localized in a region that does not physically interact with the toxins but in the intracellular ATP-binding domain instead. Our toxin binding studies have revealed that such mutation correlates with a reduction in toxin insertion into the membrane (irreversible binding) and suggests that ABCC activity as transporter is necessary for the proper action of Bt toxins.

CONTRIBUTED PAPERS Wednesday, 8:15-9:45

DIS. OF BENEFICIAL INVERTEBRATES 2

Contributed paper. Wednesday, 8:15 **128**

Nosema ceranae News: Update on Species Competition and Host-Pathogen Interaction Studies

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The apparent recent invasion of *Nosema ceranae* and its dominance over *Nosema apis* in honey bee populations in the USA and elsewhere have presented both an enigma and a treatment problem for apiculturists and scientists. Several studies, including those of our research group, have shown that *N. ceranae* produces more mature spores than *N. apis,* and we demonstrated that reproduction of *N. ceranae* recovers more quickly from fumagillin treatment than does *N. apis.* In addition, *N. ceranae* hyperproliferated in the presence of very low fumagillin concentrations in laboratory bioassays. Proteomic-level studies of fumagillin-*N. ceranae*-honey bee interactions continue and we are investigating the mechanisms of protein regulation in response to infection and fumagillin treatment. In studies of infectivity, we found that *N. ceranae* consistently has a higher IC_{50} than *N. apis.* The effect is most pronounced at 1 day post eclosion. We investigated the interaction of *N. ceranae* and *N. apis* in individual bees and found that *N. apis* produced more spores than *N. ceranae* in 62% of bees infected with equal dosages of both *Nosema* species. Mixed species infections negatively affected survival time (15-17 days) compared to single species infections (20 and 21 days for *N. ceranae* and *N. apis,* respectively) and uninfected bees (27 days). Midgut spore counts were higher for mixed species infections than for single species infections, but we did not find evidence that *N. ceranae* outcompetes *N. apis* in an individual host..

Contributed paper. Wednesday, 8:30. **129**

Influence of temperature on the development of _Nosema apis_ and _Nosema ceranae_

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Nosema apis and *Nosema ceranae* are two fungal pathogens infecting the European honey bee, *Apis mellifera*. These obligate intracellular pathogens, belonging to the phylum Microsporidia, infect epithelia cells of the midgut and elicit nosemosis. Recent studies suggested that *N. ceranae* is more virulent than *N. apis* and can lead to severe colony losses. These colony losses are so far only reported from the Southern parts of Europe. In the Northern parts (e.g., Denmark, Sweden, Finland, and Germany) *N. ceranae* could not be correlated to colony losses so far. While *N. ceranae* seems to have replaced *N. apis* in the bee population in South Europe, this is not the case for the Northern parts of Europe. Both findings suggest a climatic angle for spread, assertiveness, and virulence of *N. ceranae*. Exact whether parameters as temperature or humidity, which hinder or favor *N. ceranae* infections, are not determined so far. Spanish colleagues recently showed that *N. ceranae* has a better adaptation to complete its endogenous cycle at warmer temperatures. However, the results based on *in vivo*-infections only give a minor hint on different proliferation of both obligate intracellular pathogens exposed to different temperatures. We here present our results on the intracellular development of *N. apis* and *N. ceranae* exposed to different temperatures using our recently established cell culture model for *Nosema* spp.

Contributed paper. Wednesday, 8:45 **130-STU**

The involvement of bumblebee small interfering RNA pathway against two different bee viruses

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Wild pollinators such as bumblebees are in global decline. They share a pathogen network with other pollinators, consisting of multi-hosts pathogens and multi-pathogens hosts. Disturbance of these associations could lead toward the further host decline. Insects have developed certain immune pathways to combat viruses, of which the small interfering RNA pathway (siRNA) is important. By unveiling the interaction of the virus with the host defense pathway we can better understand the virulence of certain viruses in specific hosts. Here we use two viruses, israeli acute paralysis virus (IAPV) and slow bee paralysis virus (SBPV), representing two infection types after injection in *Bombus terrestris*, i.e. IAPV presents an overt acute infection resulting in mortality, while SBPV results in a covert persistent infection. First, to determine viral replication dynamics by following the negative and positive strands, we developed a new method in combining multiplex ligation-dependent probe amplification and qPCR. The results show both viruses experienced an exponential-plateau phase, and their replication strand were relatively low compared with genome (positive) stand. Second, both viruses increased the expression of Dicer-2 and SID, thereby activating siRNA. Finally we performed small RNAs sequencing to screen if differences in the siRNA production could explain different viral virulence.

Contributed paper. Wednesday, 9:00 **131**

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

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Terrestrial isopods are crustaceans that represent a major component of the litter ecosystem, as they mainly feed on dead plant material and participate in litter decomposition. In the isopod *Armadillidium vulgare*, genetic sex determination follows female heterogamety (ZZ males and ZW females). However, many *A. vulgare* populations harbor maternally-inherited *Wolbachia* bacterial endosymbionts. These bacteria are reproductive parasites that convert genetic males into phenotypic females, leading to populations with female-biased sex ratios. The W sex chromosome has been lost in lines infected by *Wolbachia* and all individuals are ZZ genetic males. The female sex is determined by the inheritance of *Wolbachia* by the *A. vulgare* individual. Surprisingly, some *A. vulgare* lines exhibit

female-biased sex ratios despite the lack of *Wolbachia*. In these lines, female individuals are ZZ genetic males carrying an unknown feminizing factor. To elucidate the genetic basis of female sex determination in these lines, we sequenced the genome of a female by Illumina technology. After *de novo* genome assembly, we identified a large piece of the *Wolbachia* genome transferred into the *A. vulgare* nuclear genome. The transferred genomic fragment co-segregates perfectly with the female sex in pedigrees. These results suggest that sex determination in these *A. vulgare* lines is under the control of nuclear gene(s) of bacterial origin and that bacterial reproductive parasites can drive shifts in sex determination mechanisms in animals. This research is funded by an ERC Starting Grant (EndoSexDet) to RC.

Contributed paper. Wednesday, 9:00 **132**

First characterization of a mollusk beta pore forming toxin

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Aerolysins are virulence factors belonging to the beta pore-forming toxin (b-PFT) superfamily that are abundantly distributed in bacteria. More rarely, b-PFTs have been described in eukaryotic organisms. Recently in our laboratory, a putative cytolytic protein called Biomphalysin have been characterized in the snail, *Biomphalaria glabrata*, who's primary structural features suggest that it could belong to this b-PFT superfamily. We have showed that, despite weak sequence similarities with aerolysins, Biomphalysin shares a common architecture with proteins belonging to this superfamily. A phylogenetic approach revealed that the gene encoding Biomphalysin could have resulted from horizontal transfer. Its expression seems to be restricted to immune-competent cells and is not induced by parasite challenge. Recombinant Biomphalysin showed hemolytic activity that was greatly enhanced by the plasma compartment of *B. glabrata*. We further demonstrated that Biomphalysin is able to bind to parasite and has a plasma dependent anti schistosomal activity. Surprisingly, investigation of *B. glabrata* genome reveals that this family appears to be multi-genic. More than 20 genes were identified suggesting an important role played by Biomphalysin proteins for *B. glabrata*.
These results provide the first functional description of a mollusk immune effector protein involved in killing of *S. mansoni*, agent of the second most widespread tropical parasitic disease after malaria.

Contributed paper. Wednesday, 9:30 **133-STU**

A first report of an immune-associated cytosolic PLA_2 in insects: Gene structure and function

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Eicosanoids are a group of C20 polyunsaturated fatty acids most derived from arachidonic acid (AA). A phospholipase A_2 (PLA_2) catalyses AA release from phospholipids at SN-2 position. Among three different groups of PLA2s ($cPLA_2$, $sPLA_2$, $iPLA_2$), only $sPLA_2$ (secretory type of PLA_2) has been identified as venom- or immune-associated functions. This study reports the first $cPLA_2$ (cellular and calcium-dependent PLA_2) in insects. A hemocyte transcriptome of *Spodoptera exigua* possessed 1 for $sPLA_2$, 2 for $iPLA_2$, 1 for $cPLA_2$. Expression of Se-$cPLA_2$ was inducible to bacterial challenge in hemocyte and fat body. RNA interference of Se-$cPLA_2$ expression significantly suppressed cellular immune responses of *S. exigua*. A recombinant of Se-$cPLA_2$ exhibited a specific enzyme activity influenced by p H, temperature, and calcium. Especially, Se-$cPLA_2$ was susceptible to a specific $cPLA_2$ inhibitor, but not to a specific $iPLA_2$ inhibitor. These results indicate that Se-$cPLA_2$ is a specific $cPLA_2$ and associated with immune responses.

CONTRIBUTED PAPERS Wednesday, 8:00-9:30

FUNGI 4

Contributed paper. Wednesday, 8:00 **134**

Fungal dimorphism in the entomopathogenic fungus *Nomuraea rileyi*: A search for *in vivo* produced quorum-sensing molecules

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Similar to other zoopathogenic fungi, many insect pathogenic hyphomycetes including species within the genera *Metarhizium*, *Beauveria*, *Isaria,* and *Nomuraea* exhibit a defined *in vivo* dimorphic developmental program. This program involves switching between apical to budding growth providing mycopathogens with both tissue-invasive and vegetative growth capabilities. The budding yeast-like vegetative cells absorb nutrients in the hemocoel without apparent damage to tissues allowing the insect to continue to feed and develop. The ability to switch cell phenotypes is crucial for successful *in vivo* development. *N. rileyi* exhibits a defined developmental program that involves the sequential production of cellular phenotypes designed to perform spatially and temporally unique functions. Upon reaching the nutrient-rich hemolymph the penetrant germ tube switches from an apical to a budding growth program leading to the formation of freely circulating hyphal bodies. The yeast-like hyphal bodies grow exponentially in the nutrient-rich haemolymph reaching densities that far outnumber circulating hemocytes. As a critical threshold density is achieved, these hemolymph-borne cells synchronously revert to an apical growth program forming the tissue-invasive cell phenotype. The ensuing mycelial phase produce and secrete a suite of metabolites that can modulate host development, that rapidly kill the host, and that efficiently digests insect tissue leading to the mummification of infected larvae. In this presentation investigation we detail the hyphal body to mycelial transition of *Nomuraea* in the insect host, provide evidence for quorum-sensing that is produced and released into the hemolymph, and detail the extraction and examination of the elicitors that mediate the dimorphic switch.

Contributed paper. Wednesday, 8:15 **135**

Multilocus genotyping of *Amylostereum* spp. associated with *Sirex noctilio* and other woodwasps from Europe reveal clonal lineage introduced to the US

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