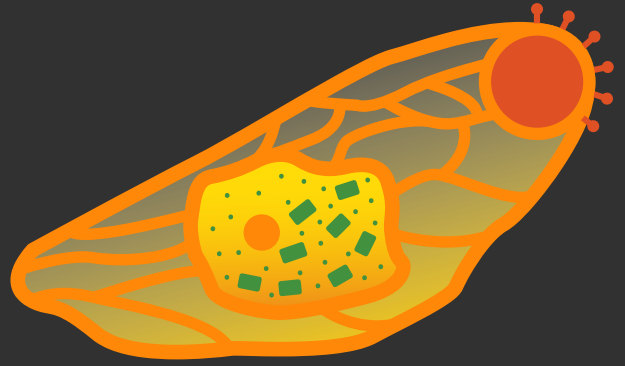


Insects:
Infection & Immunity
Online Conference
24-25 September 2020



ABSTRACT BOOK

Date	Time (UTC + 1)	Time slot	Speaker	Title
Thursday - 24th September	13:50 - 14:00	10 min	Richard Harrington	Welcome by Royal Entomological Society
	14:00 - 14:40	40 min	Luís Teixeira	Regulation of <i>Wolbachia</i> proliferation by the amplification and deletion of an addictive genomic island
	14:40 - 15:00	20 min	Daniel Leybourne	A facultative endosymbiont alters the probing behaviour of its aphid host
	15:00 - 15:20	20 min	Mariana G. Ferrarini	Setting up a Dual-RNAseq approach to unravel the modulation of symbiotic structures during the metamorphosis of the cereal weevil <i>Sitophilus oryzae</i>
	15:20 - 15:40	20 min	Cybèle Prigot	When age, gender and Wolbachia affect immune priming's protection: zoom on <i>Armadiididium vulgare</i> and the pathogenic bacteria <i>Salmonella enterica</i>
	15:40 - 16:00	20 min	Esteban J. Beckwith	A fat body-brain axis controls sickness behavior
Friday - 25th September	14:00 - 14:40	40 min	Julien Royet	Dissecting behavioral immunity in <i>Drosophila</i>
	14:40 - 15:00	20 min	Juliana N. Armache	Characterizing the global virome of <i>Apis mellifera</i> using a small RNA-based approach
	15:00 - 15:20	20 min	Crystal Vincent	Disparate regulation of the immune response drives sex differences in infection tolerance
	15:20 - 15:40	20 min	Angelina Ceballos-Escalera	The fungal communities associated with the first UK breeding population of the bark beetle <i>Ips typographus</i> include potentially invasive pathogens
	15:40 - 16:00	20 min	Arunkumar Ramesh	Constitutive activation of cellular immunity underlies the evolution of resistance to infection in <i>Drosophila</i>
	16:00 - 16:20	20 min	Alice Laciny	Of worms and shapeshifters - the phenotypic consequences of mermithid nematode infection in ants.

Regulation of *Wolbachia* proliferation by the amplification and deletion of an addictive genomic island

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Wolbachia is one of the most prevalent bacterial endosymbionts, infecting approximately 40% of terrestrial arthropod species. *Wolbachia* is often a reproductive parasite but can also provide fitness benefits to its host, as for example protection against viral pathogens. This protective effect is currently being applied to fight arboviruses transmission by releasing *Wolbachia*-transinfected mosquitoes. Titre regulation is a crucial aspect of *Wolbachia* biology. Higher titres can lead to stronger phenotypes and fidelity of transmission but can have a cost to the host. Since *Wolbachia* is maternally transmitted, its fitness depends on host fitness, and, therefore, its cost to the host needs to be controlled. Understanding this and other aspects of *Wolbachia* biology has been hampered by the lack of genetic tools. Here we developed a new forward genetic screen to identify *Wolbachia* over-proliferative mutant variants. We characterized in detail two of these new mutants, wMelPop2 and wMelOctoless, and show that the amplification or loss of the Octomom genomic region causes their over-proliferation. These results confirm previous data and expand on the complex role of this genomic region in the control of *Wolbachia* proliferation. Both new mutants shorten the host lifespan and increase antiviral protection. Moreover, we show that *Wolbachia* proliferation rate in *Drosophila melanogaster* depends on the interaction between Octomom copy number, the host developmental stage, and temperature. Our analysis also suggests that the life shortening phenotype and antiviral protection of *Wolbachia* are dependent on related, but different, properties of the endosymbiont; on the rate of proliferation and the titres at time of infection, respectively. Altogether, we demonstrate the feasibility of a novel and unbiased experimental approach to study *Wolbachia* biology, which can be further adapted to characterize other genetically intractable bacterial endosymbionts.

Dissecting behavioral immunity in *Drosophila*

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Since eukaryotes live in an environment heavily contaminated by microorganisms, it is not surprising that they have forged, over the times, extremely complex and intimate relationships. It is also expected that eukaryotes have developed mechanisms to perceive the presence of bacteria and to adapt their immune response, their physiological status or even their comportment accordingly. Many reports have shown that bacteria can interact with eukaryote nervous system, either for the benefit of the microbe that alters the host's behavior or to the benefit of the host that adapts its behavior to the infection. However, in most cases, the molecules and mechanisms underlying the dialog between bacteria and their host nervous system were not identified and their mode of action poorly understood. I will present our latest data dissecting the cellular and molecular mechanisms by which one single microbiota-derived compound, called peptidoglycan, influences the behavior of infected hosts by acting on some specific neurons.

A facultative endosymbiont alters the probing behaviour of its aphid host

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Many herbivorous arthropods frequently associate with facultative endosymbiotic bacteria, which influence arthropod physiology and fitness. In aphids, endosymbionts can increase resistance against natural enemies, enhance aphid virulence, and alter aphid fitness. However, little is known on how they influence the interactions at the aphid-plant interface. Here, we used a well characterised endosymbiont-aphid-plant system (comprising *Hamiltonella defensa*, *Rhopalosiphum padi*, and barley) and employed the electrical penetration graph technique to examine how endosymbiont infection affects the processes at the aphid-plant interface. Compared with uninfected aphids, endosymbiont-infected aphids exhibited a two-fold increase in the number of plant cell punctures, a 50% reduction in the duration of each cellular puncture and had a higher probability of achieving sustained phloem ingestion. Feeding behaviour was also altered by host plant identity: endosymbiont-infected aphids spent less time probing plant tissue, required twice as many probes to reach the phloem, and showed a 44% reduction in phloem ingestion when feeding on a wild barley relative with partial aphid resistance compared with aphids feeding on a susceptible cultivar. This study provides the first demonstration that mechanisms at the aphid-plant interface are affected endosymbiont infection and that this has consequences for aphid fitness on different quality host plants.

Setting up a Dual-RNAseq approach to unravel the modulation of symbiotic structures during the metamorphosis of the cereal weevil *Sitophilus oryzae*

Mariana Galvão Ferrarini, Nicolas Parisot, Justin Maire, Agnès Vallier, Benjamin Gillet, Sandrine Hughes, Séverine Balmand, Carole Vincent-Monégat, Anna Zaidman-Rémy et Abdelaziz Heddi.

One peculiar attribute of many insects thriving on nutritional ecological niches, including insect pests and disease vectors, is their ability to establish **long-term relationships with heritable intracellular bacteria** (endosymbionts), which supplement their diet and improve their adaptive and invasive powers. Little is known on the **modulation of symbiotic associations** throughout an insect's life cycle, especially in the case of holometabolous insects that undergo complete **metamorphosis**. To understand how symbiosis is maintained during metamorphosis, we have established a host-symbiont metatranscriptomic approach of **Dual-RNAseq**, which allows to decipher the molecular dialogue between the host and its associated endosymbionts by obtaining high-throughput data simultaneously on both symbiotic partners. We used as a model system the **cereal weevil *Sitophilus oryzae*** association with the intracellular bacteria *Sodalis pierantonius*. This endosymbiont is secluded in specialized host cells, called bacteriocytes, that group into bacteriome organs. Bacteriomes are differently organized between larvae and adults, and the endosymbionts are known to be highly critical for complete maturation of adult insects, hence providing a good model to address the question of symbiosis maintenance over metamorphosis. By combining dual-RNAseq and cell imaging, we show that the larval bacteriome dissociates at the beginning of metamorphosis and releases bacteriocytes that undergo endosymbiosis-dependent transcriptomic changes affecting cell motility, cell adhesion, and cytoskeleton organization. These bacteriocytes migrate along the midgut to clusters of stem cells, and, in parallel, the bacterial transcriptomic changes demonstrate the endosymbiont responds to the alterations occurring during metamorphosis by up-regulating virulence factor-encoding genes in accordance with a temporary symbiont infectious behavior observed by cellular imaging. Our data suggest that infection of stem cells leads to their differentiation into novel bacteriocytes that further cluster into adult bacteriomes. These findings show that **interkingdom dialogue plays a pivotal role in the morphological reorganization of the bacteriome from larval stage to adulthood**, highlighting an adaptive feature that promotes bacteriome multiplication that matches increased metabolic requirements in emerging adults.

**When age, gender and *Wolbachia* affect immune priming's protection:
zoom on *Armadillidium vulgare* and the pathogenic bacteria *Salmonella enterica***

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The protection conferred by a first infection upon a second pathogen exposure (*i.e* immune priming) is an emergent research topic in invertebrate immunity. Various species expressed immune priming, but little is known about the intrinsic factors that may influence this immune process. In our study, we explored whether age, gender and the symbiotic bacteria *Wolbachia* impact the immune priming protection in *A. vulgare* against *S. enterica*. We firstly primed young and old, symbiotic and asymbiotic males and females, either with a non-lethal dose of living *S. enterica*, LB broth or without injection (control). 7 days post-injection, we performed a LD₅₀ injection of *S. enterica* to all individuals and we monitored their survival rates. We showed that survival capacities are well influenced by the priming treatment, but the strength of protection depends also on age, gender and *Wolbachia*. Immune priming is observed in young and old asymbiotic individuals, with a decline of protection in old females, but not in male. Interestingly, symbiotic individuals harbouring *Wolbachia* expressed immune priming protection only when old, but not when young, that could be partly explained by the *Wolbachia* loads. We discuss our observed interactions both in mechanistical and evolutionnary manners.

A fat body-brain axis controls sickness behavior

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Infected animals undergo behavioural changes that are collectively called “sickness behaviours”. Some of these changes are infection-specific, but most infections cause a common suite of behavioural changes that include anorexia and disruption of sleep/wake activity cycles. These behavioural changes have significant effects on human and animal health and well-being.

Despite the importance of sickness behaviours, they are relatively poorly understood. Here, we employ *Drosophila* to study the genetic and molecular underpinnings of sickness behaviour. This model provides many benefits since several behaviours are well understood and the fly has a relatively simple and well-studied immune response, allowing us to connect infection-induced changes in behaviour to their underlying mechanisms.

We have used a newly developed behavioural profiling platform to analyse the interaction between locomotor activity and infection in *Drosophila*. We find that both pathogenic and non- pathogenic bacterial infections can cause sleep disruption. We then tested various bacterial infections and *Drosophila* immune mutants, to determine how pathogen recognition and immune pathway activation contribute to the sleep disruption we observe. We find that fat body-derived spätzle, and an active Toll signalling pathway in neurons, are necessary for the marked increase in activity triggered by bacterial recognition. We propose that pathogen sensing activates a fat body-brain axis that directly influences overt behaviour.

Characterizing the global virome of *Apis mellifera* using a small RNA-based approach

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Human activity is increasingly destabilizing ecosystems, causing incalculable and often irreparable damage to biodiversity. In the floristics field, the consequences of human action are already being observed globally, generating a significant loss in native plants and impacting commercial agriculture. One of the factors that contribute to this scenario is the decreasing number of pollinating agents, mainly insects, driven by the increased use of pesticides that are harmful to those animals. Among insects, bees are considered the main pollinators, having a global distribution and attending to a wide spectrum of plants. Besides being sensitive to pesticides, these animals are also susceptible to viral infections, and these two factors combined have led to colony collapses with consequent economic loss. Therefore, knowledge of bee virome can help the development of new strategies to control and prevent viral infections. This study aimed to analyze the collection of circulating viruses in different populations of *Apis mellifera* using a strategy based on small RNA sequencing data. Twenty-four libraries of *A. mellifera* small RNAs, originated from South Africa, USA, China, Netherlands and UK were chosen. First, the libraries were pre-processed to remove sequencing adapters and to filter sequences with bad quality (Phred <20). The second step was to remove sequences that mapped to host genome (*Apis mellifera*) or known bacterial genomes. The remaining reads were subjected to a *de novo* assembly strategy using Velvet and SPAdes. The resulting assembled contigs were characterized by sequence similarity analysis against NT and NR databases using BLAST. Viral sequences were found in 17 out of 24 libraries, and the results included some viruses known to cause high mortality rates in bee colonies, such as *Varroa destructor virus* (VDV) and *Deformed wing virus* (DWV). From all the assembled contigs, some of them showed a significant nucleotide similarity to known viruses, suggesting they are likely new strains of these viruses. In other cases, the similarity to viral sequences was limited to aminoacidic level, which suggests these might be new viral species. Spatial analysis showed that DWV is infecting bees from all over the continents, while other viruses are restricted to some regions. In addition, the contigs that could belong to new viral species were restricted to libraries from Europe. Using this approach we were able to find in our samples viruses that could have both economic and ecological importance, and possibly a new virus that infects *Apis mellifera*. Unraveling global virome of bees is an important tool to identify and monitor viruses that may cause harm to colonies. This is the first step to help prevent future outbreaks to avoid big economical and biodiversity losses.

Disparate regulation of the immune response drives sex differences in infection tolerance

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Male and female animals exhibit differences in infection outcomes. One possible source of sex-specific pathology is sex-specific costs of immune activity or pathology. Little is known about the independent effects of immune- induced versus microbe-induced pathology, and whether these may differ for the sexes. Here we compare the responses of wild-type and immunocompromised male and female flies to infection. Through measuring several metabolic and physiological outputs, we then tested whether the sexes are differentially impacted by these various sources of pathology. We show that in the fruit fly, the sexes exhibit strong differences in tolerance to bacterial infection. These data lead us to suggest a model in which male and female flies exhibit broadly similar ability to resist infection with Gram- negative bacteria, but females tend to exhibit greater tolerance of these infections because they are better at controlling the immune response and its metabolic consequences. We show that differential regulation of the Imd pathway contributes to the sex-specific pathology observed during infection. This work suggests that investigating sex differences in infection has the potential to reveal mechanisms of tolerance.

The fungal communities associated with the first UK breeding population of the bark beetle *Ips typographus* include potentially invasive pathogens

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A breeding population of *Ips typographus* has recently been encountered for the first time in the UK. This bark beetle kills millions of Norway spruce trees per year across central Europe and, benefited by climate change, it is causing even more severe outbreaks¹. However, the potential effects of an outbreak on Sitka spruce, the most commonly-planted conifer in the UK, remain largely unknown. Several species of fungi establish symbiotic relationships with bark beetles, including some fungal pathogens, but fungal assemblages are highly variable. To report which fungi may be vectored into the UK and which fungal species formed associations with these beetles, we analysed fungal assemblages from 48 *Ips typographus* specimens. To do this, we used metabarcoding of the ITS2 region of individual beetle specimens to recover a total of 416 fungal Operational Taxonomic Units (OTUs) from both developing and flying beetles. Results revealed 18 potentially invasive fungi, not present in previously surveyed British bark and ambrosia beetle populations. Among these potentially non-native OTUs, there are two Ophiostomatoid fungi: *O. bicolor*, which is frequently associated with *Ips typographus* in their native area, and *O. floccosum*. Results also suggest that at least 26 of these 416 OTUs may preferentially associate with target beetle developmental stage. Fungal communities in Norway spruce hosted more fungi categorised as symbionts, while saprotrophs were more abundant in Sitka spruce. Therefore, while some of these fungi were consistently recovered during the whole beetle life cycle, others were linked with beetle developmental stages. Taken together, these results suggest that these bark-beetles are vectoring certain fungi into the UK and probably reintroducing others that were significantly correlated with the beetle specimens but already present in the country. These results provide a comprehensive overview of the fungal communities associated with *Ips typographus* in the UK, which may be useful to inform future management decisions.

Constitutive activation of cellular immunity underlies the evolution of resistance to infection in *Drosophila*

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Organisms rely on inducible and constitutive immune defences to combat infection. Constitutive immunity enables a rapid response to infection but may carry a cost for uninfected individuals, leading to the prediction that it will be favoured when infection rates are high. When we exposed populations of *Drosophila melanogaster* to intense parasitism by the parasitoid wasp *Leptopilina boulardi*, they evolved resistance by developing a more reactive cellular immune response. Using single-cell RNA sequencing, we found that immune-inducible genes had become constitutively upregulated. This was the result of resistant larvae differentiating precursors of specialized immune cells called lamellocytes that were previously only produced after infection. Therefore, populations evolved resistance by genetically hard-wiring an induced immune response to become constitutive.

Of worms and shapeshifters – the phenotypic consequences of mermithid nematode infection in ants.

Alice Laciny

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As social insects, ants represent extremely interaction-rich biological systems shaped by tightly integrated social structures and constant mutual exchange with a multitude of internal and external environmental factors. Due to this high level of ecological interconnection, ant colonies can harbor a diverse array of parasites and pathogens, many of which are known to interfere with the delicate processes of ontogeny and caste differentiation to induce phenotypic changes in their hosts. Such parasitogenic morphologies in ants can serve as “natural experiments” that may shed light on mechanisms and pathways relevant to host development, plasticity or robustness under environmental perturbations, colony-level effects and caste evolution.

One such host-parasite system that has captivated researcher’s interest since the 18th century is that of ants and nematodes of the family Mermithidae. These large endoparasites infect ants at the larval stage and can thus disrupt development and cause multidimensional phenotypic changes ranging from shortened wings to intercaste morphology and host suicide. In this talk, I will present interesting case studies throughout history about this host-parasite association and highlight the most common phenotypic outcomes, their putative causes, and avenues for future research. I thereby aim to contribute towards highlighting the importance of parasites of social insects for both biological theory and empirical investigation and facilitate future interdisciplinary work at the interface of ecology, development and evolution.