

VIRUSES 5

Contributed paper. Wednesday, 14:00 **184****Soybean aphid viruses exploit contrasting transmission strategies**

Diveena Vijayendran, Sijun Liu, Bryony C. Bonning
 Department of Entomology, Iowa State University, Ames, IA
 50011 USA
 Address for Correspondence: bbonning@iastate.edu

The soybean aphid, *Aphis glycines* Matsumura, is an invasive pest of primary agricultural importance in North America. Following its introduction in 2000, soybean yields dropped 11%, with the costs of management and yield loss estimated to be \$1.6 billion over a 10 year period. Soybean aphids are managed primarily by application of chemical insecticides. We identified two viruses from the soybean aphid transcriptome and small RNA data that may have potential for use in soybean aphid management: Aphid lethal paralysis virus (ALPV)-Ames (Dicistroviridae) and *Aphis glycines* virus (AGV; unclassified). There is evidence for the presence of ALPV-like viruses in several different insects including the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus), the honeybee *Apis mellifera*, and Western corn rootworm *Diabrotica virgifera virgifera*. AGV has a ~5 kb single stranded RNA (ssRNA) genome and forms a 30 nm particle. The RNA-dependent RNA polymerase (RdRp) of this virus is closely related to that of *Euprosterina elaeasa* virus (Tetraviridae), while the AGV coat protein (CP) is similar to those of plant *Sobemoviruses*. Based on RT-PCR of AGV RdRp sequence, AGV-like viruses appear to be present in two other aphid species, the bird cherry-oat aphid, *R. padi* and the green peach aphid, *Myzus persicae* (Sulzer). Notably, ALPV-Ames does not appear to be vertically transmitted in the soybean aphid, while AGV is 100% vertically transmitted. The different transmission strategies of these two viruses and the implications of 100% vertical transmission will be discussed.

Contributed paper. Wednesday, 14:15 **185****Characterization of mechanisms involved in the transmission of a lepidopteran densovirus**

Cécilia Multeau¹, Doriane Mutuel², Manuela Rakotomanga²,
 Anne Kenaghan², Clément Bousquet², Rémy Froissart^{3,4},
 Nathalie Volkoff² and Mylène Ogliaastro²

¹InVivo AgroSolutions, F-06560, Valbonne, France;

²INRA, UMR 1333 DGIMI, INRA, F-34000, Montpellier, France;

³CNRS, UMR 5290 MIVEGEC, F-34394, Montpellier, France;

⁴CIRAD-SupAgro, UMR 385 BGPI, F-34398, Montpellier, France

Address for Correspondence: cmulteau@invivo-group.com

Densoviruses are small insect parvoviruses infectious per ingestion for several lepidopteran species. Their potential as microbial control agents led us to focus on the understanding of motors driving the transmission of densoviruses in the environment. Natural dynamic of densoviral infections was never investigated although several metagenomic studies revealed the presence of densoviruses in samples from various origins (feces from bats, mosquitoes, marine samples like urchins...). In this study, we qualitatively and quantitatively characterized direct and indirect mechanisms leading to the transmission of a model viral species, *Junonia coenia* densovirus, on the model host *Spodoptera frugiperda*. We showed that cannibalism of infected individuals and bites between infected and uninfected individuals are major events in the transmission of JcDNV while exposition to contaminated feces and/or regurgitations contributes to widely disseminate the

virus throughout a susceptible population. We also found that parasitoid wasps participate to indirect transmission of densoviruses although probably in a non-specific manner. Altogether, these results are a first step toward the construction of a dynamic transmission model for densoviruses.

Contributed paper. Wednesday, 14:30 **186****Discovery of circular single-stranded DNA viruses in top insect predators**

Karyna Rosario¹, Anisha Dayaram², Jessica Ware³,
 Milen Marinov², Mya Breitbart¹, Arvind Varsani²

¹College of Marine Science, University of South Florida, Florida, USA; ²School of Biological Sciences, University of Canterbury, Christchurch, New Zealand; ³School of Environmental and Biological Sciences, Rutgers University, New Jersey, USA
 Address for Correspondence: krosari2@mail.usf.edu

Viruses with circular single-stranded DNA (ssDNA) genomes that encode a replication initiator protein (Rep) are among the smallest viruses known to infect eukaryotic organisms. Additionally, their rapid evolution rates have led to the emergence of some of these viruses as serious pathogens. Recent research indicates that the host range of eukaryote-infecting circular Rep-encoding ssDNA (CRESS-DNA) viruses, which was previously thought to be restricted to plants and vertebrates, may include insects. To expand our knowledge of circular ssDNA viruses in invertebrates, this study surveyed CRESS-DNA viruses circulating among insect populations by targeting dragonflies (Eiprocta). Dragonflies are highly mobile top insect predators that accumulate viruses from their insect prey over space and time and, thus, can be used as 'sampling traps' to explore the diversity of CRESS-DNA viruses found among flying insects. Using degenerate PCR and rolling circle amplification coupled with restriction digestion, 16 CRESS-DNA viral genomes were recovered from eight different dragonfly species collected in tropical and temperate regions. Nine of the genomes are similar to cycloviruses and represent five species within this proposed genus, suggesting that cycloviruses are commonly associated with insects. Three of the CRESS-DNA viruses share conserved genomic features with the recently described fungal virus *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1. The remaining viruses are divergent species representing novel CRESS-DNA viral genera. The novelty of CRESS-DNA viruses identified in dragonflies using simple molecular techniques indicates that there is an unprecedented diversity of ssDNA viruses among insect populations.

Contributed paper. Wednesday, 14:45 **187-STU****Single-stranded DNA viruses in marine crustaceans**

Ryan Schenck¹; Karyna Rosario¹; Rachel Harbeitner¹;
 John Cannon²; Mya Breitbart¹

¹University of South Florida College of Marine Science, Tampa, Florida, USA; ²University of South Florida College of Medicine Department of Pediatrics, USA

Address for Correspondence: ryanschenck@mail.usf.edu

Metagenomic sequencing has recently revealed the ubiquity of eukaryotic circular single-stranded DNA (ssDNA) viruses in the marine environment; however, a definitive host has not been identified for most of these viruses. Through direct examination of marine shrimp and crab species, this study surveyed the diversity of circular ssDNA viruses in economically and ecologically important crustaceans, linking these newly discovered viruses to their hosts and improving our understanding of the ecological impact of ssDNA viral infection in marine crustaceans. Viral particles were partially purified from specimen homogenates through filtration, DNA was then

extracted and amplified through rolling circle amplification to enrich for small circular ssDNA templates. The concatenated circular genomes were then digested with restriction enzymes and the resulting products (~1– 4 kb) were cloned and sequenced. Thirteen distinct ssDNA viral genomes were recovered from five crab species and three shrimp species. Putative encoded proteins share less than 60% identity with known viral proteins from members of the *Circoviridae*. The detected genomes exhibit four different genomic architectures revealing an incredible diversity of ssDNA viruses in shrimp and crabs. Ongoing work aims to propagate these viruses in insect cell lines and develop a system to assess viral infectivity and modes of transmission.

Contributed paper. Wednesday, 15:00 **188**

Remarkable diversity of endogenous viruses in the genome of an isopod crustacean

Julien Théze, Sébastien Leclercq, Bouziane Moumen, Richard Cordaux, Clément Gilbert
Université de Poitiers, Laboratoire Ecologie et Biologie des Interactions - UMR CNRS 7267, Equipe Ecologie Evolution Symbiose, 86073 Poitiers Cedex 9, France
Address for Correspondence: theze.julien@gmail.com

Recent studies in paleovirology have uncovered myriads of endogenous viral elements (EVEs) integrated in the genome of their eukaryotic hosts. These fragments result from endogenization, i.e., integration of the viral genome into the host germline genome followed by vertical inheritance. So far, most studies have used a virus-centred approach, whereby endogenous copies of a particular group of viruses were searched in all available sequenced genomes. Here we follow a host-centred approach whereby the genome of a given species (the crustacean isopod *Armadillidium vulgare*) is comprehensively screened for the presence of EVEs using all viral sequences available as queries. This search and downstream evolutionary analyses revealed that 56 EVEs corresponding to 11 different viral lineages belonging to 5 viral families (*Bunyaviridae*, *Circoviridae*, *Parvoviridae*, *Nimaviridae*, *Totiviridae*) and one viral order (*Mononegavirales*) became endogenized in *A. vulgare*. We show that viral endogenization occurred recurrently during the evolution of isopods, that *A. vulgare* viral lineages were involved in multiple host-switches that took place between widely divergent taxa. Furthermore, 32 *A. vulgare* EVEs have uninterrupted open reading frames, suggesting they result from recent endogenization of viruses likely to be currently infecting isopod populations. Overall, our work shows that isopods have been and are still infected by a large variety of viruses. It also extends the host range of several families of viruses and brings new insights into their evolution. More generally, our results underline the power of paleovirology in characterizing the viral diversity currently infecting eukaryotic taxa.

Contributed paper. Wednesday, 15:15 **189**

Iteraviruses (Densovirinae) from monarch and black swallowtail butterflies and slug caterpillar moths and characterization of their expression strategies

Qian Yu, Max Bergoin, and Peter Tijssen
INRS-Institut Armand-Frappier, 531 boul. des Prairies, Laval, QC, Canada H7V1B7
Address for correspondence: peter.tijssen@iaf.inrs.ca

Iteraviruses belong to a separate genus of the Densovirinae subfamily of the Parvoviridae family and includes three densoviruses, i.e. *Casphalia extranea* Densovirus (CeDNV), *Dendrolimus punctatus* Densovirus (DpDNV) and *Bombyx mori*

Densovirus (BmDNV). In this study, we used a Sequence-Independent Single-Primer Amplification (SISPA) method to detect the pathogens of larvae from three additional insect species (*Papilio polyxenes*, *Sibine fusca* and *Danaus plexippus*), killed by some unknown pathogen. Sequencing of the clones that were obtained and BLAST analysis revealed the existence of three previously unknown densoviruses (provisionally named PpDNV, SfDNV and DppiDV). The genome of the new densoviruses were cloned into pCR2.1-topo or pBluescript(SK-) vectors. These virus sequences (including ITRs) have high identities with CeDNV and BmDNV. The identical genome organizations indicated that these three new densoviruses should be classified in the Iteravirus genus. Together with the infectious clones of CeDNV and BmDNV, we investigated the expression strategies of five different iteraviruses (PpDNV, SfDNV, CeDNV, BmDNV, DppiDV). Total RNA was obtained both from LD cell line transfected by infectious clones of the iteraviruses and virus infected larvae (*Papilio polyxenes*). RACE methods were used to identify the 5' and 3' transcription ends. The nonstructural (NS) and structural (VP) genes were located on the same strand of the genome. The NS cassette consists of two genes with NS1 and overlapping NS2. The NS2 transcripts all start at 7 nts downstream of the NS1 start codon. Transcription starts for NS1 genes are close to the AUG of NSS1. NS and VP transcripts do not overlap. The four VPs were similarly generated by leaky scanning translation of unspliced mRNA. The VP transcripts just start 2nts downstream of the poly (A) motif for NS transcripts. Interestingly, poly (A) signals for VP transcripts all overlap with the stop codons of the VP genes.

Contributed paper. Wednesday, 15:30 **190**

Remarkable genetic diversity of single-stranded DNA viruses in cultured shrimps and crickets

Hanh T. Pham, Qian Yu, Max Bergoin, Peter Tijssen
INRS-Institut Armand-Frappier, Université du Québec, Laval, QC, Canada
Address for Correspondence: peter.tijssen@iaf.inrs.ca

Single-stranded DNA viruses are among the smallest viruses and include members of the *Parvoviridae* and *Circoviridae* families (linear and circular ssDNA viruses). In the past decades, PstDNV and AdDNV have been well-known viruses that have caused a severe impact on cultured shrimps and crickets. Here, we report the discovery and genome characterization of numerous novel denso- and denso-like viruses and, for the first time, new circoviruses from these hosts. During the last years, we received many cricket samples from North America that were negative for AdDNV. However, denso-like particles have been observed by EM. Complete sequence of different viral genome have been isolated and cloned including one circular ssDNA viruses of 2.5 kb, an ambisense densovirus of 4.9 kb and a segmented brevidenso-like virus (3.3 kb). Meantime, large numbers of new ssDNA viruses were also isolated from cultured shrimp from Vietnam. Characterization of these viruses revealed 3 different, unrelated circoviruses of 1.7, 1.7 (the latter is not using the standard genetic code and may have been ingested) and 1.3 kb. We also discovered a new shrimp parvovirus of about 4.1 kb that is phylogenetically poorly related to any known parvovirus. Near-atomic structures of some cricket and shrimp parvoviruses were obtained by X-ray crystallography as well as their transcription strategy. These results demonstrate a great diversity of ssDNA-viruses infecting these economically important animals. Future work will be focused on molecular features of these viruses for a further insight into the evolution and classification of ssDNA viruses.

Contributed paper. Wednesday, 15:45 **191**

How do vine mealybug, grapevine leafroll-associated virus and grapevine interact on a molecular level?

Alicia Eva Timm¹ & Annette Reineke²

¹Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa

²Institut für Phytomedizin, Geisenheim Hochschule, Geisenheim, Germany

Address for Correspondence: aetimm@gmail.com

Vine mealybug (VMB), *Planococcus ficus*, is one of the most damaging grapevine pests in the world, largely because it is a vector of grapevine leafroll-associated virus (GLRaV). Since interactions among VMB, GLRaV and grapevine are responsible for the extent of VMB damage and GLRaV spread, we investigated the relationships among the three organisms. The effect of GLRaV infection on VMB was determined using cDNA-AFLP analysis and validated with RT-qPCR. It was found that VMB responds to GLRaV by activating only a few genes, and possibly also by endosymbiont mediation. The effect of VMB feeding on grapevine was investigated with microarray analysis and validated with RT-qPCR. Grapevine was found to respond to VMB feeding by mounting a weak response within a narrow window of time. These results are useful for understanding the interaction among the organisms in the VMB system, and limiting the damage caused in vineyards.

CONTRIBUTED PAPERS Wednesday, 14:00-16:00

BACTERIA 4

Contributed paper. Wednesday, 14:00 **192**

Analysis of the bacterial community of the insect pest *Lymantria dispar* during its life cycle

Zane Metla^{1,2,3}, Monika Maurhofer², Liga Jankevica^{1,3}

¹Plant Pathology, Institute of Integrative Biology (IBZ). Swiss Federal Institute of Technology, Switzerland; ²Laboratory of Experimental Entomology, Institute of Biology, University of Latvia, Latvia; ³University of Daugavpils, Latvia

Address for Correspondence: zane.metla@usys.ethz.ch

Gypsy moth (*Lymantria dispar*, Lepidoptera) outbreaks can cause great damage to forestry across Europe. In order to control this pest, there is need for new insecticidal bacterial strains for the development of more effective biopesticides. The insect gut microbiota represents all aspects of microbial relationships, ranging from pathogenic to obligate mutualistic interactions. Latest investigations suggest that there is competition between individual opportunistic pathogens and that they are able to upregulate the production of virulence factors according to their density within hosts, what renders them interesting for use in combination with biocontrol agents.

The objective of this work is to characterize the bacterial midgut community of *L. dispar* and to monitor 1) changes in diversity during its life cycle, 2) changes within larvae from spring to summer and 3) differences between individuals. Microorganisms were first analyzed using a culture dependent approach where midguts were extracted and plated on media. Growing bacteria were analyzed by colony characteristics - color, size, shape, opacity, margin, elevation and viscosity and then by 16S rRNA gene sequencing. In a second step bacterial midgut communities were analyzed by a culture independent method using PacBio technology to sequence full length 16S rRNA genes.

Results showed relatively simple composition of the gypsy moth midgut community. We observed differences between individual larva from the same time point and structural changes of diversity in bacterial communities over the season.

This project is conducted within the frame of the SCIEX program with ETH Zürich and the University of Daugavpils as partners

Contributed paper. Wednesday, 14:15 **193**

Contacting microbe induce grooming behaviour in *Drosophila*

Aya Yanagawa^{1,2}, Tsuyoshi Yoshimura¹, Hata Toshimitsu¹ and Frédéric Marion-Poll^{2,3}

¹Kyoto University, Uji, Japan; ²CNRS, Laboratoire Evolution, Génomes et Spéciation, Gif-sur-Yvette, France; ³AgroParisTech, Département Sciences de la Vie et Santé, Paris, France

Address for Correspondence: ayanagawa@rish.kyoto-u.ac.jp

Insects remove and clean microbes from their surface by grooming behavior, which is considered as a behavioral defense against pathogen/parasite infection in some cases. It is well known that the insects like *Drosophila melanogaster*, which live in an environment littered with bacteria, fungi and other microorganisms developing on decaying material devote a lot of time to self-grooming which seems to contribute cleaning their cuticula from external particles. The mechanisms that trigger this behavior are still ambiguous, although grooming behavior was identified in many insects. In this work, we examined if *D. melanogaster* can sense microbe in their habitat and if they conduct any hygiene behavior like grooming after they have perceived microbe. To follow the behavioral reaction, we focus on a contact chemo-stimulus, which would activate taste neurons. Microbe, microbe-related compounds and standard chemicals were used as stimuli and influence of water and mechanical stimuli were removed by using Gal4-UAS system in control experiments. Grooming seems to be specifically triggered by the activation of taste neurons since flies showed strong cleaning behavior when contacted with taste stimuli.

Contributed paper. Wednesday, 14:30 **194**

Cultivable gut bacteria of scarabs inhibit *B. thuringiensis* multiplication

Yueming Shan^{1,2}, Changlong Shu², Neil Crickmore³,

Chunqin Liu⁴, Wensheng Xiang¹, Fuping Song², Jie Zhang²

¹School of Life Science, Northeast Agricultural University, Harbin 150030, P. R. China; ²State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; ³School of Life Sciences, University of Sussex, Falmer, Brighton, UK; ⁴Cangzhou Academy of Agricultural and Forestry Sciences, Cangzhou 061001, P. R. China

Address for Correspondence: jzhang@ippcaas.cn

The entomopathogen *Bacillus thuringiensis* is used to control various pest species of scarab beetle but is not particularly effective. Gut bacteria have diverse ecological and evolutionary effects on their hosts, but whether gut bacteria can protect scarabs from *B. thuringiensis* infection remains poorly understood. To investigate this we isolated 32 cultivable gut bacteria from *Holotrichia obliqua*, *Holotrichia parallela* and *Anomala corpulenta*, and analyzed their effect on *B. thuringiensis* multiplication and Cry toxin stability. 16S rDNA analysis indicated that these gut bacteria belong to the *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes* phyla. A confrontation culture analyses of the 32 isolates against three scarab specific *B. thuringiensis* strains showed that the majority of the scarab gut bacteria had antibacterial activity against the *B. thuringiensis* strains. The Cry toxin stability analysis results showed that whilst several strains produced proteases capable of processing the scarab-specific toxin Cry8Ea, none were able to completely degrade it. These results suggest that gut bacteria can potentially affect the susceptibility of scarabs to *B. thuringiensis* and that this should be considered when considering future control measures.