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

David Duval[1,2], Richard Galinier[1,2], Guillaume Mitta[1,2], Benjamin Gourbal[1,2]
[1]CNRS, UMR 5244, Ecologie et Evolution des Interactions (2EI), Perpignan, France, [2]Université de Perpignan Via Domitia, Perpignan, France,
Address for Correspondence: david.duval@univ-perp.fr

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

Jiyeong Park and Yonggyun Kim*
Department of Bioresource Sciences, Andong National University, Andong 760-749, Korea
Address for Correspondence: lovebinwoo1@naver.com

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

Boucias, Drion[1], Liu, Shouzou[2] and Baniszewski, Julie[1]
[1]Entomology and Nematology Department, University of Florida, Gainesville FL, USA, [2]Agricultural College, Liaocheng University, Liaocheng, Shandong, China
Adress for Correspondence: pathos@ufl.edu

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

Louela A. Castrillo[1], Ann E. Hajek[1], Ryan M. Kepler[1], Juan A. Pajares[2], Iben M. Thomsen[3], György Csóka[4], Paula Zamora[5] and Sergio P. Angeli[6]
[1]Department of Entomology, Cornell University, Ithaca, NY 14853, USA, [2]Sustainable Forest Management Research Institute, University of Valladolid, Palencia, Spain, [3]Department of Geosciences and Natural Resource Management, University of

Copenhagen, Copenhagen, Denmark, [4]Department of Forest Protection, Forest Research Institute, Mátrafüred, Hungary, [5]Calabazanos Forest Health Center, Castile and Leon, Palencia, Spain,[6]Faculty of Science and Technology, University of Bolzano, Italy

Sirex noctilio is a woodwasp of Eurasian origin that was inadvertently introduced to the southern hemisphere in the 1900s and to North America over a decade ago. It attacks various *Pinus* species and cause significant mortality in pine plantations. *Sirex noctilio* is associated with a symbiotic white rot fungus, *Amylostereum areolatum*, which females inject into trees when they oviposit and which is required for survival of developing larvae. We examined the genetic diversity of *A. areolatum* isolated from *S. noctilio* and other woodwasps collected from Europe in comparison with samples from northeastern North America to determine origin of introduction(s). Multilocus genotyping of nuclear ribosomal regions and protein genes revealed two widespread multilocus genotypes (MLGs) among the European samples, one of which is present in the US. The other US *S. noctilio*-associated *A. areolatum* represented unique MLGs, although variation was primarily due to the laccase gene, with the other loci having conserved sequences. The closest relative to these US strains is a German strain with identical ITS, mtssu and tef sequences. These findings indicate multiple introductions of *S. noctilio* to North America from Europe or from Europe via South America. Our results also showed lack of fidelity between wasp hosts and *Amylostereum* species, and we found a North American woodwasp carrying an *A. amylostereum* MLG likely introduced by *S. noctilio*. These results underscore the need to study North American siricids and their fungal symbionts as *S. noctilio* continues to spread in North America.

Contributed paper. Wednesday, 8:30. **136**

Preliminary analysis of the genome sequence of *Beauveria caledonica*

Travis R. Glare[1], Aimee C. McKinnon[1] and Murray P. Cox[2]
[1]Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand, [2]Massey University, Private Bag 11 222, Palmerston North 4442, New Zealand
Address for Correspondence: travis.glare@lincoln.ac.nz

Beauveria caledonica is a pathogen of a number of insects, especially Coleoptera. Occurrence has probably been under-reported due to the morphological similarity the ubiquitous entomopathogenic fungus *Beauveria bassiana.* Recent phylogenetic studies have shown that *B. bassiana sensu lato* is really a species complex. The genomic differences between species of *Beauveria* can assist understanding of the importance of selected gene in disease and ecology of these fungi. We report on initial comparisons of the genome of *B. caledonica* strain isolated in New Zealand and *B. bassiana.* The genome was sequenced using 3 lanes of a MiSeq by NZGL (New Zealand). 15,890,840 150-bp read pairs were obtained for the 32-Mb *Beauveria* strain (~149 fold coverage). After assembly using the programme ABySS, a total of 10,951 contigs were obtained over 39 bp and an N50 of 21676, with 2827 over 500 bp. Preliminary comparisons were conducted on a range of phylogenetic, secondary metabolite and mitochondrial gene regions. Assembly of the mitochondrial genome was used to assess completeness of the coverage. The genome sequence of *B. caledonica* shows significant divergence from *B. bassiana*.

Contributed paper. Wednesday, 8:45 **137**

MALDI-TOF Mass Spectrometry: A complement to sequence-based identification technologies for major fungal entomopathogens

Richard A. Humber[1], Rogério Biaggioni Lopes[2], Marcos Faria[2]
[1]USDA-ARS Biological IPM Research, RW Holley Center, Ithaca, New York, USA; [2]Embrapa Genetic Resources and Biotechnology, Brasília, Brazil
Address for Correspondence: richard.humber@ars.usda.gov

Matrix-Assisted Laser Desorption/Ionization Time of Flight mass spectrometry has been tested and proven to be a rapid and inexpensive approach closely replicating the results of gene sequence-based analyses to identify species in such major entomopathogenic fungal genera as *Metarhizium* and *Beauveria*. While MALDI-TOF cannot replace PCR-based approaches for identifications or phylogenetic studies and cannot demonstrate relationships among fungi, it does appear to be extremely valuable for rapidly detecting anomalous isolates that need further detailed PCR-based study. This mass-spectrometric technique may be extremely valuable for ecological and population biology studies, as well as offering significant support for the efficient curation of large culture collections holding hundreds to thousands of isolates for which verified MALDI-TOF profiles are available. In comparison to the results obtained from the more routine analyses of (still) small numbers of individual genes, MALDI-TOF uses large numbers of cell proteins to group samples and, therefore, monitors much larger proportions of a total organismal genome; evidence will be presented that such a more complete coverage of the total genome suggest the existence of biogeographical groupings that may not be easily detected by PCR-based studies.

Contributed paper. Wednesday, 9:00 **138**

Transcriptomic study reveals *Pandora formicae* expressing pathogenicity related genes in final stages of host infection

Joanna Malagocka[1]; Morten N. Grell[2], Lene Lange[2], Jørgen Eilenberg[1], Annette Bruun Jensen[1]
[1]Centre for Social Evolution, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark; [2]Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Copenhagen, Denmark
Address for Correspondence: jopi@plen.ku.dk

Pandora formicae (*Entomophthorales*, Entomophthoro-mycota) is an obligate pathogen of the common red wood ant, *Formica rufa*. The fungus, similarly to other fungi of this group, enters the host body through cuticle, where it exploits nutritional resources within the haemocoel. When the infected ant is close to death, the fungus triggers a change in host behavior, manipulating it to climb a leaf (e.g. grass) or a twig. The fungus attaches the moribund host with rhizoids, the host legs grasp around the leaf or twig and the mandibles bite to vegetation and lock. Then the host dies and soon after the fungus breaks through the cuticle with conidiophores producing asexual spores. This quick transformation requires activity of several enzymes involved in cuticular breakdown, cell wall formation, and other processes. To study this, we have constructed transcriptome libraries of the last two stages: 1) when the ant is just dead with no fungal growth outside except the rhizoids, and 2) when external conidiophores are present. This first *de novo* transcriptome of an entomophthoralean fungus, in interaction with host, provides accurate insight into the plethora of genes expressed during final stages of infection, crucial for fungus transmission and reproductive success.

Contributed paper. Wednesday, 9:15 **139**

Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals putative virulence factors shared by fungal and oomycete entomopathogens

Paula F. Quiroz Velasquez, Sumayyah Abiff, Quincy B. Conway, Norma C. Salazar, Ana Paula Delgado, Jhanelle K. Dawes, Lauren G. Douma, Aurélien Tartar, Nova Southeastern University, Fort Lauderdale, FL, USA
Address for correspondence: aurelien@nova.edu

The entomopathogenic oomycete *Lagenidium giganteum* is known to infect and kill mosquito larvae and therefore has been seen as a potential biological control agent against disease vector mosquitoes. However, little is known about the pathological process of *L. giganteum* in its mosquito host. In order to detail the molecular basis of entomopathogenicity, a transcriptome analysis was initiated for *L. giganteum*, using various Next Generation Sequencing technologies. Homology searches have led to the annotation of ca. 20,000 transcripts based on significant similarity to known proteins and revealed a full complement of plant pathogenic oomycete effector orthologs. The characterization of full-length transcripts corresponding to Cellulose Binding Elicitor Lectin (CBEL), Crinkler, and elicitin proteins demonstrated that *L. giganteum* is the first described animal pathogenic oomycete to secrete canonical Crinkler and CBEL effectors. In addition, phylogenetic analyses identified a Glycoside Hydrolase 5 (subfamily 27; GH5_27) as a putative virulence factor. Genome mining indicated that GH5_27 orthologs are shared by entomopathogenic oomycetes and fungi, but virtually absent in all other oomycetes and fungi. Using PCR, GH5_27 fragments were amplified and sequenced from additional entomopathogens, suggesting that oomycete and fungi underwent convergent evolution and that GH5_27 proteins may play a crucial role in insect/microbe pathosystems. Detailing the molecular basis of entomopathogenicity may allow for the use of oomycetes and fungi as control agents against insect pests, reducing the use of insecticides that can have negative impacts on the environment and human health.

SYMPOSIUM 6 (Bacteria) Wednesday, 10:30–12:30

Structure and Function of Novel Insecticidal Toxins

Symposium. Wednesday, 10:30 **140**

Structural and biophysical characterization of Cry34Ab1 and Cry35Ab1

Matthew S. Kelker[1], Colin Berry[2], Matthew D. Baker[2], Steven L. Evans[1], Reetal Pai[1], David McCaskill[1], Joshua C. Russell[1‡], Nick X. Wang[1], J.W. Pflugrath[3], Cheng Yang[3], Matthew Wade[4], Tim J. Wess[4#], Kenneth E. Narva[1]
[1]Dow AgroSciences, LLC, Indianapolis, Indiana, United States of America; [2]Cardiff School of Biosciences, Cardiff University, Cardiff, Wales, United Kingdom; [3]Rigaku Americas Corporation, The Woodlands, Texas, United States of America; [4]School of Optometry & Vision Sciences, Cardiff University, Cardiff, Wales, United Kingdom; [‡]Current address: Department of Biochemistry, University of Washington, Seattle, Washington, United States of America; [#]Current address: Office of the Dean of Science, Charles Sturt University, New South Wales, Victoria, Australia
Address for Correspondence mskelker@dow.com

Bacillus thuringiensis strains are well-known for the production of insecticidal proteins upon sporulation and these proteins are deposited in parasporal crystalline inclusions. The majority of these insect-specific toxins exhibit three domains in the mature toxin sequence. However, other Cry toxins are structurally and evolutionarily unrelated to this three-domain family and little is known of their three dimensional structures, limiting our understanding of their mechanisms of action and our ability to engineer the proteins to enhance their function. Amongst the non-three domain Cry toxins, the Cry34Ab1 and Cry35Ab1 proteins are required to act together to produce toxicity to the western corn rootworm (WCR) *Diabrotica virgifera virgifera* Le Conte via a pore forming mechanism of action. Cry34Ab1 is a protein of ~14 kDa with features of the Aegerolysin family (Pfam06355) of proteins that have known membrane activity, while Cry35Ab1 is a ~ 44 kDa member of the Toxin_10 family (Pfam05431) that includes other insecticidal proteins such as the binary toxin BinA/BinB. The Cry34Ab1/Cry35Ab1 proteins are important solutions for control of WCR having been developed as insect resistance traits in commercialized corn hybrids for control of WCR. The structures of Cry34Ab1 and Cry35Ab1 have been elucidated to a resolution of 2.15 Å and 1.80 Å, respectively. The solution structures of the toxins were further studied by small angle X-ray scattering (SAXS) and native electrospray ion mobility mass spectrometry. We present here the first published structures from the Aegerolysin and Toxin_10 protein domain families.

Symposium. Wednesday, 10:50 **141**

Structure/function studies of Cry5B via alanine-scanning mutagenesis

Jillian Sesar[1]; Melanie Miller[1], Yan Hu[1,2], Raffi V. Aroian[1,2]
[1]Division of Biological Sciences, University of California, San Diego, CA, USA; [2]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, USA
Address for Correspondence: raroian@ucsd.edu

Cry5B is a three-domain crystal protein that attacks nematodes. In collaboration with the laboratory of Partho Ghosh, the three dimensional structure of Cry5B has been solved. Cry5B shows significant similarities with three-domain insecticidal crystal proteins in domains I and III but significant differences with insecticidal proteins in domain II. To better understand structure function relationships in Cry5B, we performed alanine-scanning mutagenesis of the entire toxin domain in which each point variant was tested in bioactivity assays with the free-living nematode *Caenorhabditis elegans*. Alanine point variants were classified into three classes—those with reduced/no bioactivity, those with relatively normal bioactivity, and those with increased bioactivity against *C. elegans*. More than 400 point variants were successfully tested. Some of those in the latter class (increased bioactivity) have been selected for further study, including fully quantitative analyses and testing their spectrum of increased action against other nematodes. Our results, as well as their implications for crystal protein – nematode interactions, will be presented..

Symposium. Wednesday, 11:10 **142**

Insights into the structures of non-3-domain toxins through structural modeling

Colin Berry
Cardiff School of Biosciences, Cardiff University, Cardiff, United Kingdom
Address for Correspondence: Berry@cf.ac.uk

A knowledge of the structure of insecticidal toxins is a major benefit in elucidating mode of action and is, therefore, fundamental for targeted mutagenesis to test mechanistic hypotheses, to alter target range and increase toxicity. Crystal structures of activated 3-domain toxins, Cyt toxins, Vip2, Mtx1 and anthrolysin are available and can be used to model structures for related proteins. There remains a significant