, D., M. Zimmer, and J. Dittmer. "The Terrestrial Isopod Microbiome: An All-in-One Toolbox for Animal-Microbe Interactions of Ecological Relevance." *Front Microbiol* 7, (2016): 1472. King, A. J., S. M. Cragg, Y. Li, J. Dymond, M. J. Guille, D. J. Bowles, N. C. Bruce, I. A. Graham, and S. J. McQueen-Mason. "Molecular Insight into Lignocellulose Digestion by a Marine Isopod in the Absence of Gut Microbes." *Proc Natl Acad Sci U S A* 107, no. 12 (2010): 5345-50. Kostanjsek, R., M. Milatovic, and J. Strus. "Endogenous Origin of Endo-Beta-1,4-Glucanase in Common Woodlouse *Porcellio Scaber* (Crustacea, Isopoda)." *J Comp Physiol B* 180, no. 8 (2010): 1143-53. Zimmer, Martin. "Nutrition in Terrestrial Isopods (Isopoda: Oniscidea): An Evolutionary-Ecological Approach." *Biological Reviews of the Cambridge Philosophical Society* 77, no. 4 (2002): 455-493.

## Keywords

Lignocellulose, terrestrial isopods, ecological interactions, microbiome, metagenomic, transcriptomic

# Regulation of the epibiotic bacterial community of the Mediterranean *Phaeophyceae Taonia atomaria* by its surface metabolome

## Authors

Ahlem Othmani<sup>1</sup>, Gérald Culioli<sup>1</sup>, Robert Bunet<sup>2</sup>, Jean-François Briand<sup>1</sup>

<sup>1</sup> Université de Toulon, MAPIEM, EA 4323, Toulon, France

<sup>2</sup> Institut Océanographique Paul Ricard, Ile des Embiez, Six-Fours-les-Plages, France

Macroalgae harbor a rich diversity of surface associated microorganisms with functions related to host health and defense. This work aims to better understand the ecological mediation between the Mediterranean ochrophyta *Taonia atomaria* and bacterial communities colonizing its surface. A specific extraction protocol dedicated to surface metabolites using a dipping technique in organic solvents was developed without causing cell lysis of the outer layer. MeOH (extraction time < 15s) was demonstrated by fluorescent microscopy to be a suitable solvent for the surface metabolome extraction. These results were validated by a metabolomic approach. The hypothesis of a chemical selection of epibiotic bacteria, which belong to a more global chemical defense strategy, is proposed following several complementary information: (i) the surface of *T. atomaria* is free of macrofouling in its natural environment, (ii) some surface-associated metabolites were able to prevent the adhesion of bacteria in a selective or non-selective manner, (iii) some surface compounds inhibited the attachment of bacteria at concentrations close to those naturally found at the surface of the algae, (iv) active surface compounds could be released into the surrounding seawater, (v) taking into account the overall chemical defense strategy, the settlement of barnacle larvae was prevented by some of the surface compounds. In addition, a seasonal monitoring of both surface metabolomes (LC-MS profiling) and bacterial communities (16S rDNA / MiSeq) was performed during the occurrence of *T. atomaria* from February to July on a collection site near Toulon (north-western French Mediterranean coast). Bacterial communities remained relatively comparable at a high taxonomic level with a clear biofilm profile related to the co-dominance of Bacteroidetes (mainly Sphingobacteria),  $\alpha$ - and  $\gamma$ -proteobacteria. However, a clear seasonal shift associated to seawater temperature increase was observed at the family level. No link appeared at the global scale between bacterial communities and surface metabolome but one may be found for specific molecules. In conclusion complementary approaches such as metabolomics and metagenomics on one hand, and in situ vs in vitro experiments on the other hand, would allow us to better characterize the relationships between *T. atomaria* and its epibiotic bacterial communities.

## References

Egan S, Harder T, Burke C, Steinberg P, Kjelleberg S, Thomas T. (2013). The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiol Rev.* 37:462-476. Othmani A., Briand J.-F., Aye M., Molmeret M. & Culioli G. (2016). Surface metabolites of the brown alga *Taonia atomaria* have the ability to regulate epibiosis. *Biofouling* 32:801-813. Othmani A., Bunet R., Bonnefont J.-L., Briand J.-F. & Culioli G. (2016). Settlement inhibition of bacteria and barnacles by molecules isolated from the Mediterranean brown seaweed *Taonia atomaria*. *J. Appl. Phycol.* 28:1975-1986.

## Keywords

*Phaeophyceae*, biofilm, epibiosis, metabolomic, metagenomic



# Context-dependency of host-microbiota interactions in *Daphnia magna*

## Authors

**Martijn Callens**<sup>1</sup>, Emilie Macke<sup>1</sup>, Koenraad Muylaert<sup>1</sup>, Peter Bossier<sup>2</sup>, Hajime Watanabe<sup>3</sup>, Ellen Decaestecker<sup>1</sup>

<sup>1</sup> Laboratory of aquatic Biology, Department of Science & Technology, KU Leuven Campus Kortrijk, Belgium

<sup>2</sup> Laboratory of Aquaculture & Artemia Reference Center, Department of Animal Production, UGent, Belgium

<sup>3</sup> Laboratory of Bioenvironmental Science, Department of Biotechnology, Osaka University, Japan

Multiple studies have indicated that host-microbiota interactions can show a high degree of context-dependency, where both the strength and direction of this interaction can depend on biotic and abiotic variables. We investigated this context-dependency using *Daphnia magna* as an experimental model system. The associated microbiota of *D. magna* is known to have an overall mutualistic relationship with its host, which is shown by the reduced growth, fecundity and longevity of *D. magna* individuals kept under germ-free conditions. We performed several experiments to quantify the strength of this interaction in different contexts. First, we experimentally assessed host-microbiota interactions under different levels of food availability. Results of these experiments showed that the relative importance of the microbiota for the host's fitness varied according to dietary conditions, with weaker interactions under low levels of food. Second, we experimentally manipulated the composition of the *D. magna* microbiota by exposure to low levels of antibiotics and subsequent transplants of these microbiota communities. Here, it was shown that transplantation of a disturbed microbiota enhanced the performance of *D. magna*, indicating the presence antagonistic interactions under normal conditions. Both results show that the host is affected on multiple levels by its associated microbiota and that the strength of host-microbiota interactions in *D. magna* can vary according to dietary and microbial conditions. These factors have to be accounted for when trying to accurately quantify the functional importance of the microbiota for *D. magna*.

## References

Martijn Callens, Emilie Macke, Koenraad Muylaert, Peter Bossier, Bart Lievens, Michael Waud, Ellen Decaestecker (2016) Food availability affects the strength of mutualistic host-microbiota interactions in *Daphnia magna*. The ISME Journal 10, 911-920; doi: 10.1098/ismej.2015.166

## Keywords

Context dependency, *Daphnia*, food availability, microbiota disturbance

# Renewing the gut microbiota of insect larvea with a bacterial community from the soil: a protocol on *Tenebrio molitor*

## Authors

**Marine Cambon**<sup>1</sup>, Jean-Claude Ogier, Marie Frayssinet, Jean-Baptiste Ferdy, Sophie Gaudriault

<sup>1</sup> INRA, 2 place Pierre Viala, 34095 Montpellier, France

The set of microorganisms associated with a given host, its microbiota, has been proposed to profoundly impact their host physiology, ecology and evolution. Demonstrating this, however, can be challenging, especially because experiments rely on individual hosts reared in controlled condition which microbiota potentially strongly differs from those of natural populations. Sampling individuals hosts in nature could sort out this issue, but poses others problems because the stage of development of sampled individuals may be difficult to assess, and because their genetic background and the environmental conditions they experienced cannot be controlled. In this study, we aimed at designing a model system, where an insect raised in controlled conditions has its microbiota renewed so that it mimics that of insects sampled in nature. For this purpose, we used two month old mealworms (*Tenebrio molitor*) reared in lab conditions, with a poorly diversified gut microbiota. We placed those insects several days in contact with soil samples, so that they ingest the bacteria naturally present in these samples. DNA was then extracted from these insects' gut, and we analyzed the V3-V4 region of the 16S ribosomal gene using metabarcoding techniques. We found that insects kept in soil for five days had a much higher gut microbial diversity. We also found insects' kept on soil samples from distinct localities have different bacterial communities. Finally, the most frequent bacterial families we found in these communities were also identified in former work where insects from natural populations have been studied. Overall, we demonstrate that it is possible to associate insects reared in controlled conditions to bacterial communities sampled from nature. These experimental associations are a promising tool to study insects gut microbiota impact on different host phenotypes, including the interaction between these insects and some entomopathogenic agents.

## Keywords

Gut microbiota, insects, metabarcoding

# How far does a holobiont extend? The effect of constructed environments on the host's microbiota

## Authors

**Felipe Campos-Cerda**<sup>1,2</sup>, Brendan Bohannon<sup>1</sup>

<sup>1</sup> Institute of Ecology and Evolution, University of Oregon

<sup>2</sup> campos\_cf@hotmail.com

Newborn animals host highly simplified microbiotas. In birds, hatching marks the onset of rapid microbial colonization from two immediate sources: parents and the nest. Two bird families (Trogon and Parrots) have diversified nesting behaviors, ranging from the use of preexisting cavities on rotten logs to digging their own cavities on termite mounds, a shift attributed to lower predation rates. We tested if termite mounds also provide better nesting climates and more stable microbial communities. We dug artificial cavities on both logs and termite mounds and measured their internal temperature and humidity. Our results showed more stable, warmer and lower humidity conditions inside termitaria. These conditions are considered to increase hatching success and nestling development. The impact of such different microclimates on the internal microbial abundances and community composition of the cavities will be determined with culture independent molecular techniques. We propose that cavity type could affect nestling survival by selecting and determining its early and potentially long term microbiota. This opens the possibility that nesting behavior can shape a host's microbial associations and those of its offspring, and this in turn suggests that nests should be considered a part of the bird-microbe-nest holobiont.

## Keywords

Environment, host's microbiota

# Assembly and disassembly mechanisms of a holobiont system

## Authors

**Isabel Casillas-Barragán**<sup>1</sup>, Marcelo Visentini Kitahara<sup>2</sup>, Márcio Reis Custódio<sup>1</sup>

<sup>1</sup> Departamento de Fisiologia Geral, IB-USP, São Paulo, Brasil

<sup>2</sup> Instituto do Mar. UNIFESP, Santos, Brasil

The cnidarians-zooxanthellae symbiosis may be impaired by bleaching. Bleaching is the loss or degradation of the pigments of endosymbiotic algae or zooxanthellae (*Symbiodinium spp.*) produced by a stressing agent. However, when this stress is transient, the holobiont system may be reestablished. In this study, we used the anemone *Exaiptasia pallida*-*Symbiodinium sp.* as a model for studying the bleaching recovery strategy. The association was morphologically assessed by histology, transmission electron microscopy and scanning electron microscopy at the tentacles, gastrovascular cavity and the excretion pellet. We propose an assembly mechanism of the holobiont related to the pattern of functional bleaching recovery found in both adult and juvenile stages. Also, we propose a disassembly mechanism of the holobiont system related to the regurgitation of free living zooxantela knitted by its own flagella based on the host and hosted membrane interaction that may suggest a proactive dispersal strategy driven by the zooxanthellae.

## Keywords

Bleaching, cnidarian recovery, functional pigmentation

# Bottlenose dolphins and killer whales harbor a unique skin microbiome that varies among individuals

## Authors

**Marlène Chiarello**, Sébastien Villéger, Corinne Bouvier, Jean-Christophe Auguet, Thierry Bouvier

UMR 9190 Marine Biodiversity, Exploitation and Conservation (MARBEC), Université de Montpellier, CNRS, IRD, IFREMER, Place Eugène Bataillon, Case 093, 34 095 Montpellier Cedex 5, France

Marine animals host diverse and species-specific microbial communities on their skin that could play a major role for their host fitness. However, most inventories of marine animals skin microbiomes have focused on corals and fishes, while the microbiome of other phylogenetic lineages, notably vertebrates, remains overlooked. Among these, one of special interest is the Cetacea, whose species descended from terrestrial mammals that went back to sea during Eocene period. Few studies have focused on wild or semi-captive cetaceans, making difficult to distinct intrinsic inter- and/or intraspecific variability in skin microbiomes from environmental effects. Assessing inter- and intraspecific variability of cetaceans skin microbiome in controlled conditions is also needed to understand the differences with skin microbiome of other marine vertebrates and terrestrial mammals. Here, using high-throughput sequencing, we assessed the skin microbiome of 8 individuals of two emblematic species, the bottlenose dolphin (*Tursiops truncatus*) and the killer whale (*Orcinus orca*), housed in captivity in controlled conditions (Marineland park, Antibes, France). Using a set of complementary alpha- and beta-diversity indices, we show that (i) cetacean skin microbiome is distinct from the surrounding planktonic communities, (ii) the 2 cetacean species host different skin microbiomes, and that (iii) inter-individual variability of the skin microbiome was higher than intra-individual variability (i.e. between body parts), within each species. The predominant microbial clades on both species were Gamma- and Alpha-proteobacteria, Actinobacteria and Firmicutes. However, Firmicutes, and especially Staphylococci, were six times more abundant on dolphin skin than on killer whale skin. Overall, the skin microbiome of those two Odontoceti cetaceans was more similar to that of the Mysticeti humpback whale than to microbiomes of fishes or terrestrial mammals.

## Keywords

Microbiome, skin, Cetacea, phylogenetic diversity

# Specificity and stability of microbial communities associated to healthy and diseased *Crassostrea gigas* oysters

## Authors

**Camille Clerissi<sup>1</sup>**, Julien de Lorgeril<sup>2</sup>, Eve Toulza<sup>1</sup>

<sup>1</sup> IHPE UMR5244, Université de Perpignan via Domitia, 58 avenue Paul Alduy, 66680, Perpignan.

<sup>2</sup> IFREMER, IHPE UMR5244, Université Montpellier 2, 2 Place Eugène Bataillon, 34095, Montpellier

Since the last decade, recurrent summer mortalities affect the exploited oyster, *Crassostrea gigas*. The pathology is multifactorial since it is induced by the combination of environmental factors (water temperature, development of viral and bacterial pathogens), physiological status and genetic backgrounds of oysters (Petton *et al.* 2013; Dégremont *et al.* 2015; Petton *et al.* 2015). Although microbiota play a crucial role for host fitness (according to the hologenome theory; Rosenberg *et al.* 2007), little is known concerning the structure of microbial communities associated to oysters, and whether they are affected by stressful conditions. To tackle these issues, we analyzed the microbiota (16S rDNA-metabarcoding) of five oyster families that showed contrasted phenotypes in terms of susceptibility to summer mortalities. First, we described the specific diversity of microbial communities, but also their functional diversity after inference of metagenomic profiles (Langille *et al.* 2013). Then, we tested if the structure of microbiota were linked to (i) the genetic background of oysters (specificity), (ii) environmental conditions (control vs. stress), and (iii) if the susceptibility status of families were positively correlated to the stability of their microbiota in various conditions.

## References

Dégremont L, Lamy J-B, Pépin J-F, Travers M-A, Renault T. New insight for the genetic evaluation of resistance to ostreid Herpesvirus infection, a worldwide disease, in *Crassostrea gigas*. PLoS ONE, 10(6): e0127917. doi:10.1371/journal.pone.0127917. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol., 31: 814-823. Petton B, Pernet F, Robert R, Boudry P. 2013. Temperature influence on pathogen transmission and subsequent mortalities in juvenile Pacific oysters *Crassostrea gigas*. Aquac. Environ. Interact., 3(3): 257-273. Petton B, Bruto M, James A, Labreuche Y, Alunno-Bruscia M-A, Leroux F. 2015. *Crassostrea gigas* mortality in France: the usual suspect, a herpes virus, may not be the killer in this polymicrobial opportunistic disease. Front. Microbiol., doi.org/10.3389/fmicb.2015.00686. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. 2007. The role of microorganisms in coral health, disease and evolution. Nat. Rev. Microbiol., 5: 355-362.

## Keywords

Oyster, health, microbiota, diversity, functional diversity, metabarcoding, metagenomic.



## Symbiont diversity and feeding strategies in insect agricultural pests

### Authors

**Maarten De Cock**<sup>1</sup>, Massimiliano Virgilio<sup>1</sup>, Anne Willems<sup>2</sup>, Peter Vandamme<sup>2</sup>, Marc De Meyer<sup>1</sup>

<sup>1</sup> Royal Museum for Central-Africa, Tervuren, Belgium

<sup>2</sup> University of Gent, Ghent, Belgium

Gut microbionts play a fundamental ecological and evolutionary role in phytophagous insects by making the plant tissue edible and by promoting adaptation between insects and host plants. SYMDIV is a collaborative project between the Royal Museum for Central Africa (Tervuren, BE) and the Laboratory of Microbiology of the University of Ghent funded by the Belgian Science Policy (BELSPO). This project, consisting of four work packages (WPs), aims at describing and comparing the gut microbiomes of phytophagous tephritid flies (Diptera, Tephritidae) with different feeding strategies (from strictly monophagous to highly polyphagous). In particular we aim at (a) comparing the 16S microbiome profiles of species such as *Bactrocera dorsalis*, *Ceratitis capitata*, etc., across multiple African and Mediterranean locations and across different host plants (WP1 and 2) (b) cultivating and isolating selected microbial strains (WP3) and (c) compiling and publishing a database of endosymbionts – host associations (WP4). A pilot methodological test is currently ongoing in collaboration with IAEA, with the objective of optimising the experimental and analytical protocols. The SYMDIV project will provide baseline data to better understand relationship between gut microbionts, host range spectrum and metabolic processes in tephritid fruit flies. We will also isolate and characterise bacterial strains that could be tested as probiotics for SIT mass rearing.

### Keywords

Tephritidae, gut microbiome, metagenomics, 16S rRNA, cultivation

# Involvement of the environment and genetic background in the composition and dynamics of microbial communities (viruses, bacteria and protists) hosted by the hemolymph of the Pacific oysters.

## Authors

**Samuel Dupont**<sup>1</sup>, B. Petton<sup>2</sup>, E. Toulza<sup>1</sup>, A. Lokmer<sup>3</sup>, C. Montagnani<sup>1</sup>, Y. Desdevises<sup>4</sup>, D. Pecqueur<sup>5</sup>, C. Salmeron<sup>5</sup>, L. Guillou<sup>6</sup>, C. Desnues<sup>7</sup>, B. La Scola<sup>7</sup>, J. de Lorgeril<sup>1</sup>, G. Mitta<sup>1</sup>, Y. Gueguen<sup>1</sup>, J.M. Escoubas<sup>1</sup>

<sup>1</sup> IHPE UMR 5244, CNRS, Ifremer, Univ. Montpellier, Univ. Perpignan Via Domitia, 34095 Montpellier, France

<sup>2</sup> Ifremer, LEMAR UMR 6539, 11 Presqu'île du Vivier, Argenton-en-Landunvez, France

<sup>3</sup> Coastal Ecology, Wadden Sea Station Sylt, Alfred Wegener Institute - Helmholtz Centre for Polar and Marine Research List auf Sylt, Germany

<sup>4</sup> Biologie Intégrative des Organismes Marins, CNRS, UMR 7232, Avenue du Fontaulé, 66650, Banyuls-sur-Mer, France

<sup>5</sup> Sorbonne Universités, UPMC Univ Paris 06, CNRS, Observatoire Océanologique de Banyuls (OOB), 66650 Banyuls/Mer, France

<sup>6</sup> Station Biologique de Roscoff, CNRS, UMR 7144, Place Georges Teissier, CS90074, 29688 Roscoff, France

<sup>7</sup> URMITE, UMR63, CNRS 7278, IRD 198, INSERM 1095, 13385 Marseille, France

Numerous studies have highlighted the importance of microbial communities in the environmental adaptability and evolution of macroorganisms that host them (hologenome concept of evolution). The microbiota of most metazoans is located on external surfaces such as skin, teguments or in cavities in contact with the outside like the digestive tract. Moreover, it is generally accepted that the circulatory system of healthy animals is sterile. Nevertheless, some marine invertebrates such as oysters escape this rule and possess an internal microbiota associated with the hemolymph (the blood of invertebrates) called hemo-microbiota. The existence of this hemo-microbiota constitutes a paradox since the hemolymph contains circulating cells (hemocytes) that play a key role in the anti-infectious response of the oyster. The hemolymph can thus be considered as an ecological niche in which immune cells and micro-organisms coexist. An attractive hypothesis is that the hemo-microbiota could be the result of a coevolution process leading to the "domestication" of a microbial community participating in the holobiont fitness. In order to explore this hypothesis, we have undertaken the characterization of the entire microbial community (viruses, bacteria and protists) living in oyster's hemolymph using global analytical approaches (metabarcoding and metagenomics). In order to determine the impact of the oyster's genetic background on the hemo-microbiota composition, we used genetically differentiated oyster families, produced in hatchery and bearing contrasted phenotypes, especially in respect to summer mortality syndrome (i.e. resistant and susceptible). These animals were then transplanted in natural environments (infectious and non-infectious) in order to study the impact of the environment on the composition and dynamics of the hemo-microbiota. Preliminary results showed that the hemo-microbiota is a complex microbial community composed of viruses, bacteria and protists. Moreover, the hemo-microbiota (bacteria and protists at least) is different from the whole oyster microbiota, suggesting that the hemolymph compartment could be considered as an ecological niche. Our results showed that the environmental factors play a major control on the composition and the dynamics of the hemo-microbiota whereas the genetic background of the animals does not seem to be significant in the shaping of microbial communities. Finally, only the viral population composition was positively correlated with the susceptibility of oysters to mortalities in the natural environment.

## Keywords

Pacific oysters, genetic backgrounds, hemolymph, microbiota

# The ecology of fly-bacteria-yeast symbiosis: laboratory interactions and their relevance to in-natura processes

## Authors

Robin Guilhot, Antoine Rombaut, Kate Howell, Anne Xuéreb, Patricia Gibert, **Simon Fellous**<sup>1</sup>

<sup>1</sup> CBGP-INRA, 34988 Montferrier sur Lez, France

The symbiosis of *Drosophila* fruit flies with yeast and bacteria is essential to insect nutrition. Recent advances are based on lab populations, where food and selective pressures on microbe transmission differ from the field. In particular, lab flies are reared without live yeast, the obligate symbiont of wild flies. Here, we investigated the symbiosis of lab *Drosophila melanogaster* with bacteria, and tested whether lab phenotypes maintain in natural conditions. Our experiments in lab nutritive medium identified some bacterial strains as strict gut symbionts. Another bacterium colonized medium surface where it was consumed by fly larvae, favoring their growth. Experiments in fruit revealed dramatically different effects of bacteria on fly phenotypes. Yeast development in the fruit, and its transmission across metamorphosis (i.e. from larvae to adults) was affected by two bacteria, probably through effects on fly physiology. In both environments, bacterial transmission across metamorphosis was rare and stochastic, a likely adaptation to lab conditions. These results indicate discoveries based on lab symbioses may not inform on field phenomena. Besides, we discovered bacterial symbionts can interfere with multiplication and transmission of yeast, the obligate symbiont of wild flies. We conclude that understanding symbiosis and its consequences to fly, bacteria and yeast dynamics in-natura will necessitate working with natural communities in ecologically relevant settings.

## Keywords

Symbiosis, empirical, *Drosophila*, ecology, evolution

# Metabolic network topology to elucidate respective roles of species in a symbiotic community

## Authors

**Clémence Frioux**<sup>1</sup>, Simon M. Dittami<sup>2</sup>, Anne Siegel<sup>3</sup>

<sup>1</sup> INRIA Rennes Bretagne Atlantique, Université de Rennes 1, Rennes, France

<sup>2</sup> Sorbonne Universités, UPMC Université Paris 6, CNRS UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff

<sup>3</sup> Institut de Recherche en Informatique et Systèmes Aléatoires (IRISA), CNRS UMR 6074, Université de Rennes 1, Rennes France

Metabolic networks are a relevant tool to study interactions within holobionts. A common weakness in ecosystems studies relying on the former, however, is related to the sometimes exotic nature of the organisms involved: interactions often occur between poorly described organisms with few available data, which may limit the applicability of quantitative flux-based techniques. In these cases, a first approach to study interactions can be based on the topology of metabolic networks to assess the producibility gains for members of ecosystems when sharing metabolic capacities. Additional manual curation based on genome annotation can then be used as a first screen to confirm or negate the hypothetical exchanges. This procedure was applied to an algal-bacterial holobiont. *Ectocarpus siliculosus* is a brown alga whose metabolic network has recently been reconstructed. It is frequently associated with the symbiotic bacterium *Candidatus Phaeomarinobacter ectocarpi*. Topology-based metabolic network analyses, in combination with additional transcriptomic datasets, shed light on putative exchanges between both in particular related to the production of algal metabolic targets. For instance, *E. siliculosus* may rely on its associated bacterium for the production of  $\beta$ -alanine, precursor of vitamin B5 for which the alga lacks an enzyme whereas the bacterium possesses the production pathway. Although experimental validation of the generated hypotheses is still pending, this example clearly demonstrates the interest of the developed approach to identify potential metabolic inter-species interactions within an ecosystem, especially when dealing with poorly-studied organisms.

## References

Dittami SM, Barbeyron T, Boyen C, Cambefort J, Collet G, Delage L, *et al.* Genome and metabolic network of “*Candidatus Phaeomarinobacter ectocarpi*” Ec32, a new candidate genus of Alphaproteobacteria frequently associated with brown algae. *Frontiers in Genetics*. 2014;5(241). Available from: [http://www.frontiersin.org/systems\\_biology/10.3389/fgene.2014.00241/abstract](http://www.frontiersin.org/systems_biology/10.3389/fgene.2014.00241/abstract). Prigent S, Collet G, Dittami SM, Delage L, Ethis de Corny F, Dameron O, *et al.* The genome-scale metabolic network of *Ectocarpus siliculosus* (EctoGEM): a resource to study brown algal physiology and beyond. *The Plant Journal*. 2014;80(2):367–381. Available from: <http://dx.doi.org/10.1111/tpj.12627>. Prigent S, Frioux C, Dittami SM, Thiele S, Larhlami A, Collet G, *et al.* Meneco, a Topology-Based Gap Filling Algorithm Applicable to Degraded Genome-Wide Metabolic Networks. *PLoS Computational Biology*, to appear.

## Keywords

Holobiont – metabolic networks – interactions – topology – gap-filling

# Coral photosymbiosis: linking phylogenetic identity to single cell metabolic activity in mixed symbiont populations

## Authors

**Béatrice Gaume**<sup>1,2</sup>, Isabelle Domart-Coulon<sup>2</sup>, Anders Meibom<sup>1,3</sup>

1 Ecole Polytechnique Fédérale de Lausanne, Laboratory of Biological Geochemistry, Switzerland

2 Muséum national d'Histoire naturelle, UMR 7245 MCAM MNHN-CNRS, Paris, France

3 Université de Lausanne, Institut des Sciences de la Terre, Switzerland

Tropical reef-building corals live in symbiosis with a wide range of micro-organisms, including unicellular Symbiodinium dinoflagellates also called *zooxanthellae*. These photosynthetic endosymbionts live inside the coral gastroderm cells. Although corals can feed on planktonic preys, the dinoflagellates significantly contribute to the nutrition of their host by transferring a large fraction (up to 90%) of photosynthates that are produced through the fixation of dissolved inorganic carbon (DIC) and nitrogen (nitrate or ammonium). Nine major clades (A-I) of Symbiodinium have been identified by molecular genetic analyses. Each clade presents distinct physiological features and the specific association between coral species and Symbiodinium clade determines the phenotype of the holobiont. Differences in the photosynthetic response to irradiance, rates of carbon fixation, and thermal tolerance can be attributed to symbiont clade. Several Symbiodinium clades can simultaneously exist within a single coral and the host can dynamically modify the proportion between dominant and background clades to adapt to changing environmental conditions. Investigating at the cellular level the metabolic exchanges between coral and Symbiodinium is of great interest to understand e.g. the bleaching process (loss of zooxanthellae) which often leads to coral death. We are aiming at quantitatively image the differential metabolic activity between symbiont clades in the same host, in the intact symbiosis. Previous studies have used mass bulk techniques to investigate the metabolism of symbionts at the colony scale. However, such studies cannot determine the specific contribution of the individual cells, as a function of their distribution in the coral host tissue. We have developed a SIMS-ISH method combining nanoscale secondary ion mass spectrometry (NanoSIMS) and in situ hybridization (ISH) for the simultaneous in situ identification of Symbiodinium genotype, and visualization of symbiont-host metabolic exchange at the level of individual cell. We focus on two reef-building coral species *Pocillopora damicornis* and *Stylophora pistillata* for which a large amount of complementary metabolic data exists. We designed specific fluorescent DNA probes to identify clade C Symbiodinium in *P. damicornis* and clade A in *S. pistillata*, and in mixed cultures. We combined the probes with pulse-chase experiments using isotopically labeled seawater (<sup>13</sup>C-bicarbonate and <sup>15</sup>N-nitrate) to attribute a particular metabolism to a specific clade. This combined method enables us to phylogenetically identify metabolically active cells from a NanoSIMS isotopic/elemental image. The precise correlation between TEM and the NanoSIMS isotopic maps allows us to follow the turnover and translocation of metabolites with sub-cellular precision in both the symbionts and the host. Due to the complex nature of the coral symbiosis, the ability to discriminate the phylogenetic identity and metabolic role of specific Symbiodinium populations in situ is crucial to understand the effects of environmental stress on the coral holobiont plasticity. Moreover, analyzing symbiotic associations in situ provides a unique insight into the spatio-temporal patterns of metabolic interactions in holobionts. This analytical breakthrough promises to open entirely new areas of research focused on understanding the dynamics of interactions between animals and the microbial world.

## Keywords

Symbiosis, corals, dinoflagellates, in situ hybridization, NanoSIMS



# Isoprenoids in plant-bacteria interactions

## Authors

**Chloé Groh**<sup>1</sup>, Claire Villette<sup>1</sup>, Hubert Schaller<sup>1</sup>, Dimitri Heintz<sup>1</sup>, Florence Ploetze<sup>1</sup>

<sup>1</sup> IBMP, 12 rue du général Zimmer, 67084-F STRASBOURG

Thanks to the recent development of the environmental microbial and genomic approaches (1, 2, 3, 4), a precise inventory of the bacterial communities interacting with many plants including *Arabidopsis thaliana* (5, 6, 7), *Nicotiana tabacum* (8, 9), or *Hordeum vulgare* (10) has been done. In addition, many of the bacterial species associated with these plants have been isolated (5, 6, 11, 12). Several studies suggest a role of some of these bacteria in the plant metabolism. This is particularly the case of isoprenoids, which are involved in essential biological processes such as cell division, photosynthesis, respiration, or immune response (13, 14). Our project aims to examine the influence of microbial communities on the plant isoprenoid metabolism, but also the role that some key isoprenoid compounds could have on the selection of the microbial community by the host plant. Biochemical mutants of *Arabidopsis thaliana* affected in isoprenoid biosynthetic pathways were genetically characterized (15). These mutants exhibit clear phenotypes of impaired growth or plastid biogenesis (15). We compared the composition of the bacterial community interacting with these plants by a metagenomics approach, isolating bacteria and identifying them. This identification requires 16S rRNA encoding gene sequencing – and mass spectrometry (MALDI) – based profiling approaches. In parallel, we currently analyze the metabolic profile of these plants. We aim to use these isolates to inoculate plants grown under controlled conditions in order to determine if there is an implication of bacteria in the plant isoprenoid metabolism, and if there is an impact of this metabolism on microbiota selection.

## References

1 Guttman *et al.*, 2014, Nature Reviews Genetics - 2 Berg *et al.*, 2014, Front. Microbiol - 3 Bulgarelli *et al.*, 2013, Annual Review of Plant Biology - 4 Lebeis, 2015, Curr. Opin. Plant Biol - 5 Bulgarelli *et al.*, 2012, Nature - 6 Lundberg *et al.*, 2012, Nature - 7 Schlaeppi *et al.*, 2014, Proc. Natl. Acad. Sci. U.S.A. - 8 Saleem *et al.*, 2015, Microb. Ecol - 9 Santhanam *et al.*, 2014, PLoS ONE - 10 Bulgarelli *et al.*, 2015, Cell Host Microbe - 11 Bai *et al.*, 2015, Nature - 12 Hardoim *et al.*, 2015, Microbiol. Mol. Biol. Rev. - 13 Wang *et al.*, 2012, Plant Physiol. - 14 Chung *et al.*, 2015, Plant Mol. Biol. - 15 Villette *et al.*, unpublished.

## Keywords

Isoprenoids, interactions, bacteria, *Arabidopsis thaliana*



# Dynamics of *Aedes albopictus* mosquito holobiont in response to temperature changes

## Authors

**Morgane Guégan**<sup>1</sup>, Florence-Hélène Tran, Guillaume Minard, Van Tran Van, Patrick Potier, Claire Valiente Moro

<sup>1</sup> UMR CNRS 5557 Ecologie Microbienne, 10 rue Raphael Dubois, 69100 Villeurbanne, France

In recent years, many examples demonstrated the importance of the microbial compartment in insect biology including its role in acclimation to environmental changes (1, 2). The tiger mosquito *Aedes albopictus* is an invasive species of Asian origin (3) and a very efficient vector of many pathogens (4). In this study, we investigated for the first time the response of the mosquito holobiont by comparing the gut microbiota dynamics in mosquitos treated or not with antibiotics for 5 days. Half of the individuals of each modality was then submitted to a temperature downshift from 28°C to 18°C. The antibiotic treatment or the temperature decrease had no effect on insects survival. However, Automated Ribosomal Intergenic Spacer Analysis Fingerprints (ARISA) revealed important modifications in the relative abundance and diversity of the gut bacterial communities in the case of both perturbations. After one month, this dysbiosis tended to return to a state of equilibrium close to that observed in control individuals. In conclusion, our results show that the mosquito bacterial microbiota is an ecosystem in fragile equilibrium and capable of resilience. High throughput sequencing of bacterial 16S rDNA gene is under analysis to identify which bacteria are impacted by abiotic perturbations. Further research is necessary to better understand the importance of the microbiota in the functioning and acclimatization of the tiger mosquito to environmental changes.

## References

- (1) Douglas AE. 2015. Multiorganismal insects: diversity and function of resident microorganisms. *Annu Rev Entomol* 60:17–34
- (2) Minard G, Mavingui P, Moro CV. 2013. Diversity and function of bacterial microbiota in the mosquito holobiont. *Parasit Vectors* 6:146
- (3) Bonizzoni M, Gasperi G, Chen X, James AA. 2013. The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. *Trends Parasitol* 29:460–468
- (4) Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Microbes Infect Inst Pasteur* 11:1177–1185.

## Keywords

Holobiont, *Aedes albopictus*, bacterial microbiota, abiotic disturbances

# Dual RNAseq to understand costs of symbiont-conferred resistance to parasitoids

## Authors

**Heidi Kaeck**<sup>1</sup>, Christoph Vorburger<sup>1,2</sup>, Alice Dennis<sup>3</sup>, Niklaus Zemp<sup>2</sup>

<sup>1</sup> EAWAG, Duebendorf, Switzerland

<sup>2</sup> ETH Zurich, Switzerland

<sup>3</sup> University of Potsdam, Germany

Infection with the bacterial endosymbiont *Hamiltonella defensa* protects black bean aphids (*Aphis fabae*) against parasitoid wasps (*Lysiphlebus fabarum*), but this protection does not come for free to the host. In the absence of parasitoids, *Hamiltonella*-infected aphids exhibit reduced lifespan and lifetime reproduction. The magnitude of these negative effects differs markedly between different *Hamiltonella* strains, which places the strains in a spectrum between pathogen-like and mutualist. However, the mechanistic basis behind the cost is still unknown. Plausible hypotheses include resource competition between host and endosymbiont, costs of immune activation, or collateral damage caused by endosymbiont-produced toxins. To obtain a functional understanding of *Hamiltonella*-induced costs, I used dual RNAseq to compare host and symbiont gene expression among aphids infected with different *Hamiltonella* strains and uninfected aphids. I show that in addition to more specific mechanisms, symbiont density also contributes to the costs of possessing *Hamiltonella*.

## Keywords

Aphid, *Hamiltonella*, cost

# Deciphering the impact of symbiotic bacteria during freshwater acclimation in the brown macroalga *Ectocarpus subulatus*: from culture dependent-approach towards integrated 'omics'

## Authors

**Hetty Kleinjan**<sup>1,2</sup>, Simon M. Dittami<sup>1,2</sup>, Christian Jeanthon<sup>3,4</sup>, Catherine Boyen<sup>1,2</sup>

<sup>1</sup> CNRS, UMR 8227, Station Biologique de Roscoff, Laboratoire de Biologie Intégrative des Modèles Marins, Roscoff, France

<sup>2</sup> Sorbonne Universités, UPMC Univ Paris 06, Station Biologique de Roscoff, UMR8227, Laboratoire de Biologie Intégrative des Modèles Marins

<sup>3</sup> CNRS, UMR 7144, Station Biologique de Roscoff, Adaptation et Diversité en Milieu Marin, Roscoff, France

<sup>4</sup> Sorbonne Universités, UPMC Univ Paris 06, Station Biologique de Roscoff, UMR7144, Adaptation et Diversité en Milieu, Roscoff, France

Similar to other (multicellular) eukaryotes, macroalgae live in close association with microbes, e.g. symbiotic bacteria, and their interactions affect the physiology of both partners. The fresh water strain (EsubFWS) of *Ectocarpus subulatus*, a small filamentous brown macroalga, tolerates a broad range of salinities compared to other Ectocarpales, yet this ability depends on its associated microbiome (Dittami et al. 2016). We aim to elucidate the functional mechanisms involved in the acclimation of the EsubFWS holobiont to fresh water, with emphasis on the metabolic interactions between the alga and the microbiome. To this aim, we first sought to create a simplified and controllable microecosystem via both the cultivation of bacterial partners and production of axenic algal filaments. Bacteria were isolated using direct-plating and dilution-to-extinction techniques, with both chemically defined and undefined low-nutrient culture media. Ground algae, algal culture medium, and cell wall extracts were used as inocula. This resulted in the isolation of 250 strains, covering 35 bacterial species, within 29 different genera phylogenetically dispersed into the Betaproteobacteria (50%), Alphaproteobacteria (27%), Gammaproteobacteria (10%), Firmicutes (5%), Bacteroidetes (5%), and Actinobacteria (3%). The effects these cultured bacteria on algal fresh water acclimation were investigated using sterilized antibiotic-treated algal filaments that were inoculated with the selected bacterial cultures: neither individual bacterial isolates nor tested artificial bacterial communities were able to restore algal tolerance to fresh water, suggesting an important role of the “uncultivable” microbiome during acclimation to freshwater. In order to generate hypotheses about the metabolic interactions between the alga and the uncultivable microbiome we chose to work with three algal-holobionts with reduced microbiomes resulting from different (mild) antibiotic-treatments. These holobionts differ in their microbiome and in their capacity to acclimate to fresh water. They will be investigated using metabolomics and transcriptomics during the change in salinity. This integrated ‘omics’ approach, as an alternative to the co-culture system, can be used to generate hypotheses about how bacterial metabolism complements the algal host during fresh water acclimation and provide us with ideas for future functional studies.

## References

Dittami, S.M. et al., 2016. Host-microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures. ISME J, 10(1), pp.51–63. Available at: <http://dx.doi.org/10.1038/ismej.2015.104>.

## Keywords

Macroalgae, microbiome, symbiotic bacteria, bacterial cultivation, metabolomics, acclimation, 'omics'

# Identification and characterization of bacterial colonization functions in the beanbug *Riptortus pedestris*-*Burkholderia insecticola* symbiosis

## Authors

**Joy Lachat**<sup>1</sup>, Quentin Nicoud<sup>1</sup>, Tsubasa Ohbayashi<sup>1,2,3</sup>, Yoshitomo Kikuchi<sup>2,3</sup>, Peter Mergaert<sup>1,2,3</sup>

<sup>1</sup> Institute for Integrative Biology of the Cell, UMR9198, CNRS, Université Paris-Sud, CEA, Gif-sur-Yvette, France

<sup>2</sup> Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Hokkaido Center, Sapporo, Japan

<sup>3</sup> Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

The phytophagous insect *Riptortus pedestris*, belonging to the suborder of the Heteroptera, is an important crop pest in Japan which feeds preferentially on soybean plants. This beanbug is associated with a bacterial symbiont, a specific *Burkholderia* species, located in a specific region of the insect's midgut called the M4 region. The M4 region is organized in crypts and constitutes the symbiotic organ where the symbiont is present extracellularly in the lumen of the crypts. This interaction promotes the growth and the development of the beanbug, and can also confer insecticide resistance towards fenitrothion. Since this bacterium is acquired horizontally, aposymbiotic insects can be reared and the bacterium can be isolated in vitro, rendering this model suitable for studying symbiotic interactions. Recently, it has been found that *Riptortus* produces antimicrobial peptides in the midgut crypts called crypt-specific cysteine-rich peptides (CCR) for which the bacterial symbiont demonstrates a high resistance profile. It has been hypothesized that these CCR peptides can participate to the specific colonization of the symbiotic organ by *Burkholderia insecticola*. In order to decipher which bacterial factors are involved in this specific colonization, we are conducting a candidate gene approach based on the implication of the bacterial membrane components, targeted by the CCR peptides. As *Burkholderia* are Gram negative species, we are interested in the lipopolysaccharide biosynthesis pathway, the hopanoid (sterol-like bacterial lipids) biosynthesis pathway and the extracytoplasmic stress response pathway. Furthermore, with a Tn-seq approach, we aim to identify which bacterial genes are essential for the CCR resistance and the insect colonization. This recent genomic application could be very efficient to pinpoint new bacterial genetic targets for future mutagenesis experiments.

## References

Kikuchi, Y., Meng, X.-Y. & Fukatsu, T. Gut Symbiotic Bacteria of the Genus *Burkholderia* in the Broad-Headed Bugs *Riptortus clavatus* and *Leptocoris chinensis* (Heteroptera: Alydidae). *Appl. Environ. Microbiol.* 71, 4035–4043 (2005). Futahashi, R. et al. Gene Expression in Gut Symbiotic Organ of Stinkbug Affected by Extracellular Bacterial Symbiont. *PLoS ONE* 8, e64557 (2013). Kikuchi, Y. & Fukatsu, T. Live imaging of symbiosis: spatiotemporal infection dynamics of a GFP-labelled *Burkholderia* symbiont in the bean bug *Riptortus pedestris*. *Mol. Ecol.* 23, 1445–1456 (2014). Chao, M. C., Abel, S., Davis, B. M. & Waldor, M. K. The design and analysis of transposon insertion sequencing experiments. *Nat. Rev. Microbiol.* 14, 119–128 (2016). Goodman, A. L. et al. Identifying Genetic Determinants Needed to Establish a Human Gut Symbiont in Its Habitat. *Cell Host Microbe* 6, 279–289 (2009).

## Keywords

Symbiosis, *Burkholderia*, antimicrobial peptides, bacterial resistance, phytophagous insects, Tn-Seq

## FRANCE GENOMIQUE: A National, world-class infrastructure for genomics and bioinformatics

**Author**

**Pierre Le Ber**

**Genoscope – CNS, Evry, France**

Created in 2012 thanks to a government grant from the “Investissements d’Avenir” program, the France Génomique (FG) infrastructure offers to the French life-sciences community access to a network facility of more than 22 sequencing and/or bioinformatics platforms that have been operational for many years and have developed one or more complementary specialties.

The missions of the France Génomique infrastructure are to provide the French scientific community (public and private sectors) with:

Leading expertise in genomics and associated bioinformatics technologies, which are crucial to stay in phase with very rapid technological evolution.

Competitively priced services in genomics and bioinformatics: The close proximity between the user and an ‘expert’ infrastructure guarantees optimal scientific exploitation of the data.

The opportunity to undertake ambitious projects of high scientific added value and strong international visibility, through the expertise and capacities offered by the infrastructure.

Thus, France Génomique aims to guarantee France a high level of competitiveness and independence in the field of genomic production and data analysis technology, at a time when technology has never been more strategic to all areas of research in the life sciences.

FG unified governance, plus shared equipment and expertise, enables to build the critical mass to remain at the cutting edge of international progress and to respond to the growing needs of genomics in terms of data processing and storage, and development of innovative tools.

# Accessing the insect holobiont through metagenomic sequencing of gut content or preserving ethanol

## Authors

**Benjamin Linard**<sup>1</sup>, Débora P. Paula<sup>2</sup>, Krister Swenson<sup>1</sup>, Fabio Pardi<sup>1</sup>, Alfried P. Vogler<sup>3,4</sup>

<sup>1</sup> Institut de Biologie Computationnelle, Laboratoire d'Informatique, de Robotique et de Microélectronique de Montpellier (LIRMM), UMR 5506, CNRS & Université de Montpellier, Montpellier, France

<sup>2</sup> Embrapa Genetic Resources and Biotechnology, Parque Estação Biológica, Brasília, Brazil

<sup>3</sup> Department of Life Sciences, Natural History Museum, Cromwell Rd, London, United Kingdom

<sup>4</sup> Department of Life Sciences, Silwood Park Campus, Imperial College London, Ascot, SL5 7PY, United Kingdom

Dna-based methods are useful to identify the gut content of arthropods, their commensal bacterial fauna, but also (non-)obligatory symbionts, associated parasites and ingested food. We recently explored the holobionts of Coleopterans (a clade which represents 25% of animal diversity) through their gut contents. Non-targeted metagenomic sequencing was used, with total DNA being extracted from independent specimen guts or from bulk samples of field specimens captured in pure ethanol. I will briefly synthesize our last work, demonstrating the potential of arthropod bulk metagenomic sequencing for the exploration of the Coleopteran holobiont. This exploration was done from three points of view [1,2,3] and showed how a) the interaction between predator and prey specimens can be detected indirectly through the sequencing of their gut symbionts, b) how novel trophic interactions can be rapidly built from such metagenomes, and c) that Coleopteran holobionts are partially transferred from the specimens to the preserving ethanol and how sequencing this media could help to associate the corresponding holobionts to specific habitats. These results also highlight why shallow metagenomic sampling of arthropod metagenomes to classical barcode-based approaches [4,5,6] is that the approach has potential for the description of complex, sometimes unexpected, symbiotic interactions. Finally, I will extend the discussion to the metagenome fraction which cannot be associated to specific clades and generally remains unexplored because of the heaviness of applying fundamental comparative genomics to such data or simply the lack of time/bioinformatic resources. Strikingly, this “dark matter” may hide some essential components of the arthropod holobiont. Notably, traces of known parasites were present in our studies and almost any microbiome may be associated a metavirome. Surely, the latter is becoming more accessible today, as shown by recent the recent explosion of viral references [6]. Yet, studying this fraction is challenging because DNA identifications made through state-of-the-art sequence aligners and phylogenetic reconstruction are difficult to scale to the high evolutionary rates of viruses. In this context, I will introduce our latest research related to alignment-free phylogenetic placements and why such approaches could accelerate the exploration of holobiont fractions which are hidden in our metagenomes.

## References

[1] Lessons from genome skimming of arthropod-preserving ethanol. Linard B, Arribas P, Andújar C, Crampton-Platt A, Vogler AP. *Mol Ecol Resour.* 2016 Nov;16(6):1365-1377.[2] Uncovering Trophic Interactions in Arthropod Predators through DNA Shotgun-Sequencing of Gut Contents. Paula DP, Linard B, Crampton-Platt A, Srivathsan A, Timmermans MJ, Sujii ER, Pires CS, Souza LM, Andow DA, Vogler AP. *PLoS One.* 2016 Sep 13;11(9):e0161841.[3] Detection and decay rates of prey and prey symbionts in the gut of a predator through metagenomics. Paula DP, Linard B, Andow D, Sujii ER, Pires CS, Vogler AP. *Mol Ecol Resour.* 2015 Jul;15(4):880-92.[4] Metagenome Skimming of Insect Specimen Pools: Potential for Comparative Genomics. Linard B, Crampton-Platt A, Gillett CP, Timmermans MJ, Vogler AP. *Genome Biol Evol.* 2015 May 14;7(6):1474-89.[5] Metagenome skimming for phylogenetic community ecology: a new era in biodiversity research. Papadopoulou A, Taberlet P, Zinger L. *Mol Ecol.* 2015 Jul;24(14):3515-7.[6] Mitochondrial metagenomics: letting the genes out of the bottle. Crampton-Platt A, Yu DW, Zhou X, Vogler AP. *Gigascience.* 2016 Mar 22;5:15.[7] Freshwater Metaviromics and Bacteriophages: A Current Assessment of the State of the Art in Relation to Bioinformatic Challenges. Bruder K, Malki K, Cooper A, Sible E, Shapiro JW, Watkins SC, Putonti C. *Evol Bioinform Online.* 2016 Jun 20;12(Suppl 1):25-33.

## Keywords

Bacterial symbionts, insect metagenomics, genome skimming, phylogenetic placement, trophic interactions



## Pacific oyster holobiont in an unknown world

### Authors

**Ana Lokmer**<sup>1</sup>, Karl Mathias Wegner

<sup>1</sup> Musée de l'Homme, France

Disease is a strong selective force, causing a rapid adaptation of holobionts to the pathogens in their environment. By virtue of their lifestyle, marine filter-feeders, such as a bivalve *Crassostrea gigas* (Pacific oyster), are constantly exposed to myriads of potentially harmful bacteria. The adaptation of both animal and its microbiota to the local biotic environment is thus an essential aspect of the oyster fitness. In addition, *C.gigas* is an important aquaculture species, whose rearing includes routine translocations between distant sites. In this way, these sessile animals get into a very unusual situation: they find themselves in a new environment, where they are exposed to yet un-encountered microbes, to which neither they nor their resident microbiota are adapted. To examine how a new biotic environment affects the Pacific oyster holobiont and its establishment at a new site, we transplanted the oysters from a population in the Southern Wadden Sea (Netherlands) to a site in the Northern Wadden Sea (Germany), sampled their microbiota in different tissues (hemolymph, mantle, gut, gills) and followed their survival over five days. We additionally treated half of the oysters with antibiotics to remove the resident microbiota, and thus disentangle the microbiota- from oyster-related effects. We found that the presence of resident, locally un-adapted microbiota increased the risk of fatal disease in translocated animals, with the highest increase in pathogenic load in the gills. To further explore how oyster-related factors contributed to the observed outcome, we are now analyzing gills gene expression during the experiment.

### Keywords

Pacific oyster, translocation, resident, pathogen, marine, biotic environment, biotic context

# Effect of early life dietary supplementation, with linseed oil, on the ruminal microbiome of lambs

## Authors

**Tamsin Lyons**<sup>1</sup>, Tommy Boland<sup>2</sup> and Evelyn Doyle<sup>1</sup>

<sup>1</sup> Environmental Microbiology Group, School of Biology and Environmental Science & Earth Institute, University College Dublin, Belfield, Ireland

<sup>2</sup> School of Agriculture and Food Science, University College Dublin, Belfield, Ireland

Ruminant livestock, such as cattle and sheep, rely wholly on microorganisms within their rumen to degrade fibrous feed and allow them to obtain nutrients from food. Ruminal microbiome structure has been shown to influence animal productivity, feed conversion efficiency and health. Diet has a significant impact on microbial community structure in the rumen and could potentially be used to manipulate the structure and create a microbiome desirable for animal productivity and health. Furthermore, Rowntree *et al*, (2010) found that inclusion of linseed oil in the diet of ruminants led to reduced enteric methanogenesis which could incur both economic and environmental benefits. The aim of this study was to determine the effect of linseed oil, administered to lambs in early life, on the host and its ruminal microbiome, and to establish if altered microbiomes persisted once administration of the supplement had ceased. Forty eight new-born lambs were blocked according to sex and allocated to one of four dietary treatments in a randomised block design; three groups were compared in this study. The control group received standard lamb meal throughout the study, the second group received lamb meal supplemented with 40 g kg<sup>-1</sup> DM of linseed oil throughout the study and the final group received the supplement pre-weaning but only standard lamb meal post weaning. At 16 weeks volatile fatty acid concentrations in the rumen were measured, ruminal microbial contents were collected and analysed, and results were compared between treatment groups. There were no significant differences in average daily weight gain between treatment groups but average daily feed intake was significantly reduced in the group receiving linseed oil, when compared to the control group. Inclusion of linseed oil in the diet throughout the study had a statistically significant effect on bacterial assemblages ( $p=0.029$ ) and archaeal assemblages ( $p=0.005$ ) in the rumen. Interestingly, when the group receiving the supplement pre-weaning only was compared to the control group, significant differences were detected in their ruminal microbial communities. This indicates that the microbiome remained altered even after supplementation had ceased.

## References

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. and Schloss, P.D. (2013), Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform, *Journal of Applied Environmental Microbiology*, 79(17), 5112-5120. Lueders, T., Manefield, M. and Friedrich, M.W. (2004) Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environ Microbiol* 6: 73-78. Rowntree, J.D., Pierce, K.M., Buckley, F., Petrie, K.J., Callan, J.J., Kenny, D.A. and Boland, T.M. (2010) Effect of either soya or linseed oil supplementation of grazing dairy cows on milk production and methane emissions, *Advances in Animal Biosciences*, 1(1), 51-51

## Keywords

Rumen, microbiome, diet, animal productivity, methane mitigation



# ***Lactobacillus plantarum* confers robustness to host fitness traits in nutritional duress**

## **Authors**

**Dali Ma**<sup>1</sup>, Maroun Bou Sleiman<sup>2</sup>, Claire-Emmanuelle Indelicato<sup>1</sup>, Bart Deplancke<sup>2</sup>, François Leulier<sup>1</sup>

<sup>1</sup> Institut de Génétique Fonctionnelle de Lyon (IGFL), Université de Lyon, Ecole Normale Supérieure de Lyon, CNRS UMR 5242, Université Claude Bernard Lyon 1, F-69364 Lyon Cedex 07, France

<sup>2</sup> Institute of Bioengineering, EPFL, Lausanne, Switzerland

Symbiosis is a pervasive theme of evolution. It has been extensively demonstrated that vertically transmitted endosymbionts can modify the host's germ-line, manipulate its reproductive behavior and thus shape its evolutionary trajectory. Horizontally acquired symbionts that constitute the host's gut microbiota, on the other hand, are known to confer fitness advantages to many somatic aspects of host's physiology during its lifetime. However, how the presence of such horizontally acquired symbiont impacts the host's phenotype under selection pressure requires more detailed study. Previously, we have demonstrated that nutritional stress triggers a profound developmental delay in germ-free *Drosophila* larvae, and mono-association with a particular member of the fly gut microbiota, *Lactobacillus plantarum* (Lp), buffers such delay. Using the same mono-association model, we demonstrate that besides promoting growth, Lp also conveys robustness to various fitness traits in different genetic background. Specifically, by studying the collection of the *Drosophila* Genetic Reference Panel (DGRP), we found that the germ-free flies raised on a low-protein diet manifest greater phenotypic variability in growth rate, maturation timing and adult allometry, and mono-association with Lp consistently buffers such variability and limits the range of phenotypic plasticity. Interestingly, a proportion of these germ-free flies also present wing pattern aberrations that are effectively masked by the presence of Lp, suggesting a hitherto unappreciated role of the gut microbiota's contribution to canalizing the invariant pattern of a developing organ. We further found that compromising reactive oxygen species (ROS) production through antioxidant treatment blocks the buffering effect of Lp on the host. In sum, we propose that in the presence of nutritional stress, members of the microbiota effectively stabilize the host's fitness traits and provides phenotypic robustness during development. Removing the gut microbiota effectively unmasks genetic differences within the host population, which can become the substrate for evolutionary forces to select upon.

## **Keywords**

Variation, mono-association, robustness, genetic diversity

# Genotype-dependent gut microbiota drives zooplankton resistance to toxic cyanobacteria

## Authors

**Emilie Macke**<sup>1</sup>, Martijn Callens<sup>1</sup>, Luc De Meester<sup>2</sup>, Koenraad Muylaert<sup>1</sup> and Ellen Decaestecker<sup>1</sup>

<sup>1</sup> Laboratory of Aquatic Biology, Department of biology, University of Leuven – Campus Kulak, E. Sabbelaan 53, B-8500, Kortrijk, Belgium

<sup>2</sup> Laboratory of Aquatic Ecology, Evolution and Conservation, University of Leuven, Charles Deberiotstraat 32, 3000 Leuven, Belgium

The gut microbiota is an important source of metabolic innovations for animal hosts, and is increasingly considered as a key factor that may drive adaptation, especially in the current context of fast and drastic environmental changes imposed by human activities and global warming. Here, we carried out a gut microbiota transplant experiment in the freshwater crustacean *Daphnia* to show that the gut microbiota mediates adaptation to cyanobacteria harmful algal blooms (cyanoHABs) in this pivotal zooplankton grazer. CyanoHABs have strongly increased in frequency and intensity as a result of eutrophication and climate warming, and pose severe threats on human and livestock health, as well as on aquatic communities. Our results revealed that genetic variation in resistance to toxic cyanobacteria in *Daphnia* is mainly mediated by genotype-dependent gut microbiota. Survival and reproduction upon cyanobacterial exposure in recipient *Daphnia* were not affected by the recipient genotype, but were strongly impacted by the donor genotype. Microbiota from resistant genotypes conferred a higher resistance to recipient *Daphnia* than microbiota from susceptible genotypes. This suggests that the *Daphnia* genotype acts indirectly on resistance to cyanobacteria, by shaping the gut microbiota. In addition, resistance was higher when donors were previously fed cyanobacteria, suggesting that gut microbiota responded to become more efficient in dealing with cyanobacteria after prior exposure. Next generation sequencing of 16S rDNA revealed that resistance was associated with changes in gut microbiota structure: cyanobacterial exposure favored bacterial taxa involved in the detoxification of cyanobacterial toxins. In concert, these results emphasize the crucial role of gut microbiota in *Daphnia* adaptation and acclimatization to cyanoHABs, and demonstrate that the host genotype interacts with the environment to shape the gut microbial community.

## Keywords

Gut microbiota, adaptation, *Daphnia*, toxic cyanobacteria

# Functional study of host - microborer interactions in the scleractinian coral holobiont

## Authors

**Anaïs Massé**<sup>1</sup>, Marie-Lise Bourguet-Kondracki<sup>1</sup>, Tribollet Aline<sup>2</sup>, Alain Paris<sup>1</sup>, Arlette Longeon<sup>1</sup>, Claude Yéprémian<sup>1</sup> Isabelle Domart-Coulon<sup>1</sup>

<sup>1</sup> Muséum National d'Histoire Naturelle, MCAM UMR7245CNRS-MNHN, Sorbonne-Universités, 63 Rue Buffon CP54, 75005 Paris, France

<sup>2</sup> Centre IRD France Nord, Institut de Recherche pour le Développement. UMR 7159, IPSL-LOCEAN (UPMC/CNRS /IRD/MNHN) 4 Place Jussieu, Tour 46-00, 5th floor, 75005 Paris Cedex, France

The coral holobiont contains diverse communities of microbial partners that are located both in the living tissues (endosymbiotic dinoflagellates, bacteria) and in the skeleton (microboring filamentous cyanobacteria, algae, and fungi). Little is known about the functional roles in living scleractinian corals of microborers actively colonizing skeletons via carbonate dissolution processes, with a few recent studies suggesting a potential ectosymbiotic role. Phototrophic microborers may indeed represent an alternative source of photoassimilates to the coral host during bleaching events (disruption of symbiosis with dinoflagellates, triggered mainly by thermal stress), improving the chances of coral survival during these increasingly frequent episodes. But, the type of transfer of photoassimilates (active vs passive) has not been determined. Moreover, the fluxes of carbon and nitrogen between microborers and the coral host have not been visualized and quantified at the individual cell level. In the present study, we aim at highlighting: (i) potential trophic roles of microboring phototrophs, especially of the Siphonales Ulvophyceae of the genus *Ostreobium* which is very abundant in living corals. In order to investigate the transfer of photoassimilates from phototrophic microborers to the coral tissue, we use pulse-chase isotopic labelling experiments in light (with <sup>13</sup>C-bicarbonate and <sup>15</sup>N-nitrate) of microborers colonizing healthy or bleached branches of *Pocillopora damicornis*. Tissue and skeletal fractions are prepared to quantify bulk isotopic enrichment using GC-IRMS and developing LC-IRMS methods. In addition, thin sections of coral branches are prepared to map isotopic enrichment with nanoscale secondary ion mass spectrometry (NanoSIMS) and quantitatively image photoassimilation and potential translocation of labeled compounds between microborers and host. (ii) molecules of the chemical communication between microborers and coral host. In order to identify small, specialized metabolites involved in this dialogue, we establish and compare the chemical profiles of microborers in situ in tissue-covered skeleton of coral branches, in cultures of microborers (*Ostreobium* strains propagated in vitro) and in primary co-cultures of the strains with coral tissues. Chemical fingerprints of organic extracts are obtained by HPLC-DAD-ELSD, MS and NMR analyses, followed by statistical analysis of overexpressed metabolites in each compartment of the holobiont. Such approaches should allow better understanding of microborer roles in living Scleractinian corals and thus their resilience capacity in the context of global change.

## Keywords

*Ostreobium*, *Pocillopora damicornis*, chemical fingerprints, isotopic labelling, in situ/in vitro approach

# A model of the evolution of endosymbiosis grounded in cross-level exchanges of energy and information

## Authors

**Hugo Mathé-Hubert**<sup>1,2</sup>, Heidi Kaech<sup>1</sup>; Christoph Vorburger<sup>1,2</sup>

<sup>1</sup> Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

<sup>2</sup> ETH Zurich, Switzerland

Life is hierarchically organized in nested levels (e.g. organelles in cells in tissues in organisms in families/groups in populations in species). As soon as one of these levels displays some heritable variation affecting its ability to reproduce itself, natural selection acts on it and shapes its “population” (e.g. organelles, cells, organisms, families/groups and species). As the whole functioning of life, the ability to reproduce itself, is determined by energy (nutrients) and information (DNA, signalling molecules, cues about the abiotic environment, allospecific and conspecifics, etc.). From a multilevel perspective, the information and the energy are also the entities exchanged between levels of organisation, making them mutually dependent (Tëmkin & Serrelli 2016). Here I present a model of multilevel selection grounded in this information and energetic framework. This model has been developed to study the evolution of endosymbiosis, but it can be easily extended to some other phenomena shaped by multilevel selection.

## References

Tëmkin I, Serrelli E (2016) "Hierarchical Dynamics Process Integration across Levels: Information and Energy in Biological Hierarchical Systems," In: *Evolutionary Theory: A Hierarchical Perspective* (eds Eldredge N, Pievani T, Serrelli E, Tëmkin I), pp. 88–153. Chicago and London.

## Keywords

Multilevel selection, endosymbiosis, individual-based model, energetic approach



# Phylosymbiosis in host-associated gut microbial communities in lower termites

## Authors

Samuel Pichon<sup>1</sup>, Veronica Chevasco<sup>1</sup>, **Caroline Michaud**<sup>1</sup>, Simon Dupont<sup>1</sup>, Anne-Geneviève Bagnères<sup>1</sup>, Franck Dedeine<sup>1</sup>

<sup>1</sup> Institut de Recherche sur la Biologie de l'Insecte - UMR CNRS 7261, Tours, France

The parallelism between host-associated microbial communities and the phylogeny of host species (as described by the term ‘phylosymbiosis’) is expected to prevail in mutually beneficial symbioses. We analyzed, based on a metabarcoding approach of the V3-V4 region of the bacterial 16S rDNA, the microbiota from 17 different colonies of 8 closely-related termite species of the genus *Reticulitermes*. These wood-feeding social insects exhibit, in their hindgut, complex nutritional mutualistic symbioses with prokaryotes and flagellated eukaryotes ensuring the degradation of lignocellulose and nutrient exchange between partners. Our results showed that *Reticulitermes* termites exhibit associations with 60-170 bacterial taxa belonging to 5 main phyla (Bacterioidetes, Spirochaete, Proteobacteria, Elusimicrobia and Firmicutes). Variation in the abundance of certain microbial taxa supports the idea that some symbionts may play specific roles among *Reticulitermes* species. However, intra-colonial microbiota variation is consistently lower than interspecific microbiota variation. This reveals significant degrees of phylosymbiosis, suggesting that gut microbial communities of these termites might be shaped by host specific factors, by host biogeography, or by both factors. In addition, we provided a comparison of microbial communities between the foraging *Reticulitermes* species and the wood-dwelling termites of the genera *Kaloterms* and *Hodotermopsis*. These results revealed that termite’s feeding habits might also significantly contribute to the gut prokaryotic microbiota of termites.

## Keywords

Co-divergence, gut microbiota, xylophagy, lower termites

# The stinkbug–Burkholderia symbiotic system: an ideal model of insect-microbe symbiosis

## Authors

**Tsubasa Ohbayashi**<sup>1,2,3</sup>, Peter Mergaert<sup>2</sup>, Yoshitomo Kikuchi<sup>1,3</sup>

<sup>1</sup> Hokkaido University, Japan

<sup>2</sup> CNRS, France

<sup>3</sup> AIST, Japan

Many insects interact intimately with bacteria inside the body. These bacteria play important roles in host survival, such as degradation of food materials, supplement of essential nutrients, and enhancement of host immunity. Recent studies have revealed such bacterial diversity and functions. However, molecular mechanisms of the specific bacterial infection are poorly understood since generally symbiotic bacteria are vertically transmitted from mother to child, commonly unculturable outside the host, and genetically unmanipulable. *Riptortus pedestris* is a notorious pest of leguminous crops, broadly distributing over south-eastern Asia. This stinkbug is symbiotically associated with *Burkholderia* sp. in the post region of the midgut, wherein symbionts promote host growth and fecundity. Unlike other symbiotic bacteria in insects, this stinkbug acquires the *Burkholderia* symbionts from ambient environment as a nymph, and these symbiotic bacteria are easy to culture and genetically manipulable outside the host, providing us a new opportunity to unveil the genetic background of insect-microbe symbiosis. I have performed detailed histological observations in the midgut and genetic approaches such as RNA-seq and colonization-deficient mutant screening. In this symposium, I would like to show recent findings underlying specific colonization mechanisms of the *Burkholderia* symbiont in the stinkbug midgut.

## References

Tsubasa Ohbayashi, Kazutaka Takeshita., Wataru Kitagawa Naruo Nikoh, Ryuichi Koga, Xian-Ying Meng, Kanako Tago, Tomoyuki Hori, Masahito Hayatsu, Kozo Asano, Yoichi Kamagata, Bok Luel Lee, Takema Fukatsu, and Yoshitomo Kikuchi (2015). "Insect's intestinal organ for symbiont sorting." PNAS 112(37): E5179–E5188.

## Keywords

Stinkbug, *Burkholderia*, partner choice, gut symbiosis

## The tree microbiome as part of the extended phenotype?

### Authors

**Anna Oliver**<sup>1</sup>, L. Newbold<sup>1</sup>, J. Taylor<sup>2</sup>, K. Schönrogge<sup>1</sup>, M. Gibbs<sup>1</sup>, A. Perry<sup>1</sup>, H. Gweon<sup>1</sup>, S. Cavers<sup>1</sup>, G. Iason<sup>3</sup>

<sup>1</sup> Centre for Ecology & Hydrology, Wallingford, UK,

<sup>2</sup> Royal Botanic Garden Edinburgh,

<sup>3</sup> Scottish Wildlife Trust

The impact of micro-organisms within host plant tissues ranges from mutualistic to pathogenic. Recent literature also discusses the role of endophytes as modifiers of plant disease. While there is evidence in some studies to suggest that endophytes might behave as enablers to pathogens, much literature emphasises the potential that endophytic microbial communities might have in suppressing and/or controlling pathogens within the community, while being adaptable over short periods of time. To date studies demonstrating control of pathogens within the microbial community are scarce and variation in microbial community structures at geographic scales and in relation to tree local adaptation is poorly understood. Trees, however, are the environment in which the endophyte community assembles and it stands to argue that heritable traits in the tree phenotype are reflected in the microbial communities they maintain. Here we present the results of a study of Scots Pine asking how effective trees are as ecological filters in determining the structure of their fungal endophyte community. We discuss what potential role the management of endophyte communities could have to mitigate threats from disease particularly in a fast changing environment.

### Keywords

Endophytes, pathogens, disease, Scots Pine

# Modeling the host-microbiota coevolution using maximum likelihood inference

## Author

**Benoît Perez-Lamarque**, H       Morlon

Institute of Biology of the Ecole Normale Sup  rieure (IBENS), Paris, France

Microbiotas play a central role in the functioning and evolution of multicellular life. Yet, we know little about how microbiotas evolve during the evolutionary history of host clades. It is commonly thought that many of these microbes are acquired from the environment during the life of the host (horizontal transmission), but that others are intimately associated with their hosts, transmitted across generations, and coevolve with them (vertical transmission). However, the lack of appropriate comparative phylogenetic method prevents us from rigorously assessing which microbes are horizontally versus vertically transmitted; and if they are vertically transmitted, to which extent. Here, we develop a simple model for the evolution of microbial communities along a host phylogeny, with the aim of identifying and quantifying vertical transmission from data that include the phylogeny of present-day hosts and the gene sequences of microbes associated with these hosts. Our model first focuses on the evolution of a particular bacterial taxon, represented by its gene sequence. The sequence evolves along the host phylogeny with a given rate of molecular evolution, is transmitted at host speciation events (vertical transmission), and experience punctual host-switching events (horizontal transmission). We put together a simulation algorithm for this model and derive its associated likelihood. Using simulations, we test the ability of the model to recover simulated parameter values and thus to identify the importance of vertical versus horizontal transmission. Finally, we apply our approach to NGS 16S microbiome data profiling from a host community.

## References

Huelsenbeck, J. *et al* (2000), "A Bayesian framework fo the analysis of cospeciation", *Evolution*. McFall-Ngai, M. *et al.* (2013), "Animals in a bacterial world, a new imperative for the life sciences", *Proceedings of the National Academy of Sciences*. Shapira, M. (2016), "Gut Microbiotas and Host Evolution: Scaling Up Symbiosis", *Trends in Ecology & Evolution*.

## Keywords

Modeling, cospeciation, coevolution

# Development of a multigenic metabarcoding of prokaryotic microbiota: application to Bacteria and Archaea of agronomical interest encountered in the rice rhizosphere of Camargue

## Authors

Isabelle Pieretti<sup>1</sup>, Jean-Claude Ogier<sup>2</sup>, Laurence Blondin<sup>1</sup>, Philippe Roumagnac<sup>1</sup>, Christian Vernière<sup>1</sup>.

<sup>1</sup> UMR BGPI, Cirad-BIOS, TA-A54/K, Campus International de Baillarguet, 34398 Montpellier Cedex 5

<sup>2</sup> UMR DGIMI Université de Montpellier, UMR 1333 DGIMI, CC54, Place Eugène Bataillon, 34095 Montpellier Cedex 05

It is our hypothesis that soil and plant microbiota are playing an eminent role in shaping both natural ecosystems and agricultural production systems. It is now widely admitted that bacterial communities show a great diversity in the rhizosphere and in the endosphere as endophytes. These microbial communities affect the fitness of both the hosts and the microbes and further play important roles in C and N cycles. Much less is known about the role played (if any) by archaea within both the plant rhizosphere and endosphere. Interestingly, a recent study has revealed a relatively high archaeal concentration in internal plant tissues (Müller *et al.*, 2015). The objective of our study is to develop a multigenic metabarcoding approach to accurately describe the biodiversity of relevant microbial communities interacting with rice paddies in the Rhône delta region. This pilot agro-ecosystem was selected because it is annually subjected to several abiotic stresses (water stress associated to rice paddies flooding, soil salinity, etc.) that are likely to enhance archaeal growth. We will retain universal prokaryotic primers and archaeal specific primers within the 16S rRNA gene, two housekeeping genes, *gyrB* (encoding the  $\beta$  subunit of the DNA gyrase) and *rpoB* (encoding the  $\beta$  subunit of the RNA polymerase) for bacteria, and the thermosome TF55 gene (encoding a type II chaperonin) for archaea. Primers evaluation will be conducted on mock cultures and by sampling rice plants during both rice paddies flooding and drying periods. This study is likely to be a first step towards better understanding the dynamics of Camargue rice paddies microbial communities in relation with abiotic constraints.

## References

Müller H, Berg C, Landa BB, Auerbach A, Moissl-Eichinger C, Berg G. 2015. Plant genotype-specific archaeal and bacterial endophytes but similar *Bacillus* antagonists colonize Mediterranean olive trees. *Front. Microbiol.* 6:138.

## Keywords

Prokaryotic microbiota, multigenic metabarcoding, rice paddies

# Does apoptosis and cell growth play a role in the host symbionts regulation in the hydrothermal vent mussel *Bathymodiolus*?

## Authors

**Bérénice Piquet**<sup>1,2,3</sup>, Bruce Shillito<sup>1</sup>, Sébastien Duperron<sup>1,4</sup>, Ann C. Andersen<sup>2,3</sup>

<sup>1</sup> Sorbonne Universités, UPMC Univ Paris 06, MNHN, CNRS, IRD, UCBN, UAG, Unité de Biologie des organismes et écosystèmes aquatiques (BOREA, UMR 7208), Equipe Adaptations aux Milieux Extrêmes, 7 Quai Saint-Bernard, Bâtiment A, 75005 Paris, France

<sup>2</sup> Sorbonne Universités, UPMC Univ Paris 06, CNRS, Unité Adaptation et Diversité en Milieu Marin (AD2M, UMR 7144) Equipe Adaptation et Biologie des Invertébrés en Conditions Extrêmes, Station Biologique de Roscoff, 29680 Roscoff, France

<sup>3</sup> Centre National de la Recherche Scientifique (CNRS), UMR 7144, Adaptation et Diversité en Milieu Marin, Station Biologique, F-29680 Roscoff, France

<sup>4</sup> Institut Universitaire de France, Paris, France

The deep sea mussels (Bivalvia: Mytilidae) house methane- and/or sulfur-oxidizing bacteria in its gills. This symbiosis makes it possible to colonize restrictive environments like hydrothermal vents and cold seeps. Bacteria use methane and/or sulfide to synthesize carbohydrates and they are the most important source of host nutrition. The density of the two types of bacteria can vary so this symbiosis is flexible (Szafranski *et al*, 2015; Duperron *et al*, 2016). The aim is to understand the mechanisms that regulate the density of bacteria in gills. What are the mechanisms involved: apoptosis, endo-exocytosis, digestion of bacteria, ...? Transcriptomic results (Guézi *et al.*, 2013) have shown that when the load of symbiotic bacteria is high in the gills of *Bathymodiolus thermophilus*, the genes involved in the inhibition of apoptosis are repressed, suggesting that when the symbiotic load is high, apoptosis is activated, and vice-versa. Using TUNEL and immunohistological techniques we have been able to follow the apoptosis and the cell growth in various zones of the gills of *B. azoricus* that have undergone different *in vivo* treatments in pressurized aquariums (sulphide, methane, heat stress, ...) and also in *B. boomerang*, which have been taken away from the hydrothermal vents in order to lose their symbionts. We compared the results of the symbiotic tissues with a non-symbiotic tissue and compared the *Bathymodiolus* mussels with *Mytilus edulis* (naturally devoid of symbionts). We can thus describe apoptosis and cell growth in the gill of *Bathymodiolus*, in relation with the presence of symbiotic bacteria, and evaluate its possible contribution to the flexibility of the symbiosis.

## References

Guézi, H. (2013) Adaptation au mode de vie symbiotique chez les moules du genre *Bathymodiolus* des écosystèmes chimiosynthétiques profonds (approche transcriptomique). Thèse de l'UPMC en Biologie, 191pp. Duperron S, Quiles A, Szafranski KM, Léger N and Shillito B (2016) Estimating Symbiont Abundances and Gill Surface Areas in Specimens of the Hydrothermal Vent Mussel *Bathymodiolus puteoserpentis* Maintained in Pressure Vessels. *Front. Mar. Sci.* 3:16. Szafranski, K.M., Piquet, B., Shillito, B., Lallier, F.H., and Duperron, S. (2015). Relative abundances of methane- and sulfur-oxidizing symbionts in gills of the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus* under pressure. *Deep Sea Res. Part Oceanogr. Res. Pap.* 101, 7–13.

## Keywords

Symbiosis, apoptosis, *Bathymodiolus*, hydrothermal vent, mussel cell growth



## How holobionts get sick: the pathobiome paradigm

### Authors

**Silvio Pitlik**<sup>1</sup>, Omry Koren<sup>2</sup>

<sup>1</sup> Weizmann Institute of Sciences, 234 Hertz St, 7610001 Rehovot, Israël

<sup>2</sup> Faculty of Medicine, Bar Ilan University, 8 Henrietta Szold St, 1311502 Safed, Israël

All living creatures are holobionts. Each holobiont comprises the host and a myriad of interacting microbes, altogether defined also as a super-organism. In health, there is a fine tuned equilibrium within the members of the microbiome and between them and the host. This relatively stable equilibrium is maintained by a high level of diversity among microbes, a delicate bio-geographic microbial distribution and a sophisticated and intricate molecular crosstalk among the multiple components of the holobiont. Pathobionts are temporarily benign microbes with the potential, under modified ecosystem conditions, to induce pathogenesis to the host. Pathobionts may be either endogenous, living for prolonged periods of time inside or on the host, or exogenous, invading the host during opportunistic situations. In both cases, the end result is the transformation of the beneficiary microbiome into a health perturbing pathobiome. We hypothesize that all diseases of holobionts, acute or chronic, infectious or non-infectious, regional or systemic, are characterized by a perturbation of the healthy microbiome into a disease pathobiome. Examples (from AIDS to Zika) will be presented to consolidate this new paradigm.

### Keywords

Holobiont, pathobiome, pathobionts, health, disease

# Genetic diversity of the symbiont as a driver of the holobiont differentiation in the temperate sea anemone *Anemonia viridis*?

## Authors

**Barbara Porro**<sup>1</sup>, C. Mallien<sup>1</sup>, T. Zamoum<sup>1</sup>, R. Christen<sup>1</sup>, P. Furla<sup>1</sup>, D. Forcioli<sup>1</sup>

<sup>1</sup> Université Nice Sophia Antipolis, UMR 7138 "Evolution Paris Seine", Équipe "Symbiose marine", 06100 Nice, France

As many Cnidarians, the sea anemone *Anemonia viridis* lives in an intimate symbiotic trophic relationship with microorganisms, in particular dinoflagellates of the genus *Symbiodinium*. The holobiont *A. viridis* – *Symbiodinium* can live in a wide geographical and habitat range, and previous studies showed that *A. viridis* harbours a genetically diverse population of *Symbiodinium* sp. which could therefore play a major adaptive role. For these reasons, the system *A. viridis* – *Symbiodinium* is a first-class model to study holobiont adaptation. Our work aims to clarify the importance of the symbiont in the response to environmental variation and will highlight the possible genotype-environment associations (GEA) at the scale of the whole holobiont. To identify the factors that drive the holobiont differentiation, we characterized the distribution of in hospite *Symbiodinium* genetic diversity by sampling 3 colour morphs of *A. viridis* (with putatively different adaptive strategies) in ecologically differentiated populations from the English Channel and the Mediterranean Sea. We assessed this genetic diversity by combining microsatellite markers for the symbiont, genotyped by NGS and RADseq markers for the animal host. This should allow us to unravel the co-evolutionary dynamics of the two partners along a geographical and/or environmental continuum.

## Keywords

Cnidarian, *Symbiodinium*, RADsequencing, microsatellite loci, mutualistic symbiosis, GEA

# Social behavior and gut-microbiota in red-bellied lemurs (*Eulemur rubriventer*): In search of the role of immunity in the evolution of sociality

## Authors

**Aura Raulo**<sup>1</sup>, Lasse Ruokolainen<sup>2</sup>, Ilkka Hanski<sup>2</sup>, Avery Lane<sup>3,4</sup>, Katherine Amato<sup>5</sup>, Rob Knight<sup>6</sup>, Steven Leigh<sup>7,8</sup>, Rebecca Stumpf<sup>8,9</sup>, Bryan White<sup>8,10</sup>, Karen Nelson<sup>11</sup>, Andrea Baden<sup>12,13,14</sup>, Stacey Tecot<sup>15, 16</sup>

<sup>1</sup> University of Oxford, UK

<sup>2</sup> Metapopulation Research Centre, Department of Biosciences, University of Helsinki, Finland.

<sup>3</sup> School of Anthropology, University of Arizona, Tucson, USA

<sup>4</sup> Department of Anthropology, Washington State University, Pullman, WA USA.

<sup>5</sup> Department of Anthropology, Northwestern University, Evanston, USA

<sup>6</sup> Departments of Pediatrics and Computer Science & Engineering, and Center for Microbiome Innovation, University of California San Diego, La Jolla, USA

<sup>7</sup> Department of Anthropology, University of Colorado, Boulder, USA,

<sup>8</sup> Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana, IL, USA

<sup>9</sup> Department of Anthropology

<sup>10</sup> Department of Animal Sciences

<sup>11</sup> J. Craig Venter Institute, 9714 Medical Center Drive, Rockville, MD 20850, USA

<sup>12</sup> Department of Anthropology, Hunter College, City University of New York

<sup>13</sup> Departments of Anthropology & Biology, the Graduate Center of City University of New York

<sup>14</sup> The New York Consortium in Evolutionary Primatology (NYCEP), New York, NY USA

<sup>15</sup> Social and Behavioral Sciences Research Institute, School of Anthropology

<sup>16</sup> Laboratory for the Evolutionary Endocrinology of Primates, University of Arizona, Tucson, USA.

Vertebrate gut microbiota form a key component of immunity and a dynamic link between an individual and the ecosystem. Microbiota might play a role in social systems as well, because microbes are transmitted during social contact and can affect host behavior. Combining methods from behavioral and molecular research, we describe the relationship between social dynamics and gut microbiota of a group-living cooperative species of primate, the red-bellied lemur (*Eulemur rubriventer*). Specifically, we ask whether patterns of social contact (group membership, group size, position in social network, individual sociality) are associated with patterns of gut microbial composition (diversity and similarity) between individuals and across time. Red-bellied lemurs were found to have gut microbiota that were relatively temporally stable and strongly social group-specific. Contrary to expectations, individual sociality was negatively associated with gut microbial diversity. However, position within the social network predicted gut microbial composition. These results emphasize the role of the social environment in determining the microbiota of adult animals. Since social transmission of gut microbiota has the potential to enhance immunity, microbiota might have played an escalating role in the evolution of sociality.

## Keywords

Microbiota, sociality, social network, cooperation, microbial transmission, stress, glucocorticoids

# Vertical and horizontal factors shape the microbiome of newborns: a midwife's perspective

## Authors

**Maïté Ribère**, François-Joseph Lapointe

Département de sciences biologiques, Université de Montréal, Canada

The question of the origin of the human microbiome is now at the heart of many scientific disputes. On the one hand, it calls into question the dogma that fetal development takes place in a sterile environment (the amniotic sac) (Aagaard *et al.* 2014). On the other hand, it challenges the long-term effects of microbial communities inherited at birth could have on our health (Decker *et al.* 2010, Marild *et al.* 2012, Kero *et al.* 2002). In recent years it was the delivery mode (natural vs. cesarean), which was first presented as the major determinant of the newborn microbiome (Dominguez-Bello *et al.* 2010). Then, the deciding effect of breastfeeding (maternal milk vs. formula) was established, in combination with the effect of delivery mode, reinforcing the idea that one should not consider them separately (Yassour *et al.* 2016, Bokulich *et al.* 2016). In this paper, we will address the different factors known to influence the microbiome of newborns and their modes of transmission, so as to study them no longer as isolated elements, but as a myriad of events that condition the shaping of the newborn microbiome.

## References

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. *Science translational medicine*, 6(237), 237ra65-237ra65. Decker, E., Engelmann, G., Findeisen, A., Gerner, P., Laaß, M., Ney, D., ... & Hornef, M. W. (2010). Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. *Pediatrics*, 125(6), e1433-e1440. Marild, K., Stephansson, O., Montgomery, S., Murray, J. A., & Ludvigsson, J. F. (2012). Pregnancy outcome and risk of celiac disease in offspring: a nationwide case-control study. *Gastroenterology*, 142(1), 39-45. Kero, J., Gissler, M., Grönlund, M. M., Kero, P., Koskinen, P., Hemminki, E., & Isolauri, E. (2002). Mode of delivery and asthma—is there a connection?. *Pediatric research*, 52(1), 6-11. Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences*, 107(26), 11971-11975. Yassour, M., Vatanen, T., Siljander, H., Hämäläinen, A. M., Härkönen, T., Ryhänen, S. J., ... & Lander, E. S. (2016). Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Science translational medicine*, 8(343), 343ra81-343ra81. Bokulich, N. A., Chung, J., Battaglia, T., Henderson, N., Jay, M., Li, H., ... & Schweizer, W. (2016). Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Science translational medicine*, 8(343), 343ra82-343ra82.

## Keywords

Vaginal microbiome, newborn, mode of delivery, breastfeeding, midwife

# Spatial metagenomics illuminate the impact of agriculture on the distribution and prevalence of plant viruses at the ecosystem scale

## Authors

**Philippe Roumagnac**<sup>1</sup>, Pauline Bernardo<sup>1</sup>, Tristan Charles-Dominique<sup>2,3</sup>, Emmanuel Fernandez<sup>1</sup>, Denis Filloux<sup>1</sup>, Philippe Ortet<sup>4,5,6</sup>, Mohamed Barakat<sup>4,5,6</sup>, Tony A. Rebelo<sup>3</sup>, Stephen Cousins<sup>3</sup>, François Mesleard<sup>7,8</sup>, Damien Cohez<sup>7</sup>, Nicole Yaverkovski<sup>7</sup>, Arvind Varsani<sup>9,10,11</sup>, Gordon W. Harkins<sup>12</sup>, Michel Peterschmitt<sup>1</sup>, Carolyn Malmstrom<sup>13</sup>, Darren P. Martin<sup>14</sup>

<sup>1</sup> CIRAD, INRA, SupAgro/UMR BGPI, TA A54/K 34398 Montpellier Cedex 5, France

<sup>2</sup> University of Cape Town, Department of Biological Sciences, Rondebosch 7701, Cape Town, South Africa

<sup>3</sup> South African National Biodiversity Institute, Kirstenbosch, Claremont 7735, Cape Town, South Africa

<sup>4</sup> CEA, DSV, IBEB, 13108 Saint-Paul-lez-Durance, France

<sup>5</sup> CNRS, UMR 6191, 13108 Saint-Paul-lez-Durance, France

<sup>6</sup> Aix-Marseille Université, 13108 Saint-Paul-lez-Durance, France

<sup>7</sup> Tour du Valat, Le Sambuc, 13200 Arles, France

<sup>8</sup> Université d'Avignon et des Pays de Vaucluse, UMR CNRS/IRD IMBE 7263/237, BP 61207 Avignon, France

<sup>9</sup> School of Biological Sciences, University of Canterbury, Christchurch, 8140, New Zealand

<sup>10</sup> Structural Biology Research Unit, University of Cape Town, Observatory, South Africa

<sup>11</sup> Department of Plant Pathology and Emerging Pathogens Institute, University of Florida, Gainesville, USA

<sup>12</sup> South African National Bioinformatics Institute, University of the Western Cape, Cape Town, South Africa

<sup>13</sup> Department of Plant Biology, Michigan State University, East Lansing, Michigan, USA

<sup>14</sup> Computational Biology Group. UCT Faculty Of Health Sciences. Observatory 7925. South Africa

Our knowledge about plant virus diversity in nature is still limited. Indeed, studies of plant-virus interactions have primarily focused on cultivated areas. This lack of knowledge about patterns of virus diversity and distribution in nature is hampering our understanding of plant virus ecology and evolution in the long term. In addition, this scarcity of knowledge does not allow to fully understand, model and predict the micro- and/or macro-evolutionary processes that are taking place across the agro-ecosystem. Consequently, it is still difficult to quantify the impact of human activities (agricultural intensification, plants transport, climate change, etc.) on host-pathogen interactions. We developed a new metagenomics approach, the so-called geo-metagenomics approach, in order to provide information about the virus biodiversity, the prevalence of unknown and asymptomatic viruses and the spatial distributions of those plant viruses in two pilot ecosystems: the Western Cape Region of South Africa and the Camargue region in France. This approach provides geographically tagged cDNA from known and unknown viruses, and further allows linking viral sequences obtained by the metagenomics approach to a specific host, and hence to geographic coordinates. The objectives of this study were to assess (i) if wild areas can be considered as reservoir of plant virus biodiversity (ii) if there exists patterns of spatio-temporal distribution of plant viruses at the agro-ecosystem scale and (ii) if ecological parameters can account for these distributions. This new approach allowed us to estimate plant virus diversity associated with both pilot agro-ecosystems. This study led to the discovery of 94 potential new plant viral species and highlighted patterns of spatial distribution of plant viral families. Plant virus prevalence associated with cultivated areas was found to be significantly greater than those associated with non-cultivated areas. Furthermore, exotic plants from South African fynbos showed significantly higher prevalence than native plants. These results emphasize the direct or indirect impact of human activity on plant virus dynamics at the agro-ecosystem scale.

## Keywords

Metagenomics, agro-ecosystem, plant viruses, diversity



# Protists are an integral part of the *Arabidopsis thaliana* microbiome

## Authors

**Melanie Sapp**<sup>3</sup>, Sebastian Ploch<sup>1</sup>, Anna Maria Fiore-Donno<sup>2</sup>, Michael Bonkowski<sup>2</sup>, Laura E. Rose<sup>3</sup>

<sup>1</sup> Biodiversity and Climate Research Centre, Frankfurt, Germany

<sup>2</sup> Department of Terrestrial Ecology, University of Cologne, Germany

<sup>3</sup> Cluster of Excellence on Plant Sciences (CEPLAS), Heinrich Heine University, Population Genetics, 40225 Düsseldorf, Germany

Although protists occupy a vast range of habitats and are known to be interacting with plants *inter alia* via disease suppression, competition with pathogens or growth stimulation their contributions to the "phytobiome" are not well described. In contrast, associations with bacterial and fungal communities have been comprehensively studied showing effects on the hosts' fitness. To bridge the gap towards a more comprehensive picture of the phytobiome, we examined cercozoan and oomycete taxa living in close association with the model plant *Arabidopsis thaliana* grown in two different soil types (loam and sand) under laboratory conditions. Soil, roots and leaves were analyzed and complemented with surface sterilized plant organs to identify endophytic micro-eukaryotes. Cercozoan taxa were identified using 18S rRNA gene metabarcoding, whereas the Internal Transcribed Spacer (ITS1) was used to determine oomycete taxa. Paired-end sequencing included cercozoan and oomycete mock communities for all barcodes. Subsequent analyses revealed a strong effect by soil type and differentiation of protist communities between plant organs, although oomycetes appeared more specialised than cercozoa. The rhizosphere harboured many oomycete plant pathogens that were mostly members of the Pythiaceae. Taxa belonging to the genus *Globisporangium* were identified as core members of the *A. thaliana* microbiome. The main *A. thaliana* associated cercozoan taxa belonged to the Glissomonadida and Cercomonadida, but also members of other orders, for example the Plasmodiophorida were found. Cercozoan communities were strongly structured by plant organ. Whilst the rhizosphere communities were strongly influenced by soil type, a phyllosphere specific community developed. Our results highlight the importance of micro-eukaryotes for the plant holobiont and deliver new knowledge on the diversity of plant-associated micro-eukaryotes.

## Keywords

Phytobiome, protists, oomycetes, cercozoa, metabarcoding



# Hunters or gardeners? Digestive mutualism in rootless aquatic carnivorous plants

## Authors

**Dagmara Sirová<sup>1</sup>**, Jiří Bárta<sup>2</sup>, Karel Šimek<sup>1</sup>, Jaroslav Vrba<sup>1,2</sup>, and Lubomír Adamec<sup>3</sup>

<sup>1</sup> Biology Centre AS CR, Hydrobiological Institute, Na Sádkách 7, 37005, České Budějovice, Czech Republic

<sup>2</sup> Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic

<sup>3</sup> Institute of Botany AS CR, Section of Plant Ecology, Dukelská 135, 37982 Treboň, Czech Republic.

The considerable similarity between microbial communities colonizing plant surfaces such as roots, and those of the animal gut is often overlooked, although this parallel provides us with many opportunities to look at the microbial as well as plant and animal ecophysiology from a different perspective (Ramírez-Puebla *et al.*, 2013). The traps of carnivorous plants are sophisticated digestive systems and represent interface environments between the supply and the demand for nutrients. In this study, we selected the miniature aquatic ecosystem in the traps of aquatic carnivorous *Utricularia* plants as our model system (Šimek *et al.*, 2016). By assessing the trap-associated microbial community structure, diversity, metabolic capabilities, as well as the nutrient regeneration potential by grazing protozoa, we gained an insight into the nutrient acquisition strategies of the *Utricularia* host plants. We conclude that the trap ecophysiological function is in many aspects highly analogous to that of the mammalian rumen and centers on the digestive mutualism involving complex consortium of diverse microorganisms which act in synergy to covert complex organic matter, often of algal origin, into a source of nutrients for plant growth.

## References

Ramírez-Puebla, S.T., Servín-Garcidueñas, L.E., Jiménez-Marín, B., Bolaños, L.M., Rosenblueth, M., Martínez, J., et al. 2013. Gut and root microbiota commonalities. *Applied and Environmental Microbiology*, 79, 2–9. Šimek, K., Pitsch, G., Salcher, M.M., Sirová D., Shabarová, T., Adamec, L., et al. 2016. Ecological traits of the algae-bearing *Tetrahymena utriculariae* (Ciliophora) from traps of the aquatic carnivorous plant *Utricularia reflexa*. *Journal of Eukaryotic Microbiology*. DOI: 10.1111/jeu.12368.

## Keywords

Digestive mutualism, carnivorous plants, plant-microbe interactions, carbon, phosphorus, bacterivory

# A hologenomic assessment of the gut microbiome of poison arrow frogs and their potential role in toxin sequestration by the host

## Authors

**Karen Siu-Ting**<sup>1</sup>, Ben Thomas<sup>2</sup>, Jamie Newbold<sup>2</sup>, Mary O'Connell<sup>3</sup>, Chris Creevey<sup>2</sup>

<sup>1</sup> Dublin City University, School of Biotechnology, Glasnevin, Dublin 9, Ireland

<sup>2</sup> Aberystwyth University, Institute of Biological, Environmental and Rural Sciences. Penglais Campus, Aberystwyth, SY23 3FD, UK

<sup>3</sup> School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK

Poison arrow frogs (of the family Dendrobatidae) secrete alkaloid toxins in their skin as defense mechanisms against predators. Numerous studies have shown that in Dendrobatids alkaloid toxins are acquired by “sequestration from diet”, i.e. uptake and storage of toxins or their chemical precursors, mostly from consumed mites and formicid ants (Saporito et al. 2004; Saporito *et al.* 2007; Dumbacher et al. 2004). It has also been shown that these frogs have evolutionary adaptations that likely reduce their sensitivity to the toxins sequestered (Tarvin *et al.* 2016). There exists the intriguing possibility that the gut microbiome of these frogs may also play a role in this process. As a holobiont consists of the organism together with its associated microbial communities (Bordenstein and Theis 2015, Theis et al. 2016) this term can be applicable to many organisms that show an effective symbiotic relationship between host and microbiome that allows phenotypic adaptation of the host (Bordenstein and Theis 2015). The aim of the present study is to assess the gut microbiome of poison arrow frogs to determine if associations exist that could be related to the digestion of a toxic diet. We analysed the gut microbiome of 3 toxin sequestering species and of 3 non-toxin sequestering species of frogs caught in the a rainforest in Peru (with 4 replicates per species). Species were selected based on sharing similar microhabitats (and therefore access to similar diets) and having relatively comparable sizes. We sequenced the bacterial 16S rRNA region for all gut microbiome samples enabling us to obtain a preliminary assessment of the microbiome composition of the 6 species studied. This information was compared with previously collected transcriptomic data from the hosts to test if the poison arrow frogs with their microbial communities could be considered a holobiont adapted to allow sequestration of toxins from their diet.

## References

Bordenstein, S.R. and Theis, K.R. (2015) Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biol*, 13(8). Dumbacher, J. P. *et al.* (2004) Melyrid beetles (Choresine): A putative source for the batrachotoxin alkaloids found in poison-dart frogs and toxic passerine birds. *PNAS*, 101, 15857–15860. Saporito, R. A. *et al.* (2004) Formicine ants?: An arthropod source for the pumiliotoxin alkaloids of dendrobatid poison frogs. *PNAS*, 101(21), 8045-8050. Saporito, R. A. *et al.* (2007) Oribatid mites as a major dietary source for alkaloids in poison frogs. *PNAS* 104(21), 8885-8890. Tarvin, R. *et al.* (2016) Convergent substitutions in a sodium channel suggest multiple origins of toxin resistance in poison frogs. *Mol Biol Evol*, 1068-108. Theis, K.R. *et al.* (2016) Getting the hologenome concept right: An eco-evolutionary framework for hosts and their microbiomes. *mSystems*, 1(2).

## Keywords

Poison arrow frogs, gut microbiome, toxin sequestration

# The effect of urbanisation on the gut microbiota on the house sparrows

## Authors

**Aimeric Teyssier**<sup>1,2</sup>, Noraine Salleh Hudin<sup>1</sup>, Lieze Rouffaer<sup>1</sup>, Diederik Strubbe<sup>1</sup>, Luc Lens<sup>1</sup>, Joël White<sup>2</sup>

<sup>1</sup> Terrestrial Ecology Unit, Department of Biology, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium

<sup>2</sup> EDB (Laboratoire Evolution et Diversité Biologique), ENFA-CNRS-UPS, UMR 5174, 118 route de Narbonne, 1062, Toulouse Cedex 9, France

The gut microbiota performs a number of essential functions for its host, such as a central role in digestion, immunity and protection against pathogens of the host. The composition and the diversity of the gut microbiota are expected to be shaped not only by the characteristics of the host but also the environment in which the host lives. However, little is known on the effects of anthropogenic habitat alterations on the gut microbiota of natural populations. Here we examined the impact of urbanisation at different spatial scales on the gut microbiota of a passerine bird, the house sparrows (*Passer domesticus*). We performed a large scale study by sampling the gut microbiota of 36 populations of sparrows from sites in Belgium with varying degrees of urbanisation, ranging from rural habitats to city centres. Overall, we find that urbanisation is associated to lower gut diversity and to changes in taxonomic composition. Interestingly, we also find that local urbanisation does not impact the microbiota in the same way according to the landscape urbanisation levels. Given the importance of the gut microbiota for host fitness, these urban-related modifications could have negative consequences on the hosts and potentially contribute to the recent decline of the house sparrows populations in cities.

## Keywords

Gut microbiota, urbanisation, house sparrows

# An integrative approach to decipher the summer mortality syndrome affecting Pacific oysters

## Authors

Julien De Lorgeril<sup>1</sup>, Aude Lucasson<sup>1</sup>, Bruno Petton<sup>4</sup>, **Eve Toulza<sup>1</sup>**, Philippe Haffner<sup>1</sup>, Delphine Destoumieux<sup>1</sup>, Christian Chaparro<sup>1</sup>, Richard Galinier<sup>1</sup>, Camille Clerissi<sup>1</sup>, Caroline Montagnani<sup>1</sup>, Jean-Michel Escoubas<sup>1</sup>, Agnès Vergnes<sup>1</sup>, Marc Leroy<sup>1</sup>, Lionel Degrémont<sup>2</sup>, Adeline Pérignon<sup>3</sup>, Marianne Alunno-Bruscia<sup>4</sup>, Pierre Boudry<sup>4</sup>, Frédérique Le Roux<sup>5</sup>, Yannick Gueguen<sup>1</sup>, Guillaume Mitta<sup>1</sup>

<sup>1</sup> UMR Interactions Hôtes Pathogènes Environnements

<sup>2</sup> Station Ifremer La Tremblade

<sup>3</sup> CRCM Comité Régional de Conchyliculture de Méditerranée

<sup>4</sup> UMR Laboratoire des Sciences de l'Environnement Marin

<sup>5</sup> UMR Biologie Intégrative des Modèles Marins

Mass and recurrent mortality events of complex etiology have increasingly affected invertebrates of ecological and/or economical interest such as honeybees, corals and bivalves over the past years. The main oyster species farmed in France and worldwide, *Crassostrea gigas*, is no exception to this rule. It suffers from devastating summer mortality outbreaks, whose severity has dramatically increased since 2008. It particularly affects juvenile stages (< 1 year) throughout French coasts decimating up to 90 % of spats in some farms, resulting in significant economic losses. This phenomenon is due to a combination of direct and indirect factors. Among indirect factors, the intensification of the production, worldwide transport of animals, anthropogenic impacts or climate change in coastal areas have been suspected to weaken the animals and increase the frequency of outbreaks thus contributing to the emergence of the phenomenon. Several factors have been involved in the expression of the juvenile mortalities syndrome and underscored the complexity of the pathosystem: (i) the disease depends on the temperature of the seawater potentially modifying both host physiology and composition/function of its associated microbiota; (ii) the susceptibility of oysters to potential pathogens (herpes viruses, vibrios) depends on the genetics of oysters and their age; (iii) a species/strain of a single microorganism is not sufficient to cause this disease suggesting a polymicrobial nature of the syndrome; (iv) different dynamics of oyster mortalities are observed according to the production site. Altogether these data suggest that abiotic factors, human activities, oyster genetics, their age and life history, their immune status and of course, the pathogens are involved and might influence each other. However, the relative weight of these factors, their interaction patterns, their dynamics during pathogenesis remained largely unknown, which makes the disease difficult to understand, predict and control. To open a way out of this crisis and find solutions for a sustainable oyster aquaculture, it is necessary to decipher this complex pathosystem. To achieve this goal, we developed an integrated multidisciplinary research program in an attempt to link together different levels of understanding of the disease. It focused on the dynamic study of the "oyster holobiont" (oyster and associated microbiota, including pathogens) during the pathogenesis. We selected two highly contrasted full-sib families, one susceptible and the second resistant to implement an experimental design reproducing the natural route of the infection and we followed the temporal dynamics of the microbiota, the pathogens and the response of the oyster by kinetics using a combination of approaches. This allowed us to decipher the sequence of events leading to the breakdown of holobiont homeostasis, the pathogen development and oyster mortalities.

## Keywords

*Crassostrea gigas*, mass mortalities, integrative approaches, holobiont health



# Molecular interactions between endophytic bacteria and fungi from brown algae in the context of quorum sensing

## Authors

**Anne Tourneroche**<sup>1</sup>, Raphael Lami<sup>2</sup>, Soizic Prado<sup>1</sup>

<sup>1</sup> Molécules de Communication et Adaptation des Micro-organismes, MCAM UMR 7245 CNRS/MNHN 57 rue Cuvier (CP54) 75005 Paris

<sup>2</sup> Laboratoire de Biodiversité et Biotechnologies Microbiennes, LBBM USR3579 UPMC-CNRS Observatoire de Banyuls, Avenue du Fontaulé, 66650 Banyuls Sur Mer

Marine brown algae are key primary producers and form a specific habitat strongly impacting coastal marine life [1]. In addition, the aquaculture of brown algae represents a fast-growing economic sector [2]. Worryingly, this algoculture expansion is associated with an increase of infectious diseases which may have strong economic and ecological impacts [3]. Besides of the phycopathogens, macroalgae also harbor microorganisms which may be involved in development, defense, and nutrient supply of the host-algae [1]. In this context, we aim at exploring the microbial interactions within the endomicrobiota of *Saccharina latissima* and its impact on the algal fitness. Our recent results highlighted that metabolites produced by endophytic fungi could inhibit the bacterial quorum sensing (QS). QS allows bacteria to coordinate gene expression according to cell density via the production, and detection, of chemical mediators. This intercellular signaling system especially controls virulence gene expression in many bacterial species. Thus, we hypothesized that the QS inhibiting compounds produced by fungi may interfere with this bacterial coordinated gene regulation and prevent the emergence of some bacterial phenotypes deleterious for the host. In this context, bacterial and fungal strains were isolated from the internal tissues of the brown algae *Saccharina latissima* and identified. Some bacterial strains have shown a QS capability while fungal strains have displayed a capacity to modulate this bacterial QS. A multidisciplinary approach is in progress to decipher these interspecific interactions within the endomicrobiota of *S. latissima* and assess possible impacts on host.

## References

[1] S. Egan *et al.* (2013) FEMS Microbiol. Rev. 37:462–476. [2] G. Kerlero de Rosbo *et al.* (2014) Technical Report ADEME., pp.164. [3] C. Gachon *et al.* (2010) Trends in Plant Sci., 15: 633–640.

## Keywords

Fungal-bacterial communication, quorum-sensing inhibition, endophytes, phycopathogens, brown algae, host-microbiota interactions

# Water-bear with me for a moment: the tardigrade microbiota

## Authors

**Matteo Vecchi**<sup>1</sup>, Irene Newton<sup>2</sup>, Lorena Rebecchi<sup>1</sup>, Agnese Ferrari<sup>1</sup>, Roberto Guidetti<sup>1</sup>

<sup>1</sup> Department of Life Sciences, University of Modena and Reggio Emilia. Via Campi 213/D, 41125 Modena, Italy

<sup>2</sup> Department of Biology, Indiana University. Jordan Hall 221, 1001 E. 3rd St., 47405 Bloomington, IN, USA

Symbiotic associations of metazoans with bacteria are ubiquitous and virtually no animal is axenic (McFall-Ngai *et al.*, 2013). The microbial community associated with animal hosts (the microbiota) has been characterized for many phyla (e.g. Porifera, Cnidaria, Nematoda, Arthropoda, Chordata, Annelida, Mollusca), but we are far from a complete understanding of the currently recognized diversity of animal hosts. Tardigrada (also known as “water bears”) is a phylum of micrometazoans famous for their ability to undergo cryptobiosis (i.e., ametabolic states of life in response to adverse environmental conditions, Guidetti *et al.* 2011), and studied for their phylogenetic position with respect to the Arthropoda and *Onychophora* phyla in the evolution of the Panarthropoda, a clade within the Ecdysozoa (moulting animals, Aguinaldo *et al.*, 1997). However, we know very little about the association between tardigrades and bacteria (Vecchi *et al.*, 2016). Here, we designed an experiment to determine if tardigrades are colonized by a specific microbial community and to identify their potential symbionts. Using 16S rRNA gene amplicons (V3-V4), we characterized the microbiome of six tardigrades species from Europe (Italy, Sweden) and Antarctica, spanning the two classes Heterotardigrada and Eutardigrada. In tandem, we also characterized the bacteria associated with the substrates (mosses, lichens, freshwater sediments) on which these tardigrades were found, allowing us to rule out environmental contamination as a contributor to the tardigrade microbiome. The tardigrade microbiotas consistently differ from that of the substrates and are species-specific. Interestingly, we identified differences in the bacterial community composition when the same species of tardigrade was collected from different samples, suggesting that as in other animal phyla, diet and environment may play a role in shaping the microbiota of water bears. Finally, we identified two putative Rickettsiales symbionts in two tardigrade species. Our results suggest that like all other animals, tardigrades have their own microbiota that differs among species. Future work will be directed to better characterize the bacterial OTUs closely associated with tardigrades, to understand how the microbiome is acquired and maintained, and to identify which organs house these bacteria.

## References

Aguinaldo AMA, Turbeville JM, Linford LS, Rivera MC, Garey JR, Raff RA & Lake JA. (1997). Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature*, 387(6632), 489-493. Guidetti R, Altiero T & Rebecchi L. (2011). On dormancy strategies in tardigrades. *Journal of Insect Physiology*, 57(5), 567-576. McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, Douglas AE *et al.* (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences*, 110(9), 3229-3236. Vecchi M, Vicente F, Guidetti R, Bertolani R, Rebecchi L & Cesari M. (2016). Interspecific relationships of tardigrades with bacteria, fungi and protozoans, with a focus on the phylogenetic position of *Pyxidium tardigradum* (Ciliophora). *Zoological Journal of the Linnean Society*, 178(4), 846-855.

## Keywords

Tardigrada, microbiome, Rickettsiales, 16S, metabarcoding



# Structuring genetic and taxonomic diversity in gut microbes

## Authors

**Chloé Vigliotti**<sup>1,2</sup>, Eric Baptiste<sup>1</sup>, Philippe Lopez<sup>1</sup>, Anthony Herrel<sup>2</sup>, Michel Habib<sup>3</sup>

<sup>1</sup> UMR 7138 Evolution Paris Seine, Université Pierre et Marie Curie, 75005 Paris cedex, France

<sup>2</sup> UMR 7179, CNRS/MNHN, Département d'Ecologie et de Gestion de la Biodiversité, Paris Cedex, France

<sup>3</sup> LIAFA, UMR 7089 CNRS & Université Paris Diderot - Paris 7, France

Structuring genetic and taxonomic diversity in gut microbes of lizards affected by a quick dietary change 35 years ago, ecologists introduced 10 insectivorous lizards from the islands of Pod Kopiste to that of Pod Mrcaru[1]. *Podarcis sicula* on Pod Mrcaru became omnivorous (80% herbivorous)[2] and changed in morphology[3]. However, changes of their gut microbiome/microbiota were not investigated. We sequenced 32 samples from guts of insectivorous and omnivorous lizards by Illumina Miseq to test whether (i) changes in microbial communities[4] and (ii) functional acquisition/loss of microbiome gene families were associated with the dietary shift[5]. We used multivariate analyses and innovative network models (reads similarity, unifracs networks) to analyze variations in microbiota/microbiomes. During this dietary shift, the abundance of few microbial taxa (e.g. genera *Clostridium* sensu stricto and *Treponema* are more abundant in omnivorous than in insectivorous lizards.) and few key metabolic genes changed (the biotin enzyme of the biotin metabolism pathway, involved in legume degradation). Thus specific changes in the microbiome correlate with major changes in the hosts phenotypes. The use of this additional vertebrate non mammalian model, with a rapid generation time, provides a novel perspective to assess the generality of findings regarding dietary shift effects in human microbiome studies.

## References

- [1] Nevo, &al (1972), Notes on experimental introductions. *Oecologia* 10
- [2] A.Herrel, & al. (2008), *PNAS*
- [3] A.Herrel, (2007), Cambridge University Press
- [4] Lawrence A. David & al. (2014), *Nature*
- [5] I.L. Brito & al.(2016), *Nature*.

## Keywords

Microbiome, microbiota, dietary shift, genetic diversity

# The microbiome of prominent Brassicaceae: health issues for plants and humans

## Authors

**Birgit Wassermann**<sup>1</sup>, Daria Rybakova<sup>1</sup>, Gabriele Berg<sup>1</sup>

<sup>1</sup> Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, 8010 Graz, Austria

Human health is not only a consequence of our own genetics; much more it relies on the complex relationship with our inherent microbiome, our diet and the microorganisms associated with it. Therefore, it is all the more important to extend distinctive knowledge of the microbiota inhabiting raw eaten vegetables and fruits. The microbiome of cruciferous vegetables, each purchased as supermarket and farmer's market equivalent, was investigated in the present study. The following subjects were pursued in depth: i) the overall microbiota inhabiting edible vegetable parts and the differences due to the point of sale, ii) Brassica-associated bacteria with active myrosinase, suggested to provide cancer preventing properties for humans and iii) putative biological control agents for an ecologically friendly and sustainable treatment of Brassica crops. The microbiome of all Brassica samples consisted of 10,458 OTUs, represented by 257 bacterial orders. Almost 80% were shared by all vegetables investigated. Those taxa were defined as the core microbiome, composed of 76% Proteobacteria, 19% Bacteroidetes and 2% of each Actinobacteria, Verrucomicrobia and Firmicutes. A significant crop-specific influence of the purchase origin was observed, while the overall composition of the microbiome was found to be rather driven by the vegetable genotype. According to co-occurrence network analysis, less abundant taxa play a key role for the microbial community. Supermarket vegetables contained more bacterial genera with described human pathogenic or opportunistic strains while the amount of plant beneficial taxa was higher in farmer's market equivalents. Four strains of *Pseudomonas* and *Serratia plymuthica*, isolated from farmer's market vegetables, were identified to suppress plant pathogenic fungus *Verticillium longisporum* and to reveal plant growth promoting properties. Myrosinase-active *E. cloacae* KS50 was isolated from turnip cabbage; enzymatic activity was determined by different experimental approaches. Overall, the data show a high microbial diversity in prominent Brassica vegetables with a strong potential to influence plant health as well as human health.

## Keywords

Brassicaceae, vegetable microbiome, bacterial myrosinase, glucosinolates, biological control agents

# Host-microbiome interactions in a changing world: studying the impacts of urbanisation and climate change on the gut microbiota of two wild species

## Authors

**Joël White**<sup>1</sup>, Aimeric Teyssier<sup>2</sup>, Elvire Bestion<sup>3</sup>, Julien Cote<sup>4</sup>

<sup>1</sup> ENSFEA - UMR Evolution et Diversité Biologique, Castanet-Tolosan, France

<sup>2</sup> University of Ghent, Belgium Luc Lens, University of Ghent, Belgium

<sup>3</sup> University of Exeter, United Kingdom

<sup>4</sup> University of Toulouse 3, France

Human activity is now recognised as being the dominant cause of most contemporary environmental changes, leading to alterations in ecosystem functioning, community dynamics and generally impacting the fitness of organisms. A vast majority of such organismal studies focus on the effects of anthropogenic perturbations on individuals *per se*. However, the recent surge in microbiome studies has brought about a paradigm shift by which individuals cannot truly be considered independently of the bacterial communities they host. The gut microbiome for instance, is involved in host digestion or nutrient synthesis, protection against pathogens and more widely shapes host immunity, life history traits and even behaviour, underlining its vital importance for host fitness. The determinants of microbiome composition and diversity are thought to be multiple, being related to host traits, but also to factors linked to the host's environment. Thus any major changes in the environment, and in particular anthropogenic perturbations, are highly likely to alter host-associated microbiomes leading to potential dysbioses and adverse effects on hosts. Here we present work investigating the respective impact of two major anthropogenic environmental perturbations, urbanisation and climate change, on the gut microbiota of two host species, House sparrows and Common lizards. Our wide-scale study on sparrow populations provides evidence that urbanisation reduces the overall bacterial diversity of the gut microbiota and induces changes in taxonomic and functional composition. Using a unique experimental setup, the Metatron, we show that a 3°C increase in ambient temperature induces a 34% decrease in gut microbiota diversity of common lizards and leads to differential taxonomic and functional compositions as compared to the control treatment. We further show that these changes of microbiota in warmer climates are associated to lower subsequent survival of the hosts. Both these studies provide evidence that anthropogenic alterations of the environment impact microbiome diversity and structure; which could potentially increase direct detrimental effects of these perturbations on hosts, producing synergic negative effects. Overall our results highlight the importance of accounting for host-microbiome interactions when examining the response of organisms to anthropogenic environmental stressors.

## Keywords

Gut microbiota, bird, lizard, anthropogenic perturbations, global change, illumina, 16S metabarcoding

# Can symbiotic bacteria influence mate choice in a vertebrate?

## Authors

**Danielle J. Whittaker**<sup>1,2</sup>, Samuel P. Slowinski<sup>3</sup>, Osama M. Alian<sup>4</sup>, Andrew D. Winters<sup>4</sup>, Ellen D. Ketterson<sup>3</sup>, Kevin R. Theis<sup>1,4</sup>

<sup>1</sup> BEACON Center for the Study of Evolution in Action, Michigan State University

<sup>2</sup> Department of Integrative Biology, Michigan State University

<sup>3</sup> Department of Biology, Indiana University

<sup>4</sup> Department of Immunology & Microbiology, Wayne State University

Symbiotic microbes influence host phenotypes in myriad ways including producing or altering chemical signals important in social and reproductive behavior 1,2. Microbes are known to influence chemical signals in vertebrates, particularly mammals 3,4, but no study has yet demonstrated their specific role in mate choice, though the phenomenon has been documented in insects 5. Studies of multiple bird species have recently demonstrated that volatile compounds present in preen oil, secreted by the uropygial gland and spread by the birds onto their feathers, communicate information about the sender's species, sex, and breeding condition 6-8. In the dark-eyed junco (*Junco hyemalis*), we have previously demonstrated that these chemical signals predict reproductive success 9. The junco uropygial gland harbors a very diverse and rich bacterial community, and several of these bacterial genera are known to produce volatile compounds, including those found in junco preen oil 10. We examined whether these symbionts influence the production of preen oil volatiles involved in mate choice by experimentally testing the relationship between resident bacteria associated with the preen gland and the production of preen oil volatile compounds in captive dark-eyed juncos. We injected a broad spectrum antibiotic into the preen glands of the treatment group, and saline in the control group, for 5 days, and sampled the microbiotas and volatile compounds before and after treatment. We found that, compared to the control group, birds in the treatment group had significantly lower concentrations of volatile compounds related to reproductive success. We will also discuss the experimentally induced changes in the microbiotas of the treatment group, particularly in relation to the microbiotas of mated pairs of free-living juncos with varying levels of mate fidelity.

## References

- 1 Archie, E. A. & Theis, K. R. Animal behaviour meets microbial ecology. *Anim. Behav.*82, 425-436 (2011).
- 2 Ezenwa, V. O. & Williams, A. E. Microbes and animal olfactory communication: Where do we go from here? *BioEssays*36, 847-854 (2014).
- 3 Theis, K. R. et al. Symbiotic bacteria appear to mediate hyena social odors. *P. Natl. Acad. Sci. USA*110, 19832-19837, doi:10.1073/pnas.1306477110 (2013).
- 4 Leclaire, S., Nielsen, J. F. & Drea, C. M. Bacterial communities in meerkat anal scent secretions vary with host sex, age, and group membership. *Behav. Ecol.*25, 996-1004 (2014).
- 5 Sharon, G. et al. Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *P. Natl. Acad. Sci. USA*107 (2010).
- 6 Whittaker, D. J. et al. Songbird chemosignals: volatile compounds in preen gland secretions vary among individuals, sexes, and populations. *Behav. Ecol.*21, 608-614 (2010).
- 7 Whittaker, D. J. et al. Role of testosterone in stimulating seasonal changes in a potential avian chemosignal. *J. Chem. Ecol.*37, 1349-1357 (2011).
- 8 Soini, H. A., Whittaker, D. J., Wiesler, D., Ketterson, E. D. & Novotny, M. Chemosignaling diversity in songbirds: chromatographic profiling of preen oil volatiles in different species. *J. Chromatogr. A*1317, 186-192 (2013).
- 9 Whittaker, D. J., Gerlach, N. M., Soini, H. A., Novotny, M. V. & Ketterson, E. D. Bird odour predicts reproductive success. *Anim. Behav.*86, 697-703 (2013).
- 10 Whittaker, D. J. & Theis, K. R. in *Chemical Signals in Vertebrates 13* (eds Bruce A. Schulte, Thomas E. Goodwin, & Michael H. Ferkin) 105-117 (Springer International Publishing Switzerland, 2016).

## Keywords

Behavior, chemical communication, birds, olfaction



