develope in to the 4th stage larvae, that leaves slugs. Later they mature and reproduce in the soil. Despite the fact this nematode is a parasite of snails in helicicultre and also an invasive slug *Arion vulgaris* (syn. *A. lusitanicus*), that is one of the most serious pest in agriculture and horticulture, the knowledge about morphology and ecology of this nematode are very poor. We performed some studies of this nematode with a goal to provide new information about morphology, phylogeny and ecology of this species. This work brings, above all, the complete redescription of *A. appendiculatum*, include molecular biological characterisation suggesting high intraspecific variabily in ITS region. Results of ecological studies provided new information about the saprobic life cycle and natural prevalence, but also show that, in standard conditions, *A. appendiculatum* has very week influence on mortality and feeding activity of slugs *A. vulgaris*, while in other stressful conditions it might be an important agent controling population density. But we concede that this can be also strongly influenced by bacterial associates, even though the role of bacteria in nematode development is questionable.

Poster / Nematodes. Wednesday, 16:30. **NE-20**

Osmotic stress tolerance and infective juvenile production of entomopathogenic nematodes subject to fast host-desiccation treatments

Jaime Ruiz-Vega[1], Teodulfo Aquino-Bolaños[1], Juan R. Delgado-Gamboa[2] and Carlos I. Cortés-Martínez[2]
Becarios [1]COFAA-IPN y [2]PIFI-IPN
Laboratory of Biological Control, CIIDIR U. OAXACA, IPN; Calle Hornos 1003, Santa Cruz Xoxocotlan, Oax., México, 71230
Address for correspondence: jvega@ipn.mx

Entomopathogenic nematodes (EPN) are being used commercially in several countries for the control of soil dwelling pests. However, their effectiveness is affected by environmental stresses such as low soil moisture. An alternate method for ensuring nematode's survival and infectivity is to apply them in the cadavers of *Galleria mellonella* used to reproduce them. It has been reported that the IJ's emerging from cadavers have increased infectivity and higher tolerance to low soil moisture and high temperatures. To determine the optimum time post infection and intensity of desiccation for higher IJ's production and their effects on osmotic stress tolerance in these EPN a laboratory experiment was carried out. Our results showed that timing to start desiccation (2, 4 and 6 days post-infection) and intensity (1, 2 and 4 days in a desiccator) affected weight reduction, especially in *S. glaseri*, which resulted in higher death rates of the IJ's. The total number of nematodes, however, was not related to the opportunity or intensity of the stress treatments, but to nematode species and initial weight of the hosts. In an evaluation of survivorship in a 30 % PEG-8000 solution, pre-conditioned *Heterorhabditis bacteriophora* showed a significantly higher tolerance to osmotic stress than *Steinernema glaseri* and showed an increase in tolerance 100 % larger than the observed with the last nematode species. The higher percent of survivorship was obtained with IJ's from hosts where desiccation treatments initiated 2 days post-infection in both EPN.

Poster / Nematodes. Wednesday, 16:30. **NE-21**

Assessing entomopathogenic nematode population genetics: a research and teaching approach

Abigail Lewis, Logan Jefferson, Glen Stevens, Michaela Gazdik
School of Natural Sciences and Mathematics, Ferrum College, Ferrum, VA, USA
Address for Correspondence: gstevens3@ferrum.edu

While entomopathogenic nematodes (EPN) are important components of ecosystems, relatively little is known about the genetics of individual EPN populations in natural settings. We are combining an attempt to answer the question "How related are EPN found in natural settings?" with an integration of EPN into an undergraduate Genetics course module on population genetics. We used Random Amplified Polymorphic DNA (RAPD) approaches, and are working with lab maintained geographic isolates of EPN to identify appropriate primers and develop methodology. We have tested our technique by first assessing the genetic variability of a single geographic isolate of a single EPN species, and then exposed waxworms to a combination of geographic isolates of that species. We then assessed the genetic variability of the IJs that emerged from "mixed-isolate" waxworms. RAPD has been effective at identifying markers for individual geographic isolates, and for assessing the population genetics from "mixed-isolate" populations. RAPD is also a standard technique taught in Genetics labs, meaning that a high throughput of samples is possible and that undergraduates are exposed to real-world questions in the classroom. Once this technique has been fully developed for laboratory isolates, we plan to move this research effort into the local ('natural') environment, where we will answer the original question regarding the population genetics of local EPN isolates pre- and post-infection. This may improve our understanding of how natural populations are structured, and hopefully will provide insight that is relevant to the use of these organisms for biological control.

VIRUSES

Poster / Viruses. Wednesday, 16:30. **VI-1**

High-level Expression of Foreign Protein Using the Partial Polyhedrin-fused Baculovirus Expression System

Sung Min Bae[1]; Tae Young Shin[1]; Jae Bang Choi[1]; Yeon Ho Je[2]; Byung Rae Jin[3]; Soo Dong Woo[1]
[1]Department of Agricultural Biology, Chungbuk National University, Chungju, Korea; [2]Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Korea; [3]College of Natural Resources and Life Science, Dong-A University, Busan, Korea
Address for Correspondence: sdwoo@cbnu.ac.kr

Polyhedrin is the major component of the nuclear viral occlusions produced during replication of the baculovirus *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). To enhance the production efficiency of foreign protein in baculovirus expression system, the effects of various polyhedrin fragments were investigated by fusion expressing them with the enhanced green fluorescent protein (EGFP). Recombinant viruses were generated to express EGFP fused with polyhedrin fragments based on the previously reported minimal region for self-assembly and the KRKK nuclear localization signal (NLS). The marked increase of EGFP production by these fusion expressions was confirmed through protein and fluorescence intensity analyses. Among the fusion-expressed protein in nucleus and cytoplasm, the most hyper-expression was observed in the fusion of amino acids 19 to 110 and 32 to 59 of polyhedrin. The marked increase of production of several other foreign proteins was proved by the fusion expression with these polyhedrin fragments. This study suggests a new option for higher expression of useful foreign recombinant protein by fusion expression with the partial polyhedrin in baculovirus.

Poster / Viruses. Wednesday, 16:30. **VI-2**

A natural recombinant between *S. frugiperda* MNPV and *S. litura* NPV

Gloria Barrera[1], Laura Villamizar[1]; Manuel Alfonso Patarroyo[2], Oihane Simón[3], Primitivo Caballero[3], Mariano Belaich[4], Daniel Ghiringhelli[4]
[1]Centro de Biotecnología y Bioindustria (CBB), Corpoica, Bogotá, Colombia, [2]Fundación Instituto de Inmunología de Colombia (FIDIC), Bogotá, Colombia, [3]Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Navarra, España, [4]Laboratorio de Ingeniería Genética y Biología Celular y Molecular – Area Virosis de Insectos, Universidad Nacional de Quilmes, Argentina
Address for Correspondence: gbarrera@corpoica.org.co

A Colombian SfMNPV isolate (SfCOL) and its genotypic variant (SfCOL-A) have shown great potential as active ingredients for a biopesticide product to control the fall armyworm *S. frugiperda* in Colombia. The complete genomic sequence of SfCOL-A was determined and analyzed in the present study, consisting of 134,239 bp, encoding 144 putative open reading frames. Gene synteny maps showed great colinearity with genomes of other sequenced SfMNPVs. SfCOL-A genome displayed a ~1470 bp deletion localized within the main variable region among SfMNPV geographical isolates and their genotypic variants previously described. Interestingly, a ~2970 bp sequence block insertion, carrying two ORFs which lacked any similarity with previously described SfMNPV genes, was also found in this region.The highest identity values and codon usage similarity within the inserted sequence suggested the idea of a recombination event between SpltNPV-II (or a similar virus) and a wild type Colombian SfMNPV. Two bioinformatics approaches (relative similarity and bootscanning analysis) were used to explore the recombination hypothesis. Both analyses supported the hypothesis, showing a recombination event involving the C_{term} region of the *chitinase* ORF and the N_{term} region of the *gp37* ORF. This event resulted in the deletion of a genomic region including the *Homologous Region 3* (HR3) and the *Sf23* ORF; and an insertion of ~2970 bp carrying the splt020 and splt021 ORFs from SpltNPV-II. Breakpoints seemed to be localized within the frames of *chitinase* (near position 21,471) and *gp37* (near position 24,443) genes in SfCOL-A, restoring the integrity of both frames. These results suggested a natural recombination between heterologous baculoviruses involving genes that encode non-essential proteins and affect the viral phenotype.

Poster / Viruses. Wednesday, 16:30. **VI-3**

Host specificity and PIFs based phylogeny of Betabaculovirus isolates from Gelechiidae family

Juliana Gómez[1], Laura Villamizar[1]; Gloria Barrera[1]; Cecilia Turco[2], Mariano Belaich[2], Daniel Ghiringhelli[2]
[1]Centro de Biotecnología y Bioindustria (CBB), Corpoica, Bogotá, Colombia; [2]Laboratorio de Ingeniería Genética y Biología Celular y Molecular – Area Virosis de Insectos, Universidad Nacional de Quilmes, Argentina
Address for Correspondence: gbarrera@corpoica.org.co

Tecia solanivora, *Phthorimaea operculella* and *Tuta absoluta* are insect species belonging to the potato tuber moth complex (Lepidoptera: Gelechiidae) and are considered the main pests of potato and tomato crops. In this sense, baculovirus constitutes a useful tool for the biological control of these insects. In the viral cycle, a protein complex known as PIF (*Per Os* Infectivity Factors) are responsible for the virus entry into the mid gut cells determining the host range. In the present work the heterologous host infection of three viral isolates (recovered from moths of each insect species *T. solanivora, P. operculella* and *T. absoluta*) was determined by oral inoculation of each specie larvae with occlusion bodies (OBs). Infection by each of the three Granuloviruses in the three different host species produced a fatal disease. Additionally, comparative sequence analysis of *pif* genes was assessed. Seven pairs of degenerated primers were designed to amplify sequences of *p74*, *pif1*, *pif2*, *pif3*, *odv-e28*, *odv-e56* and *pif6*. The PCR products were cloned and sequenced. Comparative analysis of *pif* sequences of three isolates revealed high similarity with *Phthorimaea operculella* granulovirus (PhopGV) previously reported in the Genbank. The topology of phylogenetic tree using concatenated deduced aminoacid sequence of seven PIFs was consistent with previously published trees for Baculoviruses using three conserved genes or complete genomes. The three isolates evaluated were grouped with PhopGV. These results suggest a potential use of Granuloviruses isolated from different species of Gelechiidae family for biological control in heterologous species and showed the utility of *pif* (core genes) for phylogenetic studies in Baculoviruses.

Poster / Viruses. Wednesday, 16:30. **VI-4**

Diagnosing the unknown – advancing the taxonomy of aquatic invertebrate viruses

Kelly S. Bateman[1], Grant D. Stentiford[1] and Monique M. van Oers[2]
[1]European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK; [2]Laboratory of Virology, Wageningen UR, Radix Building, Droevendaalsesteeg 4, 6708 PB Wageningen, Netherlands.
Address for correspondence: kelly.bateman@cefas.co.uk

Few viruses from marine invertebrates, in particular crustaceans, have been assigned to a virus family with certainty because biochemical, biophysical and immunological data are incomplete or lacking, essentially due to the lack of crustacean cell cultures. Particularly, we have very limited information on viral infections in non-commercial crustacean species or in other invertebrates that may be living in the same environment. Crustacean viruses have so far been tentatively assigned to families based upon morphological and developmental characteristics and the location within the cell. The need to complete full characterisations and harmonise the naming of new viruses using International Committee on Taxonomy of Viruses (ICTV) guidelines is evident throughout the literature with many different names or abbreviations being used to describe the same virus. We present the identification of a virus infection from wild caught *Crangon crangon* (brown shrimp), the optimisation of viral purification techniques from these samples, and the application of next generation sequencing to characterise the viral genome. Similarities between crustacean viruses and those described in other invertebrates including insects may assist in classification of this novel virus. The data obtained will be also be used to develop a diagnostic tool.

Poster / Viruses. Wednesday, 16:30. **VI-5**

Proteomic analysis of the occluded Tipula oleracea nudivirus (ToNV)

Annie Bézier[1], Grégoire Harichaux[2], Julien Gaillard[3], Karine Musset[1], Valérie Labas[2], Elisabeth A. Herniou[1].
[1]Institut de Recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université François Rabelais, 37200 Tours, France; [2]Laboratoire de Spectométrie de masse, Plateforme d'Analyse Intégrative des Biomolécules et des Phénomique des Animaux d'Intérêt Bio-agronomique. UMR INRA 0085-CNRS 7247-UFR-IFCE, 37380 Nouzilly, France; [3]Laboratoire de Biologie Cellulaire, Microscopie Electronique, Faculté de Médecine, Université François Rabelais, 37032 Tours, France
Address for correspondence: annie.bezier@univ-tours.fr

The *Nudiviridae* family has been recently established by the International Committee on Taxonomy of Viruses (ICTV). Although six fully sequenced genomes are now available in databases and protein profile from nudivirus particles were mainly characterized by polyacrylamide gel electrophoresis, only few direct matches have been published between genomic and proteomic data to the exception of the major occlusion body protein (mOBp) from Penaeus monodon nudivirus (PmNV) and four nucleocapsid proteins from Helicoverpa zea nudivirus 2 (HzNV-2). Function of nudiviral predicted proteins is still inferred from what is known from their baculovirus sister-group and the occluded nature of virions remains incidental to the *Nudiviridae* family. Tipula oleracea nudivirus (ToNV) is one of the causative agents of crane fly nucleopolyhedrosis. The dsDNA virus genome was recently sequenced. Phylogenetic analysis revealed ToNV is related to Betanudivirus clade representatives and distantly related to another Diptera-infecting nudivirus representative, the Drosophila innubia nudivirus (DiNV). Electronic microscopies showed occlusion bodies are irregularly shaped and measure from 2 to 5 µm in length and 2 µm in mid-diameter. They are filled with rode-shape virions containing a single nucleocapsid within a tri-layered envelope. Quantitative proteomic analysis using on-line nanoflow liquid chromatography tandem mass spectrometry (nanoLC-MS/MS) revealed ToNV occlusion bodies are composed of 47 viral proteins, of which the most abundant are the functional homolog of baculovirus major occlusion bodies proteins and the homologs to two HzNV-2 predicted ORFs corresponding to virion structural proteins.

Poster / Viruses. Wednesday, 16:30. **VI-6**

Nucleopolyhedrovirus and Microsporidia in Winter Moth (*Operophtera brumata, L.*) and Bruce Spanworm (*O. bruceata, Hurst*) populations in the Northeast US

Hannah J. Broadley [1,2]; Joseph S. Elkinton [1,2]; John P. Burand[3]; Lina Tian [3]; Leellen F. Solter[4]
[1] Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts Amherst, USA; [2] Department of Environmental Conservation, University of Massachusetts Amherst, USA; [3] Department of Microbiology, University of Massachusetts Amherst, USA; [4] Department of Entomology, University of Illinois, USA
Address for Correspondence: hbroadley@cns.umass.edu

The winter moth (WM, *Operophtera brumata, L.*), a polyphagous geometrid affecting mainly deciduous tree species was accidently introduced to the Northeast United States from Europe in the 1990s. Although WM has been in a continuously outbreaking population since its introduction, the native congener, Bruce spanworm (BSW, *O. bruceata, Hurst*) rarely exhibits outbreaks. We propose that this difference in population dynamics exists because BSW is experiencing a different set of pathogens, which exist at a higher prevalence. Field collected WM and BSW larvae were reared in the lab and percent mortality was noted. Cadavers were examined microscopically for evidence of *Microsporidia* and nucleopolyhedrovirus (NPV) infections. DNA was extracted from BSW samples that were positive for NPV, and amplified by PCR to detect and characterize polyhedrin gene sequences. Of 433 BSW larvae, 51.5% did not survive to the pupal stage while only 1.1% of the 15,677 WM larvae died prior to pupation. BSW had a higher prevalence of Microsporidian infection than WM (63.0% compared to 3.3%) while WM experienced a high prevalence of NPV (93.3% compared to 14.1%). Polyhedrin sequence from BSW was only 88% identical to that of OpbrNPV, indicating that the NPV infecting these insect species are different. In conclusion, WM and BSW are experiencing different pathogens and at a different prevalence. Understanding the controls of epizootics on BSW may provide valuable insight into possible biological controls for WM.

Poster / Viruses. Wednesday, 16:30. **VI-7**

Regulation and activation of two effector caspases that affect Sindbis virus replication in *Aedes aegypti* mosquitoes

Ning Huang, A. Lorena Passarelli, and Rollie J. Clem
Division of Biology, Kansas State University, Manhattan, KS
Address for Correspondence: rclem@ksu.edu

The yellow fever mosquito (*Aedes aegypti*) proteins CASPS18 and CASPS19 are closely related effector caspases that are hypothesized to be involved in midgut escape of Sindbis virus (SINV). Silencing CASPS18 and 19 expression results in decreased SINV titer in *A. aegypti* following oral infection, while overexpression of CASPS19 by recombinant SINV in *A. aegypti* causes increased virus replication. Furthermore, levels of the midgut basal lamina proteins collagen IV and laminin are increased in infected mosquitoes when CAPS18/19 are silenced, and decreased by overexpression of CASPS19, consistent with a role in midgut escape. CASPS18 lacks a typical caspase active site motif (QACRG) and has no enzymatic activity, but is able to directly enhance the activity of CASPS19. To investigate the mechanism of enhancement, we examined whether the two proteins interact and found that CASPS18 co-immunoprecipitated with CASPS19 when expressed in Sf9 cells. Under these conditions, both CASPS18 and 19 underwent a proteolytic processing event that released the small subunit. The intact CASPS19 catalytic site was required for processing of both proteins. Recombinant purified CASPS18 enhanced the activity of purified active CASPS19 in vitro, but was not able to induce the activation of unprocessed CASPS19, indicating that the enhancement of CASPS19 activity by CASPS18 occurs after CASPS19 activation. Recombinant CASPS19, alone or with CASPS18, could not directly cleave collagen IV or laminin, suggesting that the effect of these caspases on the levels of midgut basal lamina proteins is not through direct cleavage, but may instead be an indirect effect.

Poster / Viruses. Wednesday, 16:30. **VI-8**

Proteomic analysis and *in vivo* diferential gene expression of *Trichoplusia ni* granulovirus (TnGV)

Angeles Bivián Hernández; Ingrid Zanella-Sainz;
Paloma Dávila-Alvarez, J. Eleazar Barboza-Corona;
Fabiola León-Galván; M. Cristina Del Rincón-Castro
Food Department, Division of Life Sciences, University of Guanajuato, Irapuato, Gto. México
Address for Correspondence: cdelrincon@ugto.mx

Nucleopolyhedrovirus (NPV) and *Granulovirus* (GV) infections on *Trichoplusia ni* larvae is regulated by the expression of the virus genes. There are very few reports about the differential expression of the baculovirus genes in the host. This report deals with the proteomic analysis to detect the differential expression of viral genes. Also, macroarrays were prepared to analyze total proteins from infected *T. ni* larvae with the granulovirus TnGV, which were compared with those from non-infected larvae, obtained at different post-infection (p.i.) periods. When the expressed proteins from infected and non-infected larvae were compared, no significant change in the protein pattern was observed at 24 hs p.i.; however, when compared at 48, 72, 90, and 120 hs p.i., differential protein bands were detected in the infected larvae, not present in the non-infected larvae. Additionally, substractive libraries were constructed in order to identify those genes expressed differentially at different p.i. periods. Libraries were obtained with 36, 21, 16, 13, and 23 clones at 24, 48, 72, 96, and 120 hs p.i., respectively. In these macroarrays a decrease of the hybridization intensity was observed as the p.i. periods were increasing. This observation may suggest that, due to the TnGV infection in the *T. ni* larvae tissues, the expression of normal proteins of the host decreased. That is, there might be an expression repression of the larval

genes. This report it sets the bases to understand the induction and repression mechanisms of the insect genes, when a GV infection occurs..

Poster / Viruses. Wednesday, 16:30. **VI-9**

Recombinant Iridovirus IIV-6 expresing the Cn-10 neurotoxin from *Centruroides noxius* scorpion

Flor C. Arellano-Villagómez[1]; Jorge E. Ibarra[2]; M. Cristina Del Rincón-Castro[1]
[1]Food Department, Division of Life Sciences, University of Guanajuato, Irapuato, Gto. México. [2]CINVESTAV-IPN Unidad Irapuato, Irapuato, Gto. México
Address for Correspondence: cdelrincon@ugto.mx

Was established the methodology to obtain a recombinant Iridovirus *in vivo,* using the microparticle bombardment. Genes encoding for the green fluorescent protein GFP, and the protein Cn10, was cloned into the 295L gene Iridovirus, strain IIV -6. Were standardized optimal conditions for micro-projectile bombardment cotransfection of the vector DNA TOPO-295L-GFP- Cn10 and wild DNA from Iridovirus IIV-6, being the ratio of 3:1 (vector: wild DNA) the more useful for obtained recombinant Iridovirus. Recombinant Iridovirus IIV-6, was obtained by co-transfection of vector DNA TOPO-295L-GFP-Cn10 and wild DNA Iridovirus IIV-6, using the technique of biolistic to co-infecting *Galleria mellonella* larvae. This recombinant Iridovirus expressed both proteins, the GFP and Cn10. Furthermore, using fluorescent microscopy, were detected a green fluorescent staining in few portions of fat tissue of *G. mellonella* larvae. The potential expression of GFP and Cn10 proteins, was corroborated by SDS-PAGE, restriction analysis and PCR. This is the first report of the production of a recombinant Invertebrate Iridovirus, expressing a reporter gene (GFP) and a virulence gene (Cn10) and represents a model system for the genetic improvement of Invertebrate Iridovirus. More studies are needed at the molecular level, such as the sequencing of the genome of recombinant Iridovirus IIV-6 and performing Western Blot tests to detect, for one hand, the insertion of both genes (GFP and Cn10) into the genome of IIV-6 Iridovirus, and on the other hand, to verified the correct expression of both proteins in the tissues of the infected insect larvae..

Poster / Viruses. Wednesday, 16:30. **VI-10**

Genomic sequencing and analysis of Sucra jujuba nucleopolyhedrovirus

Xiaoping Liu, Feifei Yin, Zheng Zhu, Dianhai Hou, Jun Wang, Lei Zhang, Hualin Wang, Zhihong Hu, Fei Deng
State Key Laboratory of Virology, Virus Resource and Bioinformatics Center, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P.R. China
Address for Correspondence: df@wh.iov.cn

The complete nucleotide sequence of Sucra jujube nucleopolyhedrovirus (SujuNPV) was determined by 454 pyrosequencing. The SujuNPV genome was 135,952 bp in length with an A+T content of 61.34%. It contains 131 putative open reading frames (ORFs) covering 87.9% of the genome. Among these ORFs, 37 were conserved in all completely sequenced baculovirus genomes, 25 conserved in lepidopteran baculoviruses, 64 were found in other baculoviruses, and 5 were unique to SujuNPV genome. Seven homologous regions (*hrs*) were identified in the SujuNPV genome which can be classified into two groups. SujuNPV was identified to contain several duplicated or multiple copy genes, as it contains two copies of helicase, DNA binding protein gene (*dbp*) and *cg30,* 3 copies of inhibitor of apoptosis gene (*iap*), and 4 copies of baculovirus repeated ORF (*bro*). Phylogenetic analysis suggest that SujuNPV belongs to a subclade of group II alphabaculovirus, interestingly different from other baculoviruses, all the nine members of this subclade contain a second copy of *dbp*.

Poster / Viruses. Wednesday, 16:30. **VI-11**

Functional analysis of exonuclease gene (012L) of *Chilo* iridescent virus

Yeşim Aktürk Dizman[1,2], Cemal Sandallı[2], Zihni Demirbağ[2] and Remziye Nalçacıoğlu[1]
[1]Karadeniz Technical University, Faculty of Sciences, Department of Biology, Trabzon, Turkey [2]Recep Tayyip Erdoğan University, Faculty of Arts and Sciences, Department of Biology, Rize, Turkey
Address for Correspondence: akturkyesim_53@hotmail.com

Chilo iridescent virus (CIV) encodes an open reading frame (ORF 012L) homologous to exonuclease II of *Schizo-saccharomyces pombe*. In the current study, we focused on the characterization of *exonuclease* gene of CIV. The target gene was cloned into the pET28a vector, expressed in *E. coli* strain BL21 (DE3) Lys with an N-terminal His tag and purified to homogeneity by using Ni-NTA affinity chromatography. Biochemical characterization of the purified CIV-exonuclease protein (CIV-Exo) confirmed that this viral protein is a functional 5'-3' exonuclease that digests 3'-biotin-labelled oligonucleotides and linear double-stranded DNA molecules from their 5'-termini in a highly processive manner. CIV-Exo has also a potent endonuclease activity in vitro. The CIV-Exo converted supercoiled plasmid DNA (replicative form I, RFI) into the open circular form (RFII) and then open circular form into linear form (RFIII). Both exonuclease and endonuclease activities of CIV-Exo are optimal at pH 8.0 in the presence of 10 mM $MgCl_2$, 2 mM dithiothreitol and 100 µg BSA ml^{-1}.

Poster / Viruses. Wednesday, 16:30. **VI-12**

Identification of a new multiple nucleopolyhedrovirus isolated from the Jasmine moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) in Egypt

Regina G. Kleespies[1], Yongjie Wang[2], Said El Salamouny[3], Mona Awad[3], Essam Agamy[3], Ramadan Salama[3] and Johannes A. Jehle[1,2]
[1]Institute for Biological Control, Julius Kühn Institute, Darmstadt, Germany; [2]Agricultural Service Station Palatinate, Neustadt/Weinstr., Germany; [3]Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt
Address for Correspondence: ssalamo@clemson.edu

A new multiple nucleopolyhedrovirus was isolated from diseased larvae of the jasmine moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) in Egypt. The virus caused typical symptoms of a baculovirus infection, and it was possible to propagate the causative agent in larvae of the homologous host. Light microscopy studies showed polyhedral occlusion bodies (OBs). Electron microscopy of ultrathin sections of polyhedral OBs showed multicapsid virions identifying the virus as a multiple embedded nucleopolyhedrovirus. Therefore, this virus was termed *Palpita unionalis* multiple nucleopolyhedrovirus (PaunNPV). The identity of the isolated virus was confirmed by sequencing of a 452 bp fragment of the *polyhedrin (polh)* gene that was amplified using degenerate primers. Blast search showed that it was closely related to *polh* genes in *Dirphia peruvianus* NPV, *Pterolocera amplicornis* NPV, and *Nepytia phantasmaria* NPV. A neighbour-joining phylogenetic tree was constructed based on the predicted amino acid sequences of the *polh* genes of the selected closely related NPVs. Phylogenetic distances suggested that PaunNPV should be considered to belong to a novel species within the genus *Alphabculovirus*. Preliminary bioassay data showed that the virus was active against either 2nd or 4th instars of jasmine moth. The calculated

LC_{50} was $1.3x10^3$ and $3.1x10^3$ OBs/ml for the tested 2nd and 4th instars, respectively. The study reports a new baculovirus that might be used as a promising agent for biological control of the jasmine moth.

Poster / Viruses. Wednesday, 16:30. **VI-13**

A single baculovirus for the production of recombinant Adeno-Associated Virus 8 vectors

Lionel Galibert; Aurélien Jacob; Bérangère Bertin; Marjorie Boutin Fontaine; Delphine Bonnin; Christophe Lecomte; Christel Rivière; Otto-Wilhelm Merten
Genethon, 1bis, rue de l'Internationale, 91002 Evry, France
Address for correspondence: galibert@genethon.fr

We have developed a single baculovirus, named "Monobac", for the production of recombinant Adeno-Associated Virus vectors of serotype 8 (rAAV8) using the Sf9 cell/baculovirus system. In an AcMNPV bacmid devoid of the *chitinase* and *cathepsin* a genes, the AAV *rep2* and *cap8* genes have been inserted at the *egt* locus, while the recombinant AAV was cloned in the Tn7 site. This system was used for the production rAAV8 encoding the human γ-sarcoglycan gene, of clinical interest for the treatment of LGMD2C (γ-sarcoglycanopathy) myopathy disease. Enhanced rAAV8 productivity was observed in the cell culture and was maintained after purification, compared to production system based on the use of 2 baculoviruses. The produced rAAV8 capsids displayed a reduced degradation profile of the capsid proteins VP1/VP2 due to the elimination of the baculovirus *cathepsin* protease gene. This optimized system allows the production of an improved quantity of rAAV vectors with improved vector quality, resulting in enhanced infectivity of the rAAV. .

Poster / Viruses. Wednesday, 16:30. **VI-14**

Determining the role of P10 during baculovirus infection through the development of novel mutants in *Autographa californica* multicapsid nucleopolyhedrovirus

Leo Graves[1], Farheen Raza[1], Sarah L. Irons[1]; Robert D Possee[1,2] & Linda A King[1]
[1]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford UK, [2]Oxford Expression Technologies Ltd, Oxford, UK.
Address for Correspondence: 12083983@brookes.ac.uk

P10 is a fibrous protein that forms complex networks of filaments and a distinct perinuclear tubular structure around the nucleus during the later stages of infection of cells with baculovirus. Previous research has suggested possible roles of P10 in nuclear stability, polyhedron formation and cell lysis, but distinct functional roles for the protein have yet to be determined. In order to investigate the role of P10 during infection, a variety of *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) mutants have been constructed, which include a *p10* deletion and associated rescue virus, phosphorylation mutants and a virus in which the AcMNPV *p10* coding region has been replaced with that from *Spodoptera frugiperda* (Sf)NPV. Mass spectrometry was used to confirm the phosphorylation of P10 serine 93. Mutation of serine 93 to alanine affected the structure of P10 tubules as evidenced by confocal microscopy. The distinctive tubular structure surrounding the nucleus that is observed in wild-type virus infected cells failed to form correctly. Circular dichroism analysis confirmed a distinct change in the protein secondary structure. These data suggest that phosphorylation plays a key role in P10 function. Replacement of the AcMNPV *p10* coding region with that from SfNPV resulted in a virus with low budded virus titre and aberrant rearrangement of microtubules in comparison to AcMNPV-infected cells, suggesting that the SfNPV P10 may be affecting microtubules and translocation of nucleocapsids to the plasma membrane for budding.

Poster / Viruses. Wednesday, 16:30. **VI-15**

Evaluation of the transcriptional transactivation of betabaculovirus regulatory elements in transformed cell lines by alphabaculovirus transcription factors

Santiago Haase[1], M. Leticia Ferrelli[1]; Matías L. Pidre[1]; Alicia Sciocco-Cap[2], Víctor Romanowski[1]
[1]IBBM-UNLP-CONICET, La Plata, AR; [2]IMyZA-INTA, Castelar, AR;
Address for Correspondence: santiagohaase@biol.unlp.edu.ar

The narrow host range of baculoviruses is one of their advantages for sustainable pest control of a single insect species with minimal or no effect on non-target organisms. However, the limited host range may be less desirable from the economical point of view since more than one pest can be present at the same time on most crops. Engineering baculovirus genomes with an expanded host range by design would be an answer to this type of scenarios. Nevertheless, genetic determinants of host range have not been widely characterized and the mechanisms of host recognition are still not well understood. In this context, the generation of hybrid or chimaeric baculoviruses may be an empirical approach to generate viruses with expanded host range. The expression of viral genes in this context will require the transcriptional transactivation of their promoters by heterologous transcription factors (TFs). However, the recognition of baculovirus promoters in different species has not been systematically studied so far. The aim of our work is to evaluate the transcriptional transactivation of betabaculovirus promoters by alphabaculovirus TFs. It has been noted before that late promoters require the replication of the DNA to be activated. Therefore, we generated stably transformed cell lines expressing the red fluorescent protein (DsRed) as a reporter gene under the control of immediate-early, early and late gene promoters of the Anticarsia gemmatalis nucleopolyhedrovirus (AgMNPV) and Epinotia aporema granulovirus (EpapGV), respectively. These cell lines were infected with AgMNPV to evaluate the transcriptional transactivation of these promoters. Our results showed that the AgMNPV transcription factors activate early and late EpapGV promoters.

Poster / Viruses. Wednesday, 16:30. **VI-16**

***Enhancin Genes of Lymantria dispar* NPV Do Not Increase Potency Via Metalloprotease Activity**

Kelli Hoover[1], James Slavicek[2], Algimantas P. Valaitis[2,3], Nancy Hayes-Plazolles[2], and Elizabeth McCarthy[1]
[1] Department of Entomology, Penn State University, University Park, PA USA, [2] USDA Forest Service, Delaware, OH USA, [3]Retired
Address for Correspondence: kxh25@psu.edu

The baculovirus encoded enhancins characterized so far are metalloproteases that digest proteins in the peritrophic matrix (PM) of their host midgut, increasing viral potency in many systems. *Lymantria dispar* NPV has two *enhancin* genes (E1 and E2) that are distributed in the ODV envelopes, placing them in a position to interact with the PM and the and possibly midgut cells. Deletion of either *enhancin* or both reduces viral potency 2-fold and 12-fold, respectively, compared to wildtype. Removal of the PM with optical brightener treatment did not alter these differences in potencies, suggesting that the enhancins do not affect the PM. The results of an *in vitro* PM digestion assay found that although the PM was degraded, it was not affected by inhibitors of metalloproteases, whereas treatment with serine protease inhibitors showed little or no PM degradation. Mutant LdNPV viruses were generated by altering the region that encodes the zinc binding site of the metalloprotease; this region of E1 and E2 was either deleted or altered by homologous amino

acid substitution to attempt to retain a functional enzyme. Bioassays showed that the deletion or alteration of just the zinc binding site of the metalloprotease, but not the entire enhancin gene, did not change viral potency. For example, a construct with E1 deleted/E2zinc modified had the same potency as E1deleted/E2 intact. These results suggest that the enhancins of LdNPV do not improve viral potency through the activity of metalloproteases, but appear to have a different mechanism, which has yet to be identified..

Poster / Viruses. Wednesday, 16:30. **VI-17**

A Cypovirus VP5 Displays the RNA Chaperone-like Activity that Destabilizes RNA Helices and Accelerates Strand Annealing

Jie Yang, Jiamin Zhang, Yuehua Kuang and Yuanyang Hu
State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, China.
Address for Correspondence: yyhu@whu.edu.cn

For double-stranded RNA (dsRNA) viruses in the family Reoviridae, their inner capsids function as the machinery for viral RNA (vRNA) replication. Unlike other multishelled reoviruses, cypovirus has a single-layered capsid, thereby representing a simplified model for studying vRNA replication of reoviruses. VP5 is one of the three major cypovirus capsid proteins and functions as a clamp protein to stabilize cypovirus capsid. Here, we expressed VP5 from Helicoverpa armigera cypovirus-5 (HaCPV-5) in a eukaryotic system and determined that this VP5 possesses RNA chaperone-like activity, which destabilizes RNA helices and accelerates strand annealing independent of ATP. Our further characterization of VP5 revealed that its helix-destabilizing activity is RNA specific, lacks directionality, and could be inhibited by divalent ions, such as Mg2+, Mn2+, Ca2+ or Zn2+, to varying degrees. Furthermore, we found that HaCPV-5 VP5 facilitates the replication initiation of an alternative polymerase (i.e. reverse transcriptase) through a panhandle-structured RNA template, which mimics the 5'-3' cyclization of cypoviral positive-stranded RNA. Given that the replication of negative-stranded vRNA on the positive-stranded vRNA template necessitates the dissociation of the 5'-3' panhandle, the RNA chaperone activity of VP5 may play a direct role in the initiation of reoviral dsRNA synthesis..

Poster / Viruses. Wednesday, 16:30. **VI-18**

A recombinant *Autographa californica* nucleopolyhedrosis virus expressing a Cyt1A/GFP chimera in *Trichoplusia ni* larvae

Miguel A. Salas-Marina [1], Cristina Del Rincón-Castro [2] and Jorge E. Ibarra [1]
[1]CINVESTAV-Irapuato, Irapuato, GTO, Mexico; [2]División de Ciencias de la Vida, Universidad de Guanajuato, Irapuato, GTO., Mexico
Address for Correspondence: jibarra@ira.cinvestav.mx

A novel approach was followed in order to achieve the expression of the Cyt1A toxin of *Bacillus thuringiensis* in a recombinant strain of AcNPV, trying to increase its virulence. First, the reporter GFP protein was used as a means to identify recombinant viruses; and second, biolistisc was used to achieve co-transfection. The recombinant construction pAccyta-GFP, containing the *B. thuringiensis* gene *cyt1A* fused with the GFP gene under the control of the p10 promoter from the pacuW31 vector was generated. Successful co-transfection by biobalistics was achieved with the AcNPV genome, when neonate *Trichoplusia ni* larvae were bombarded with DNA-coated gold micro-projectiles. Treated larvae showed the typical NPV infection symptoms, although only a thorough inspection detected fluorescent points in the fat body. Microscopic corroboration indicated that a recombinant AcNPV (AcNPV-cyt1a-GFP) was generated, showing glowing polyhedra in the infected cells, under fluorescence microscopy. This observation may indicate that the putative chimeric protein is incorporated into the polyhedron structure during its integration. Interestingly, the nuclei holding the recombinant polyhedra appeared less compact than those holding the wild-type polyhedra. A series of purification cycles of AcNPV-cyt1a-GFP rendered larvae showing fluorescence throughout the whole body. Preliminary observations indicate that AcNPV-cyt1a-GFP kills larvae faster than the wild-type strain; however, accurate LT_{50}s are still to be estimated.

Poster / Viruses. Wednesday, 16:30. **VI-19**

iLOV baculovirus: Using a novel small fluorescent protein for imaging virus proteins during infection

Farheen Raza[1], Sarah Irons[1], Leo Graves[1], Stan Botchway[2], Robert Possee[1,3], Linda King[1]
[1] Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK; [2] Central Laser Facility, STFC, Harwell, UK; [3] Oxford Expression Technologies, Oxford, UK
Address for Correspondence: sarahirons@brookes.ac.uk

Imaging of living cells is now a common place approach in cell biology and virus research, however addition of conventional fluorescent proteins such as green fluorescent protein (GFP) and its derivatives, can lead to alterations in the location and behaviour of target proteins. Such problems in mis-targeting have previously been observed on fusion of GFP to *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) P10, thus hindering imaging of P10 dynamics during virus infection of insect cells. Here we report use of iLOV, a small (~11kDa) genetically encoded fluorescent protein based on the LOV domain of plant phototropin 2, fused to P10 in AcMNPV. Expression of the P10-iLOV fusion during infection showed presence of filaments and nuclear structures, comparable to those seen in previous immunofluorescence images. We have also looked at the fluorescence lifetime of iLOV in P10 structures, where we established that the P10-iLOV fusion shows a very long fluorescence decay of ~4ns, compared to ~2.5ns for GFP. This work shows the successful use of iLOV in the baculovirus system, and provides an opportunity to tag proteins where GFP has previously failed. In addition the long fluorescence lifetime makes iLOV a promising candidate for use in protein interaction studies using Förster resonance energy transfer-fluorescence lifetime imaging (FRET-FLIM).

Poster / Viruses. Wednesday, 16:30. **VI-20**

Expression analysis of the *nsd-2* gene encoding the putative densovirus receptor in the midgut

Katsuhiko Ito [1], Hiroko Tabunoki [1], Takeshi Yokoyama [1], Keiko Kadono-Okuda [2]
[1]Tokyo University of Agriculture and Technology, Tokyo, Japan; [2] National Institute of Agrobiological Sciences, Ibaraki, Japan
Address for Correspondence: katsuito@cc.tuat.ac.jp

Bombyx mori densovirus type 2 (BmDNV-2) is a pathogen that replicates only in the midgut columnar cells and causes fatal disease in the silkworm. The resistance to BmDNV-2 is determined by a single gene, *nsd-2*, which is characterized as non-susceptibility irrespective of the viral dose. Previously we have identified *nsd-2* by positional cloning and found that this gene encodes a putative amino acid transporter which might work as a receptor for BmDNV-2. In this study, we investigated the relationship between the part of the midgut expressing *nsd-2* and the BmDNV-2 infection. To investigate the expression pattern of *nsd-2* in the midgut, we divided the midgut into three parts, anterior, middle, and posterior part, and performed the RT-PCR analysis with total RNA isolated from each part. *nsd-2* transcript

was strongly expressed in the posterior part of the midgut. However the expression levels of *nsd-2* were very low or no-detection in the anterior and middle parts. This regional expression pattern of *nsd-2* was common to all the investigated silkworm strain. On the other hand, the BmDNV-2-derived transcript was clearly detected in the posterior part of the midgut, but significantly lower in the anterior and middle parts. These results suggested that BmDNV-2 infection depended on the expression levels of *nsd-2* in the midgut. In insects, there is little information regarding the host's own factors in virus infection, therefore, we expect that our result will contribute to understanding the infection mechanism of insect virus. .

Poster / Viruses. Wednesday, 16:30. **VI-21**

Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua*

Agata K. Jakubowska [1]; Melania D´Angiolo [1]; Rosa M. González Martínez [1]; Anabel Millán Leiva[1]; Arkaitz Carballo [2;] Rosa Murillo [2]; Primitivo Caballero [2]; Salvador Herrero[1]
[1]Department of Genetics, Universitat de València, Dr Moliner 50, 46100 Burjassot, Spain; [2] Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, 31192 Mutilva Baja, Navarra, Spain
Address for Correspondence: sherrero@uv.es

Viral covert infections in invertebrates have been traditionally attributed to sublethal infections that did not reach enough viral titer to establish an acute infection. Recent studies are revealing that, although true for some viruses, other viruses may follow the strategy of establishing covert or persistent infections without producing the death of the host. In the last years, a large number of viruses causing covert infections in all type of hosts have been identified, mostly due to the revolution in the sequencing technologies. The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae) is a worldwide pest that causes significant loses to agricultural and ornamental plant industries. A comprehensive transcriptome analysis of the larval stage of *S. exigua* revealed the presence of an important number of unigenes belonging to novel RNA viruses, most of them from the order *Picornavirales*. In order to characterize *S. exigua* viral complex, we have completed the genomic sequences of three picorna-like viruses, two of them representing new members of the family *Iflaviridae* and a third one defining a new family. Additional studies have been performed to determine their morphology, infectivity, tissue distribution and abundance in the larval hosts. Influence of these viruses on the insect fitness as well as their effect on other viral and bacterial entomopathogens used for the control of this pest is also discussed.

Poster / Viruses. Wednesday, 16:30. **VI-22-STU**

A novel baculovirus-derived promoter with high activity in the Baculovirus Expression System

Maria Martinez-Solis [1]; Silvia Gomez-Sebastian [2]; Jose M Escribano [3]; Agata K. Jakubowska [1]; Salvador Herrero [1]
[1] Department of Genetics, Universitat de Valencia, Burjassot, Spain; [2] Alternative Gene Expression S.L. (ALGENEX), Centro Empresarial, Parque Científico y Tecnológico de la Universidad Politécnica de Madrid, Campus de Montegancedo, 28223 Pozuelo de Alarcón, Madrid, Spain; [3] Departamento de Biotecnología, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Autovía A6, Km 7.5, 28040 Madrid, Spain
Address for Correspondence: maria.martinez-solis@uv.es; sherrero@uv.es

In this work, we describe a novel baculovirus promoter for heterologous protein expression in insect cells using the baculovirus expression system. The promoter sequence is derived from the *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) genome and it was identified as potential promoter after transcriptional studies of the SeMNPV interaction with its host. First, an open reading frame (ORF) of SeMNPV was identified between the most highly abundant sequences in the transcriptome of *S. exigua* larvae infected with SeMNPV. Moreover, microarray-derived data showed high transcriptional activity of that ORF at different time points during the infective process. Different regions upstream of that ORF were tested for their promoter activity in the AcMNPV baculovirus expression system. Their ability to drive the expression of the GFP protein was compared against the polyhedrin (polh) conventional promoter in different cell lines, Sf21, Hi5, and Se301 and larvae from *S. exigua* and *Trichoplusia ni*. Although we found high levels of GFP expression with several regions, the strongest promoter activity was defined by 120 nt upstream the translation start site. GFP expression was up three times higher than the expression obtained with the polh promoter. Additionally, we also tested the activity for the combination of this sequence of 120 nt with the polh promoter revealing an additive effect over the polh promoter activity. This new promoter improves the conventional baculovirus expression system, allowing a considerable increase in the ability of producing large quantity of recombinant protein.

Poster / Viruses. Wednesday, 16:30. **VI-23**

Construction and Characterization of a Recombinant Invertebrate Iridovirus

Arzu Ozgen[1], Hacer Muratoglu[2], Zihni Demirbag[1], Just M. Vlak[3], Monique M. van Oers[3], Remziye Nalcacioglu[1]
[1]Karadeniz Technical University, Faculty of Science, Department of Biology, 61080, Trabzon, Turkey; [2]Karadeniz Technical University, Faculty of Science, Department of Molecular Biology and Genetics, 61080, Trabzon, Turkey; [3]Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
Address for Correspondence: remziye@ktu.edu.tr

This study describes the construction and characterisation of a recombinant Chilo iridescent virus (family *Iridoviridae*) encoding the green fluorescent protein (GFP). We showed that homologous recombination is a valid method to make CIV gene knockouts and to insert foreign genes. The CIV 157L gene, putatively encoding a non-functional inhibitor of apoptosis (IAP), was chosen as target for foreign gene insertion. The *gfp* open reading frame preceded by the viral *mcp* promoter was inserted into the 157L locus by homologous recombination in *Anthonomus grandis* BRL-AG-3A cells. Recombinant virus (rCIV-Δ157L-*gfp*) was purified by successive rounds of plaque purification it was confirmed by PCR, sequencing and restriction analysis. One-step growth curves for recombinant and wild-type CIV were similar. Also slot blot analysis showed that DNA's of both recombinant and wild-type CIV started replication at the same time. Hence, CIV157L can be inactivated without altering the replication kinetics of the virus. Consequently, the CIV 157L locus can be used as a site for insertion of foreign DNA, e.g. to modify viral properties for insect biocontrol.

Poster / Viruses. Wednesday, 16:30. **VI-24**

RNA interference and insect-virus interactions

David Neunemann; David G. Heckel, Heiko Vogel
Max Planck Institute for chemical ecology, Jena Germany
Address for Correspondence: dneunemann@ice.mpg.de

Gene silencing via dsRNA has become a powerful tool to explore functional genomics in a wide variety of eukaryotic organisms.

However, RNA interference (RNAi) especially in Lepidoptera is not straight-forward and as efficient as in other insects and it is difficult to establish robust methods. So far, several potentially limiting factors for RNAi in Lepidoptera have only been proposed for *Bombyx mori*. An important role in the somewhat random success of RNAi in Lepidoptera could be the tissue-specific gene silencing effects, and also how the dsRNA is delivered to that tissue. To address this highly variable RNAi efficiency, we focused on the RNAi pathway (miRNA-pathway and siRNA-pathway) genes, and genes related to dsRNA transport or spreading in the Lepidopteran *Helicoverpa armigera* and *Heliothis virescens.* When analyzing RNAi-related gene expression levels in different larval tissues, we found that R2D2 is transcribed at very low levels in all tissues except testes, whereas Loquacious is transcribed at very high levels in all tissues. These results suggest that, despite appropriate design, dsRNAs could fail to enter the siRNA pathway, and to knock-down genes of interest due to the observed very low levels of R2D2. As the siRNA pathway is also known as the "antiviral pathway" and defends the organism against RNA and DNA viruses, we also aim at analyzing RNAi related genes (in vivo and in vitro) to an infection with *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Helicoverpa armigera* single nucleopolyhedrovirus (HaSNPV) - both as wild type and modified forms.

Poster / Viruses. Wednesday, 16:30. **VI-25**

Studies on existing and new isolates of *Cryptophlebia leucotreta* granulovirus (CrleGV) on FCM populations from a range of geographic regions in South Africa

John K. Opoku-Debrah [1,4]; Martin Hill [1]; Sean Moore [1,2]; Caroline Knox [3]
[1]Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa; [2]Citrus Research International, P. O. Box 20285, Humewood, Port Elizabeth, 6013, South Africa.; [3] Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa; [4]River Bioscience (Pty) Ltd, P.O. Box 20388, Humewood, Port Elizabeth, 6013, South Africa
Address for Correspondence: papajohny@gmail.com

Considering the possibility of some geographic populations of the false codling moth, *Thaumatotibia leucotreta* (Meyrick) developing a reduced susceptibility to the baculovirus biopesticides, Cryptogran and Cryptex, as was the case with codling moth (CM), *Cydia pomonella* (L.) to the codling moth virus (CpGV) in Germany, the search for new isolates of the *T. leucotreta* baculovirus (CrleGV) become eminent. Here we report on the successful induction of a latent baculovirus infection in five geographic populations of *T. leucotreta* and the subsequent recovery of five new CrleGV isolates. These include the Ado, Cit, Mbl, Nels and MixC isolates. These isolates were shown to be genetically different from each other and from the commercial isolates, Cryptex and Cryptogran, using restriction enzyme analysis. The new isolates have been named CrleGV-SA Ado, CrleGV-SA Cit, CrleGV-SA Mbl, CrleGV-SA Nels and CrleGV-SA Mix isolates. Sequence analysis of the *granulin* and *egt* genes of all isolates revealed single nucleotide polymorphisms (SNPs) in both genes. Significantly, SNPs in the *egt* genes of these isolates resulted in a change in amino acid sequence. DNA profiles from RFLPs, as well as phylogenetic analysis based on *granulin* and *egt* sequencing showed the presence of two CrleGV-SA genome types. Cryptex and CrleGV-SA Ado, CrleGV-SA Cit, CrleGV-SA Mbl and CrleGV-SA Mix have been placed as members of Group one CrleGV-SA, and Cryptogran and CrleGV-SA Nels isolate placed into Group two CrleGV-SA. Studies on the comparative biological activity of the isolates also revealed significant differences between the relative potencies of the viral isolates against *T. leucotreta* from the Ado and MixC colonies..

Poster / Viruses. Wednesday, 16:30. **VI-26**

Effects of the baculovirus fibroblast growth factor on Sindbis virus replication

Wenbi Wu, Rollie J. Clem, and A. Lorena Passarelli
Division of Biology, Kansas State University, Manhattan, KS 66506, USA
Address for Correspondence: lpassar@ksu.edu

Fibroblast growth factors (FGFs) are conserved among vertebrate and invertebrate organisms and function in cell proliferation, cell differentiation, tissue repair, and development. Many baculoviruses encode functional viral fibroblast growth factor (vFGF) homologs that stimulate cell motility of insect cells and activate host FGF receptors. During baculovirus infection of midgut lepidopteran cells, expression of vFGF leads to caspase activation and remodeling of tracheal epithelial cell basal lamina. Tracheal cell basal lamina remodeling results in structural discontinuities that allow baculovirus midgut escape. We hypothesized that vFGF would assist in midgut escape of the arbovirus Sindbis virus (SINV) during infection of mosquitoes. We first verified that vFGF stimulated cell motility in two mosquito cell lines, C6/36 and Aag2. Utilizing an alphavirus transducing system for SINV, we then constructed recombinant SINVs expressing *vfgf* (MRE/vFGF, TE/vFGF), and control viruses with the same insert in antisense orientation (MRE/vFGFas, TE/vFGFas). TE-based viruses replicate in cell cultures but poorly infect mosquito midguts, while MRE-based viruses infect midguts efficiently. Replication of each vFGF-expressing virus and its control virus was similar in both cell lines. Female *Aedes aegypti* mosquitoes orally infected with each of the recombinant viruses had no significant replication differences, measured by determining infectious viruses in individual mosquitoes, mosquito midguts, or carcasses. Thus, it does not appear that expressing vFGF affects SINV replication and dissemination.

Poster / Viruses. Wednesday, 16:30. **VI-27**

Sensitivity and vertical transmission of nuclearpolyhedrovirus in various populations of gypsy moth *Lymantria dispar*

Olga Polenogova [1], Alexandr Ilyinykh [1], Dmitriy Kurenschikov [2], Philipp Ilyinykh [3], Elena Imranova [2], Alexandr Baburin [2]
[1] Institute of Systematics and Ecology of Animals Siberian Branch of Russian Academy of Sciences, Novosibirsk, RUSSIA; [2] Institute of Water and Ecological Problems Far Eastern Branch of Russian Academy of Sciences Kim-Yu-Chena, Khabarovsk, RUSSIA; [3] State Research Center of Virology and Biotechnology "Vector", Novosibirsk, RUSSIA
Address for Correspondence: ovp0408@yandex.ru

The gypsy moth is known as the most biologically and economically important pest species. Nucleopolyhedrovirus (LdNPV) is one of the key factors that influence on the gypsy moth population density. The different sensitivity of larvae gypsy moth LdNPV from different nature population was registered. This sensitivity of insects may depend on percentage of occult virus in insects populations. High susceptibility of larvae to virus was registered in population with high level (91 ± 7%) occult virus as compared to population with lower level (48 ±5%) of occult virus. In addition for detection of virus transmission during several generations the larvae of parents generation were infected with high (modeling of epizootic) or low doses (modeling of sporadic death) of the LdNPV. Enhanced insects mortality caused by spontaneous virus infection in three progeny generations has been shown for parents infected by both doses of virus compared to non infected control. The level of occult virus was in 2-fold decreased to third generation for all cases. However occult virus has been detected up to sixth generations just in case of parents'

infected with high dose of virus. Possibly exogenous insect virus may be activator of viral infection and lead to epizootic. However sometimes exogenous virus produces transgenerational occult form.

Poster / Viruses. Wednesday, 16:30. **VI-28**

Establishment of SeMNPV Persistent Infection and Screening of Persistent Infection Associated Genes in Baculovirus

Weng Qingbei[1], Li Min[1], Yang Kai[2], Pang Yi[2]
[1] School of Life Sciences, Guizhou Normal University, Guiyang, China; [2] State Key Laboratory of Biocontrol and Institute of Entomology, Sun Yat Sen University, Guangzhou, China
Address for Correspondence: wengqingbei@gznu.edu.cn

Persistent baculovirus infection is observed in insect populations. Persistent infection can be transformed to a replicative and infective state and plays an important role in epizoology of baculoviruses. However, the molecular mechanism of baculoviral persistence is unknown. *Spodoptera exigua multiple nucleopolyhedrovirus* (SeMNPV) was serially undiluted passaged in Se301 cells to reduce virulence. Upon infection of Se301 cells with the SeMNPV up to passage 8, a few cells survived even if most of cells died due to virus infection. The surviving cells were passaged and designated as P8-Se301 cells. The cells continually released infectious progeny virus and show a typical character trait of baculovirus persistent infection. Using limited dilution method, a cell clone was isolated and designated as P8-Se301-C1. The cells were morphology similarly to the Se301 cells, and no polyhedra or viral particles were observed. However, incomplete SeMNPV genomes and low level SeMNPV transcripts presented in P8-Se301-C1 cells. It was suggested that a latent-like viral infection is present in the P8-Se301-C1 cells. To screen and identity the persistent infection associated genes in baculovirus, The total protein was extracted and isolated through 2-D gel electrophoresis, the differential expression were analyzed between the P8-Se301-C1 cells and the healthy Se301 cells. It would provide a basis for further exploring the molecular mechanisms of baculoviral persistence. .

Poster / Viruses. Wednesday, 16:30. **VI-29-STU**

Larvicidal activity of an ascovirus from *Spodoptera litura* against parasitoid wasps

Shiori Sagawa, Eiko Arai, Maki Inoue, Yasuhisa Kunimi, Madoka Nakai
Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Japan
Address for Correspondence: madoka@cc.tuat.ac.jp

When the endoparasitoid *Cotesia kariyai* (Hymenoptera: Braconidae) parasitizes *Mythimna separata* larvae infected with *M. separata* entomopoxvirus (MySEV), the parasitoid larvae die in the infected host. Death is caused by a 28-kDa polypeptide, named parasitoid killer toxin (PKT), which is encoded by the MySEV genome and secreted from MySEV-infected fat body cells into the hemolymph of an infected larva. *pkt* gene homologues are found not only in entomopoxviruses but also in other insect viruses including granuloviruses, nucleopolyhedroviruses and ascoviruses (AVs). AVs are double-stranded DNA viruses and mainly infect noctuid larvae, producing symptoms that include stunted growth and opaque white hemolymph. A unique characteristic of AVs is their poor *per os* infectivity; in nature, AVs are transmitted by the ovipositors of female parasitoid wasps. Since AV transmission thus coincides with wasp oviposition, parasitoid wasp larval mortality in an AV-infected host has sometimes been attributed to the AV, although no known mechanism explains such larvicidal activity. To elucidate whether the *pkt* homologue in AVs is involved with this larvicidal phenomenon, we sequenced *pkt* homologue in an AV isolated from *Spodoptera litura* in Japan, and found that its predicted amino acid sequence displayed identity with MySEV PKT in a 750-bp partial sequence. Hemolymph from AV-infected larvae showed larvicidal activity against *C. kariyai* and *Microplitis* sp. (Braconidae) larvae. These results suggest that PKT expressed from the AV genome can cause death of braconid parasitoid larvae in hosts infected with the AV isolate.

Poster / Viruses. Wednesday, 16:30. **VI-30**

"11K" genes family *sf68*, *sf95* and *sf138* modulate transmissibility and insecticidal properties of *Spodoptera frugiperda* multiple nucleopolyhedrovirus

Inés Beperet [1]; Oihane Simón [1]; Trevor Williams [2]; Miguel López-Ferber [3];Primitivo Caballero [1,4]
[1] Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain; [2] Instituto de Ecología AC, Xalapa, Mexico; [3]LGEI, Ecole de Mines d' Alès, Alès, France; [4] Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain
Address for Correspondence: oihane.simon@unavarra.es

The "11K" protein family is notable for having homologs in both baculoviruses and entomopoxviruses. These genes are classified as either type 145 or type 150, according to their similarity with *ac145* or *ac150* of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). One homolog to *ac145* (*sf138*) and two homologs to *ac150* (*sf68* and *sf95*) are present in *Spodoptera frugiperda* multiple nucleopolyhedro-virus (SfMNPV). Recombinant viruses lacking *sf68*, *sf95* or *sf138* (Sf68null, Sf95null and Sf138null, respectively), and the respective repair viruses, were generated from a bacmid comprising the complete virus genome. Occlusion bodies (OBs) of the Sf138null virus were ~15-fold less pathogenic to insects, which was attributed to a 100-fold reduction in ODV infectious titer/OB. Inoculation of insects with Sf138null OBs in mixtures with an optical brightener failed to restore the pathogenicity of Sf138null OBs to that of the parental virus, indicating that the effects of *sf138* deletion on OB pathogenicity were unlikely to involve an interaction with the gut peritrophic matrix. In contrast, deletion of *sf68* and *sf95* resulted in a slower speed-of-kill by ~7%, and a concurrent increase in the total production of OBs/larva. Phylogenetic analysis indicated that *sf68* and *sf95* were not generated after a duplication event of the *ac150* gene. We conclude that type 145 genes modulate primary infection process of the virus, whereas type 150 genes appear to have a role in spreading systemic infection within the insect.

Poster / Viruses. Wednesday, 16:30. **VI-31**

Characterization of two ORFs undergoing positive selection in a genotype of *Chrysodeixis chalcites* single nucleopolyhedrovirus from the Canary Islands

Oihane Simón [1]; Leopoldo Palma [1]; Alexandra Bernal [1]; Delia Muñoz [2]; Trevor Williams [3]; Primitivo Caballero [1,2]
[1] Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain; [2] Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain; [3] Instituto de Ecología AC, Xalapa, Mexico;
Address for Correspondence: oihane.simon@unavarra.es

The availability of genome sequences of different genotypes from a single isolate could help explain phenotype differences related to changes at genome level. Here we report the complete genome sequence of five genotypes of a *Chrysodeixis chalcites* single NPV isolate from the Canary Islands (named ChchSNPV-TF1-A, -B, -C, -G and -H). The whole genome sequences of the

ChchSNPV-TF1 genotypes are 99% identical to the previously reported ChchSNPV strain from The Netherlands (ChchSNPV-NL). ChchSNPV-TF1-A, -B, -C, -H genomes did not present ORF 53 of unknown function that is unique to ChchSNPV genomes. Major regions of variability among ChchSNPV genomes was identified in the *hoar* and *bro-d* genes. In an effort to identify genes potentially involved in virulence or in determining population level adaptations, selection pressure analysis was performed. Five ORFs were identified as undergoing positive selection; *chch55* (*bro-a*), *chch65* (*chitinase*), *chch69* (*bro-b*), *chch143* and *chch144*, the last two of which are of unknown function. Strong selection for *bro* and *chitinase* genes indicates that viral replication and liquefaction processes are critical points at which adaptation acts during transmission of these viruses. Among the unknown ORFs, *chch143* exhibits a high degree of similarity with the metalloprotease superfamily and with the previously characterized *sf29* of *Spodoptera frugiperda* multiple nucleopolyhedrovirus involved in ODV packaging. Experiments are in progress to determine the function of *chch143* and *chch144* in the transmission of ChchSNPV.

Poster / Viruses. Wednesday, 16:30. **VI-32**

Genome sequence and organization of a *Betabaculovirus* pathogenicto cassava hornworm, *Erinnyis ello ello* (Lepidoptera: Sphingidae)

Daniel M. P. Ardisson-Araújo [1]; Fernando Lucas Melo [1]; Miguel S. Andrade [1]; William Sihler [2]; Sonia N. Báo[1]; Bergmann M. Ribeiro[1]; Marlinda L.Souza [2]
[1] Laboratory of Baculovirus, Cell Biology Department, University of Brasília, 70910-900, Brasília, DF; Brazil. [2]Embrapa Genetic Resources and Biotechnology, Biological Station Park, 70770-917, Brasília, DF, Brazil.
Address for correspondence: marlinda.souza@embrapa.br

The cassava hornworm, *Erinnyis ello ello* (Lepidoptera: Sphingidae), is a very severe pest in cassava (*Manihot esculenta*) due to its worldly geographical distribution and high capacity of leaf consumption. It is also the most serious pest of the rubber tree (*Hevea brasiliensis*) in Latin America. The *Baculovirus Erinnyis* has been shown to be an economically viable and safe biopesticide for controlling this pest in South America. In the present work the complete sequence of the *Erinnyis ello granulovirus* (ErelGV) genome was determined. The viral DNA was extracted from a viral isolate collected in South Brazil, in 1986. Analysis by transmission electron microscopy showed granular occlusion bodies with single virions inside the protein matrix, confirming that this pathogen is a *Betabaculovirus*. The genome is 102,759 bp with G+C content of 38.7%, being larger than the previous estimation of 90,000 ± 5,000 bp based on restriction mapping for a Colombian isolate. A total of 130 putative ORFs were found encoding at least 50 amino acids. Eight of these were shown to be unique (*ErelOrf-11*, *ErelOrf-15*, *ErelOrf-27*, *ErelOrf-53*, *ErelOrf-59*, *ErelOrf-70*, *ErelOrf-90*, *ErelOrf-102*), and all the predicted protein had no significant similarity to any other sequences in GenBank. ErelGV is closely related to *Choristoneura occidentalis granulovirus* (ChocGV) and *Pieris rapae granulovirus* (PiraGV). No typical homologous regions (hrs), *cathepsin* or *chitinase* genes were detected. *Alphabaculovirus* horizontal gene transfer, such as *he65* and *p43* homologous genes, was found. Moreover, a nucleotide metabolism-related gene and two genes acquired probably from *Densovirus* were also detected.

Poster / Viruses. Wednesday, 16:30. **VI-33-STU**

Analysis of genetic interactions among four non-essential genes of BmNPV

Hitomi Taka [1], Chikako Ono [2], Masanao Sato [3], Shin-ichiro Asano[1], Hisanori Bando[1]
[1] Graduate School of Agriculture, Hokkaido University, Sapporo, Japan; [2] Research Institute for Microbial Diseases, Osaka University, Suita, Japan; [3] Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology, Okazaki, Japan
Address for Correspondence: h-taka@abs.agr.hokudai.ac.jp

Nucleopolyhedroviruses (NPV) produce copious amounts of polyhedrin (Polh) by the end of a replication cycle and form a lot of polyhedra in the nuclei of infected-host insect cells. This characteristic feature of NPV is a beneficial trait as the gene expression vector. To further develop baculoviral applications, deep insight into the functions and interactions of viral gene products concerning the explosive expression of Polh is necessary.We constructed a library of single gene knockout BmNPVs and showed that 86 out of 141 viral genes were dispensable for expression of the polyhedrin gene and production of infectious viral progenies (Ono et al., 2012). However, it has not been examined how these non-essential genes in combinations contribute to the viral infection. We then started a study to understand the genetic interactions among the non-essential genes. In this present study, we constructed BmNPVs lacking multiple non-essential genes and analyzed the expression of EGFP under the control of the polyhedrin gene promoter. Synergistic, compensatory, and additive relationships were observed in the genetic interaction analysis between pairs of adjacent genes in the *orf11-12-13-14* gene cluster. The results in this study revealed complex genetic interactions among the non-essential genes of BmNPV.

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Comparative fitness of a granulovirus mutant possessing larger occlusion bodies than wild type *Adoxophyes orana* granulovirus

Haruaki Uchida, Yasuhisa Kunimi, Maki Inoue, Madoka Nakai
Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Japan
Address for Correspondence: madoka@cc.tuat.ac.jp

During host infection, the virus particles of baculoviruses become embedded within a large proteinaceous occlusion body (OB). Outside the host, the OB protects virus particles from environmental factors including ultraviolet light (UV). However, the mechanism(s) that determine the morphology (size and shape) of OBs are not understood. We isolated a novel mutant of *Adoxophyes orana* granulovirus (AdorGV) from an *A. honmai* larva in a tea field in Japan. This mutant AdorGV produced cube-shaped OBs with edges of approximately 1.0 µm, whereas a wild type (WT) AdorGV isolated in the UK produces typical ellipsoidal OBs of approximately 0.5 µm in length. According to its full genome sequence, the mutant AdorGV was closely related to WT AdorGV. Since such giant OBs should be more costly for the virus to produce than the smaller WT AdorGV OBs, the mutant AdorGV may exhibit a trade-off in production fitness to acquire other adaptive traits. In this study, the UV tolerance of mutant AdorGV was compared to that of WT AdorGV. The persistence of the mutant AdorGV was four times longer than that of the WT AdorGV. The UV tolerance of OB-derived virus particles of mutant and WT AdorGV showed no significant difference. Thus, we elucidate that mutant AdorGV have high UV tolerance to produce giant OBs. This trait may be trade-off of some cost of mutant AdorGV such as production cost of giant OBs.

Poster / Viruses. Wednesday, 16:30. **VI-35**

Granulovirus detection in larvae of sugarcane borers *Diatraea* spp. (Lepidoptera: Pyralidae) in Colombia

Cristian Guzmán, Diana Pinzón, Carolina Ruiz, Juliana Gómez, Carlos Espinel, Gloria Barrera, Laura Villamizar
Centro de Biotecnología y Bioindustria (CBB), Corpoica, Bogotá, Colombia
Address for Correspondence: lvillamizar@corpoica.org.co

Panela is a solid piece of unrefined sucrose obtained from evaporation of sugarcane juice, a very important industry and source of employment in Colombia. Panela yield depends of sucrose content in sugarcane, characteristic seriously affected by the presence of the stem borers complex, difficultly controlled by chemical insecticides, being an alternative the use of biological agents as granuloviruses. In this sense, the objective of this work was to isolate granulovirus naturally infecting *Diatraea* spp. larvae in sugarcane crops for panela production in Colombia. Larvae were collected from three different production areas and maintained in quarantine until dead. A total of 445 larvae were collected, 227 in Boyacá, 130 in Santander and 88 in Nariño. From collected larvae, 39 individuals died showing disease symptoms. Five dead larvae showed fungal mycelium growth and 34 presented sings of viral infection, which were analyzed by granulin gen QPCR, complete granulin gen PCR with degenerated primers and Dot Blot by using polyclonal antibodies for granulin produced in hen eggs. Two samples from Boyacá and two samples from Santander were positive by molecular and immunological methods, being three detected by QPCR and Dot Blot simultaneously and one from Boyacá positive by the three evaluated techniques. Only the 0.89% of collected larvae evidenced viral infection by granulovirus, which were detected by using very low volumes of crude samples. All methods showed to be promising for detecting granulovirus in field samples and four detected virus will be amplified in the insect for a further characterization and biopesticide development.

Poster / Viruses. Wednesday, 16:30. **VI-36**

Earthworm-mediated dispersal of baculovirus occlusion bodies in soil: a laboratory study

Dennis A. Infante-Rodríguez [1]; Delia Muñoz [2]; Jorge Valenzuela [1]; Trevor Williams [1]
[1] Instituto de Ecología AC, Xalapa, Mexico; [2] Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain
Address for Correspondence: trevor.inecol@gmail.com

Soil is an important environmental reservoir of baculovirus occlusion bodies (OBs). The multiple nucleopolyhedrovirus (SfMNPV, genus *Alphabaculovirus*) of *Spodoptera frugiperda* has attracted attention as a potential biological insecticide for control of this pest in maize and sorghum crops in the Americas. This study examined the potential role of the earthworm *Eisenia fetida* as a possible disperser of SfMNPV OBs in a model laboratory system. A soil incorporation bioassay technique was calibrated using *S. frugiperda* second instars that fed on an OECD artificial soil (70% sand, 20% kaolin, 10% peat) contaminated with SfMNPV OBs ($5x10^4$ - $5x10^9$ OBs/ml). The LC_{50} value was estimated at 2.3×10^8 OBs/ml. The gut pH of *E. fetida* was estimated to be pH 5.0-6.0 using pH indicators. Earthworms burrowed 22.5 cm into experimental soil in a 72 h period. Earthworms redistributed SfMNPV OBs vertically by up to 22 cm in artificial soil over periods of 1, 7 and 15 days. Incubation of earthworms in OB treated soil for 7 days did not significantly affect the insecticidal activity of the OBs compared to OBs in soil in the absence of earthworms ($P>0.05$). This represents a previously unrecognized mechanism of baculovirus dispersal in the environment that is likely to have important implications in the persistence of OB populations in soil reservoirs.

Poster / Viruses. Wednesday, 16:30. **VI-37-STU**

Effects of rearing temperature on the susceptibility of larvae of the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae) to *A. honmai* nucleopolyhedrovirus

Takeshi Yamaga, Madoka Nakai, Maki Inoue, Yasuhisa Kunimi
Laboratory of biological control, Graduate School of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu city, Tokyo 183-8509, Japan
Address for Correspondence: kunimi@cc.tuat.ac.jp

Many environmental factors, such as ultraviolet, humidity and temperature, can affect the susceptibility of insect hosts to entomopathogens. Among these factors, temperature is one of the most important factors for both insect susceptibility and multiplication of entomopathgens in the host. The smaller tea tortrix, *Adoxophyes honmai*, is one of the most important pests of tea plants in Japan and occurs four or five times in a year. In addition, larvae of *A. honmai* live in a wide range of temperature from 0°C to 35°C. Here, we examined the effects of high temperature on the susceptibility of *A. honmai* larvae to *A. honmai* nucleopolyhedrovirus (AdhoNPV). Fifth instar larvae of *A. honmai* were exposed to AdhoNPV by the modified droplet feeding method and reared on artificial diet at 25°C, 28°C, 31°C or 34°C. The susceptibility of *A. honmai* larvae was reduced with an increase in rearing temperature. No AdhoNPV-infected larvae were observed when larvae were reared at 34°C. The infection rates of *A. honmai* fifth instar larvae that were reared at 34°C were significantly lower than those of larvae that were reared at 25°C, 28°C and 31°C when budded viruses of AdhoNPV were injected.

Poster / Viruses. Wednesday, 16:30. **VI-38**

Characterization of Nodaviral Protein A Revealed RNA Synthesis and Terminal Nucleotidyl Transferase Activity

Zhaowei Wang, Xi Zhou, Dong Li and Congyi Zheng
State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, China
Address for Correspondence: cctcc202@whu.edu.cn

Nodaviruses are a family of positive-stranded RNA viruses with a bipartite genome of RNAs, RNA1 and RNA2. Protein A, which is recognized as an RNA-dependent RNA polymerase (RdRP), is encoded by genomic RNA1 and functions as the sole viral replicase protein responsible for its RNA replication. Although nodaviral RNA replication has been studied in considerable detail, the mechanism(s) governing the initiation of nodaviral RNA synthesis have not been determined. In this study, we characterized the RdRP activity of Wuhan nodavirus (WhNV) protein A and Flock House virus(FHV) in detail and determined that these nodaviral protein A initiates RNA synthesis via a de novo mechanism. Moreover, we uncovered that both of WhNV protein A and FHV protein A possess terminal nucleotidyl transferase (TNTase) activity. We subsequently found that the TNTase activity of WhNV protein A and FHV protein A could function in vitro to repair the 3' initiation site, and may function as a rescue and protection mechanism to protect the 3' initiation site, and ensure the efficiency and accuracy of viral RNA synthesis. Furthermore, we determined the cis-acting elements for RdRP or TNTase activity at the 3' end of positive- or negative-strand RNA1. Altogether, our study establishes the de novo initiation mechanism of RdRP and the terminal rescue mechanism of TNTase for WhNV and FHV protein A, and represents an important advance toward understanding nodaviral RNA replication.