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

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

Assessing entomopathogenic nematode population genetics: a research and teaching approach
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Poster / Nematodes. Wednesday, 16:30. NE-20

High-level Expression of Foreign Protein Using the Partial Polyhedrin-fused Baculovirus System
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Polyhedrin is the major component of the nuclear viral occlusions produced during replication of the baculovirus Autographa californiae 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-infection. This may improve the production efficiency of foreign protein in baculovirus expression system, 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.
A Colombian SIMNPV isolate (SICOL-A) and its genotypic variant (SICOL-V) 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 SICOL-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 SIMNPVs. SICOL-A genome displayed a ~1470 bp deletion localized within the main variable region among SIMNPV 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 SIMNPV 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 SpHNPV-II (a wild type virus) and a wild type Colombian SIMNPV. Two bioinformatics approaches (relative similarity and bootstrapping analysis) were used to explore the recombination hypothesis. Both analyses supported the hypothesis, showing a recombination event involving the C terminus region of the chitinase ORF and the N terminus region of the gp37 ORF. This event resulted in the deletion of a genomic region including the Homologous Region 3 (HR3) and the 2I23 ORF; and an insertion of ~2970 bp carrying the splt020 and splt021 ORFs from SpHNPV-II. Breakpoints seemed to be localized within the frames of chitinase (near position 21,471) and gp37 (near position 24,443) genes in SICOL-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.
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 *Panaeus 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* innubria 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.

**Post/ 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**

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The winter moth (WM, *Operophtera brumata, L.*), a polyphagous geometer 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 conger, 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 *ObrNPV*, 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.

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

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

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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 *CASPS18/19* 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.

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

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

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*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, subtractive 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 IV-VI expressing the Cn-10 neurotoxin from Centruroides noxius scorpion**

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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 IV-V. Were standardized optimal conditions for micro-projectile bombardment cotransfection of the vector DNA TOPO-295L-GFP-Cn10 and wild DNA from Iridovirus IV-VI, being the ratio of 3:1 (vector: wild DNA) the more useful for obtained recombinant Iridovirus. Recombinant Iridovirus IV-V, was obtained by cotransfection of vector DNA TOPO-295L-GFP-Cn10 and wild DNA Iridovirus IV-VI, using the technique of biologic 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 IV-VI and performing Western Blot tests to detect, for one hand, the insertion of both genes (GFP and Cn10) into the genome of IV-VI 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**

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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**

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Chilo iridescent virus (CIV) encodes an open reading frame (ORF 012L) homologous to exonuclease II of Schizo-saccaromyces 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 (RFII). Both exonuclease and endonuclease activities of CIV-Exo are optimal at pH 8.0 in the presence of 10 mM MgCl₂, 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**

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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 pterophorius 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 Alphabaculovirus. Preliminary bioassay data showed that the virus was active against either 2nd or 4th instars of jasmine moth. The calculated
LC50 was 1.3x10^2 and 3.1x10^2 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.

### VI-13

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

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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 the 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 quality of rAAV vectors with improved vector quality, resulting in increased infectivity of the rAAV.

### VI-14

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

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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.

### VI-15

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

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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 Antarsica gemmatalis nucleopolyhedrovirus (AgMNPV) and Epinoia aperosa 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.

### VI-16

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

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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 enhancer gene, did not change viral potency. For example, a construct with E1 deleted/E2 zinc modified had the same potency as E1 deleted/E2 intact. These results suggest that the enhancers 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

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For double-stranded RNA (dsRNA) viruses in the family Reoviridae, their inner capsid function as the machinery for viral RNA (vRNA) replication. Unlike other doublestranded 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 to stabilize cypovirus capsid. Here, we expressed VP5 from Helioverpa 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 helixdestabilizing 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 Trichoposphia ni larvae

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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, biolistic was used to achieve co-transfection. The recombinant construction pAccya-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 biolistics was achieved with the AcNPV genome, when neonate Trichoposphia 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 LT50s 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

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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 behavior 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 studies. 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

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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
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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 Ilitaviiridae 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 silkworm and baculovirus expression system
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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. Finally, 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 a green fluorescent protein (GFP) was compared against the polyhedrin (poh) conventional promoter in different cell lines, S212, 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 poh promoter. Additionally, we also tested the activity for the combination of this sequence of 120 nt with the poh promoter revealing an additive effect over the poh 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
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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 Antheronomus granarius cells. Recombinant virus (rCIV-157L-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
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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 straightforward 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 FCN populations from a range of geographic regions in South Africa**

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Considering the possibility of some geographic populations of the false codling moth, *Thaumatobitia 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**

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Riboflavin 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 alphanavirus 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 nucleopolyhedrovirus in various populations of gypsy moth *Lymnaedra dispar***

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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**  
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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 epizootology of baculoviruses. However, the molecular mechanism of baculoviral persistence is unknown. **Spodoptera exigua** multiple nucleopolyhedrovirus (SeMNPV) was serially unidiluted 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 identify 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**  
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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 larva 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  
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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 nucleopolyhedrovirus (SIMNPV). 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 OD 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-kil by ~7%, and a concurrent increase in the total production of OBs/infarv. 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**  
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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 genotypes. 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 st29 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-33-STU
Analysis of genetic interactions among four non-essential genes of BmNPV
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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.

Poster / Viruses. Wednesday, 16:30. VI-34 STU
Comparative fitness of a granulovirus mutant possessing larger occlusion bodies than wild type Adoxophyes orana granulovirus
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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.
Granulovirus detection in larvae of sugarcane borers *Diatreaeae spp.* (Lepidoptera: *Pyralidae*) in Colombia

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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, characteristically seriously affected by the presence of the stem borers complex, difficulty 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 *Diatreaeae spp.* larvae in sugarcane crops for panela production in Colombia. Larvae were collected from three different production areas and methodically sampled. Soil was aerated and analyzed by SfMNPV OBs in a model laboratory. This represents a significant advance toward understanding the environment that is likely to have important implications in the persistence of OB populations in soil reservoirs.

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

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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 entomopathogens in the host. The smaller tea tortrix, *Adoxophyes honmam*, 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. honmam* 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. honmam* larvae to *A. honmam* nucleopolyhedrovirus (AdhoNPV). Fifth instar larvae of *A. honmam* 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. honmam* 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. honmam* 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.

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

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Nodaviruses are a family of positive-stranded RNA viruses with a bipartite genome consisting of RNA1 and RNA2, which is recognized as an RNA-dependent RNA polymerase (RdRP), is encoded by genomic RNA1 and functions as the sole viral replicate 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 initiation site, indicating that it acts 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 WhNV protein A, and represents an important advance toward understanding nodaviral RNA replication.