Westfälische Wilhelms-Universität Münster

1st Nosema Workshop

of the DFG-SPP 1399

host-parasite coevolution

29th-30th January 2013,

Münster

Dear attendees,

Welcome to the 1st Nosema Workshop of the DFG-SPP 1399 "Host parasite coevolution". The goal of this workshop is to stimulate new collaborations between SPP groups working with the microsporidium Nosema and with a diverse range of hosts: *Tribolium castaneum*, *Apis sp*., *Harmonia axyridis* etc. Furthermore, the aim is to parallelize bioinformatics workflows (e.g. regarding gene expression analysis), enable cross species comparative analyses and start new projects (e.g. regarding the expression of microRNAs upon infection with Nosema). As a guest speaker, we have invited Martin Embley from Newcastle who is an expert on the evolution of microsporidia. Furthermore, Robert Hirt from the same institute will present results on the genome of the microsporidium Trachipleistophora hominis. Additionally, independent of our workshop, there will be a presentation by Marc Rehmsmeier from Bergen about “Tools for the prediction of microRNA targets” and by Thomas Junker, a famous publicist and biology historian, about “Evolution and the meaning of life”. We are looking forward to a very exciting workshop!

Sarah Behrens (Organizer of the Workshop)

Erich Bornberg-Bauer (Coordinator of the SPP-Bioinformatics Platform)

Joachim Kurtz (Spokesperson of the SSP)

Programme

Tuesday, 29th January 2013	
12.30 pm	Arrival and Lunch at Ristorante "La gondola d'oro"/Mensa Aasee
1.45 pm	Welcome Address and Introduction
2 pm	Martin Embley (Lecture Hall): "Unraveling the chimeric origins of eukaryotes: trees, genomes and organelles"
3 pm	Robert Hirt: "Comparative genomics and evolutionary processes shaping microsporidia protein coding capacity"
3.30 pm	Coffee Break
4 pm	IEB Seminar by Marc Rehmsmeier, Bergen: "Tools for the prediction of microRNA targets" Abstract (Lecture Hall)

5.15-6.00 pm	Andreas Vilcinskas: “Parasites as biological weapons: how microsporidia might be facilitating the invasive success of the ladybird *Harmonia axyridis*” and: Gender- and stressor-specific microRNA expression in *Tribolium castaneum*
6.00-6.30 pm	Qiang Huang: “Molecular analysis of the *Nosema ceranae* infection tolerance in the honey bee, *Apis mellifera*”
7 pm	Dinner at the traditional Westphalian restaurant “Großer Kiepenkerl”

Wednesday, 30th January 2013	
8.30-9.00 am	Charlotte Rafaluk: “Experimental evolution – when host and parasite go extinct”
9 am	“The Growth of Evolutionary Thought” Lecture by Thomas Junker: “Evolution and the meaning of life” (Lecture Hall) alternatively: Discussion (Seminar Room)
10 am	Coffee Break
10.30 am	Robert Paxton: “Nosema in honey bees: a brief overview”
10.40 am	Chris Mayack: “The physiological effects of a *Nosema ceranae* infection in its host the honeybee”
11.10 am	Myrsini E Natsopoulou: “Competition between the intracellular pathogens *Nosema apis* and *Nosema ceranae* in the midgut of the honey bee (*Apis mellifera*)”

11.40 am	Vincent Doublet: “Interaction between *Nosema ceranae* and viruses in honey bees (*Apis mellifera*)”
12.10 am	Dino P. McMahon: “Impact of single- and co-infection of injected Deformed Wing Virus (DWV) and *Nosema ceranae* on honey bees”
12.40 pm	Lunch at Ristorante "La gondola d'oro"/Mensa Aasee
2-3 pm	Discussion

Abstracts

Vincent Doublet:

Interaction between *Nosema ceranae* and viruses in honey bees (*Apis mellifera*).

Nosema ceranae is an emergent pathogen of the European honey bee (*Apis mellifera*). This microsporidian first appeared in Europe and America in the 1990's and is now spread almost all over the world. First mentioned as one of the main factors of recent worldwide honey bee colony losses, it is rather accepted now that infections by *N. ceranae* alone are unlikely to lead to honey bee decline. However, in combination with other stressors, *N. ceranae* infection can markedly reduce the lifespan of adult honey bees and thus might compromise the survival of a colony. It has been shown recently that experimental Nosema infection combined with contamination by pesticides increases the rate of mortality of adult bees due to an interaction among both factors (Alaux et al. 2010; Vidau et al. 2011). Using a similar experimental paradigm, we artificially co-infected bees *per os* with *N. ceranae* and two common honey bee viruses; black queen cell virus (BQCV) and deformed wing virus (DWV), widespread pathogens that infect honey bee ventricular tissue. Interestingly, these viruses did not interact in the same way with the microsporidian. On the one hand, BQCV and Nosema seem to interact synergistically, as co-infected bees have a much greater rate of mortality than single-pathogen infected bees. On the other hand, co-infection by DWV and Nosema did not lead to an increased host mortality, but rather suggest competition between the two pathogens, characterized by a lower number of virus particles in ventricular cells when Nosema infection were already established. These observations support the idea that co-infection by multiple pathogens could be a significant factor of honey bee decline. But the causal mechanisms of interaction between them are yet to be defined.

Martin Embley:

Unraveling the chimeric origins of eukaryotes: trees, genomes and organelles

The universal tree of life as typically shown in textbooks divides life into three domains: the Archaebacteria, Eubacteria and Eukaryotes. Two main criticisms have been made of this tree. Firstly, that it may be incorrect because the models used to make it are overly-simplistic and secondly, that in any case its history represents – given the frequency of gene transfers - only a very small number of genes (less than 1% of the genes on any one genome). I will discuss recent work addressing both of these issues. In the second part of my seminar, I will discuss work on investigating the functions of the minimal mitochondria of parasitic protozoa (including Microsporidia). These organelles no longer make their own ATP but nevertheless they have been retained and presumably still fulfil important functions for the parasite host cell. Thus, in addition to helping us to understand parasite biology these investigations have the potential to help us understand why mitochondria, even when they don't make ATP, are apparently essential components of all eukaryotic cells.

Robert Hirt:

Comparative genomics and evolutionary processes shaping microsporidia protein coding capacity.

The Microsporidia have evolved a distinctive and highly successful strict intracellular parasitic life style. Initially discovered as important insect pathogens they are now also recognised as human pathogens when the immune system is either disturbed or not fully developed. Microsporidia also became the centre of attention for evolutionary biologist investigating the early stages of eukaryotes evolution due to microsporidia perceived simple cellular organisation and 18sRNA gene phylogenies. However molecular phylogenies of a few protein coding genes followed by the analysis of the genome sequence of *Encephalitozoon cuniculi* (2.9 Mbp, Katinka et al. 2001) indicated that these parasites are related to fungi, demonstrating that their relatively simple cellular and genome architecture originated through reductive evolution rather then representing a "primitive bauplan". The sequencing of the relatively larger genome of *Trachipleistophora hominis* (~9 Mbp, Heinz et al. 2012) isolated from an AIDS patient, and possibly of zoonotic (insect) origin, has provided an important new reference point to investigate the tempo and mode of the reductive, and expansive, evolution that is shaping microsporidia gene coding capacity. Comparative genomics of microsporidia also provides exciting opportunities to guide experimental work to study host-microsporidia interactions, for which very little is currently known at the molecular and cellular level. Selected aspect of the analyses of the genome of *T. hominis* and how these data might benefit our work on characterising the molecular basis of microsporidia strict intracellular life style will be presented.

Qiang Huang:

Molecular analysis of the *Nosema ceranae* infection tolerance in the honey bee, *Apis mellifera*

Nosema ceranae has been recently introduced into the honey bee *Apis mellifera* as a novel microsporidian gut parasite. We combined the quantitative genetic and population genetic analysis to locate the genetic region involved in *N. ceranae* infection tolerance. Four QTLs were identified significantly reducing the *N. ceranae* spore load and explaining 20.4% of total spore load variance. Moreover a selective sweep was revealed within the major QTL region. The genetic variability of the swept loci was not only reduced in relation to the flanking markers within the selected strain, but also significantly reduced compared to the unselected honey bee strain. The consistent results indicated the existence of a genetic basis of *N. ceranae* infection resistance and suggested the positive selection driving the success of the selective breeding.

Dino McMahon:

Impact of singe- and co-infection of injected Deformed Wing Virus (DWV) and *Nosema ceranae* on honey bees.

Increases in colony losses pose a significant threat to honey bee populations. Data are presented from a laboratory experiment investigating the impact of two emerging pathogens on honey bees. Adult worker bees were challenged by injecting with Deformed Wing Virus (DWV) and feeding with *Nosema ceranae*, either in isolation or in combination. Treatment with DWV had the largest single impact on honey bee mortality. Analyses of the effects of single- and co-infection on pathogen growth are presented. Investigations into host gene expression responses are currently ongoing.

Chris Mayack:

The physiological effects of a *Nosema ceranae* infection in its host the honeybee.

The temperature sensitive microsporidian *Nosema ceranae* is a relatively new parasite that has recently jumped hosts from the Asian honeybee (*Apis cerana*) to the western honeybee (*Apis mellifera*). *N. ceranae* appears to be more widespread and outcompeting the former microsporidian that has typically been found in western honeybees, *Nosema apis*. This particular scenario, where a closely related parasite is now infecting a new host, is a unique opportunity to study whether behavioral changes observed from infection are a result of a co-evolved parasitic manipulation or due to an incidental by-product from infection. I will discuss how the behavioral fever observed in infected honeybees is not necessarily precluded from natural selection acting on it as it increases reproductive potential for *N. ceranae*, but due to the relatively new parasite-host relationship it is unlikely to be considered a parasitic manipulation. Energetic stress, the incidental by-product from infection, is actually beneficial for *N. ceranae* due to its broader thermal tolerance in comparison to *N. apis*, and therefore this may be the mechanism responsible for seeing the increased prevalence and higher parasite loads of *N. ceranae* around the world.

Myrsini Natsopoulou:

Competition between the intracellular pathogens *Nosema apis* and *Nosema ceranae* in the midgut of the honey bee (*Apis mellifera*).

Nosema apis and *Nosema ceranae* are two microsporidian species that are pathogens of honey bees (*Apis sp.*). Both species are obligate intracellular pathogens that infect the epithelial cells of the ventriculus of adult bees. While *N. ceranae* was believed to be restricted to Asian honey bees (*Apis cerana*), recent studies have demonstrated the widespread emergence of *N. ceranae* as a parasite of western honey bees (*A. mellifera*) at least since 1997. Moreover, due to its widespread distribution and high prevalence, it has been suggested that *N. ceranae* is replacing *N. apis* (native to *A. mellifera*), at least in some host populations. In this study we compare the growth of these microsporidia after challenging individual honey bees with a single or with co-infection. Sequential as well as mixed in vivo infection trials were performed using newly emerged bees, and infection levels were determined 14 days post treatment by quantitative PCR. We test and present findings on the symmetry of the competitive interaction between the original versus emerging infectious microsporidian pathogen of the honey bee *A. mellifera*.

Robert Paxton:

Nosema in honey bees: a brief overview

Following its first description in the early 1900's, *Nosema ap*is was the only microsporidian described from the western honey bee (*Apis mellifera*), to which it is largely restricted. Unlike many other congeners, *N. apis* exhibits extreme tissue tropism, only infecting host ventricular cells. Parasitism shortens host lifespan. A second microsporidian, *Nosema ceranae*, was first described in the Asiatic honey bee *Apis cerana* in China in 1996. Since 2006 it has been associated with *A. mellifera* throughout the native range of *A. mellifera* (Europe, Africa, Near East) and in every other location of the world to which *A. mellifera* has been imported (Americas, Far East, Australasia) as well as in other bee species e.g. bumble bees (*Bombus spp.*). *Nosema ceranae* also exhibits extreme tissue tropism (host ventricular cells) and has been blamed as a cause of recent high colony losses. I shall briefly review the biology of these two pathogens with respect to the SPP.

Charlotte Rafaluk:

Experimental evolution – when host and parasite go extinct

Paranosema whitei is a natural parasite of the red flour beetle *Tribolium castaneum*, with both having previously been used in several successful co-evolution experiments together. *T. castaneum* is a particularly interesting organism in terms of host-pathogen interactions as it not only possesses an internal immune system but is also capable of secreting compounds into its environment with broad antimicrobial effects, providing it with an additional line of defence. Furthermore, it has been previously shown that the presence of these compounds in the environment provide fitness benefits to the beetles when *P. whitei* is also present. With the initial aim of testing the effect of *P.whitei* on the evolution of the different components of the beetles' immune system over time, I have been running an evolution experiment with *P. whitei* at 3 different environmental concentrations. This has, however, resulted in complete host extinction in all cases after only 2-4 generations of evolution. I am currently testing whether this is a consequence of an increase in environmental pathogen concentration, which is potentially allowed for by my experimental set-up, or whether it is a consequence of an increase in virulence of the pathogen.

Andreas Vilcinskas & Heiko Vogel:

Parasites as biological weapons: how microsporidia might be facilitating the invasive success of the ladybird Harmonia axyridis.

The harlequin ladybird *Harmonia axyridis* has emerged as an invasive species that outcompetes and threatens native ladybird species and displays high pathogen and parasite resistance. We discovered that *H. axyridis* has become resistant against microsporidia, obligate intracellular parasites it harbors in its hemolymph. These microsporidia wil kill native ladybirds such as *Coccinella septempunctata* both upon predation of *H. axyridis* eggs or larvae and upon experimental injection of spores isolated from hemolymph of *H. axyridis* . The identified novel microsporidia species can, therefore, be considered as a biological weapon against intraguild predators which may contribute to the remarkable invasive success of the harlequin ladybird. We also purified in hemolymph of *H. axyridis*, a secondary metabolite called harmonine. This compound displays a strong activity against a broad spectrum of pathogens and parasites. Strikingly, next generation-sequencing of its immunity-related transcriptome elucidated an impressive diversification of antimicrobial peptides of *H. axyridis*. These findings invite studies addressing trade-offs and fitness-costs in investment in either innate immunity or chemical defenses. Using an advanced set of methods and combining complementary analyses at biochemical (bioassays, proteomics), molecular (RNA-Seq, epigenetics analysis) and cellular (cell lines & toxicity assays) levels, we aim to decipher the coevolution between *H. axyridis* and its microsporidia, with emphasis on host adaptations providing resistance or tolerance against its own biological weapons

Participants

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Organization

Venue

Institute for Evolution and Biodiversity

Westfälische Wilhelms-Universität

Hüfferstrasse 1

48149 Münster

Directions: http://ieb.uni-muenster.de/contact

Accommodation:

Hotel Jellentrup

Hüfferstr. 52

48149 Münster

http://www.hotel-jellentrup.de

Arrival time: 1pm - 10 pm (luggage drop-off: 7am-10 pm)

Lunch and dinner:

(Please note that the SPP will only cover the dinner at Großer Kiepenkerl (meal+ 1 drink); Lunch will be at your own expenses)

Mensa am Aasee, Bismarckallee 11, 48151 Münster

Restaurant “Großer Kiepenkerl”, Spiekerhof 45, 48143 Münster

Ristorante "La gondola d'oro", Hüfferstraße 34, 48151 Münster

Venue, Hotel, Restaurant etc. on goole maps

Contact person:

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