

VIRUSES 4

Contributed paper. Wednesday, 10:30 **154****Mamestra configurata nucleopolyhedrovirus-A transcriptome from infected host midgut**Martin A. Erlandson¹, B. Cameron Donly², David A. Theilmann³, Dwayne D. Hegedus¹, Cathy Coutu¹ and Douglas Baldwin¹¹Saskatoon Research Centre, AAFC, Saskatoon, SK, S7N 0X2, Canada; ²Southern Crop Protection & Food Research Centre, AAFC, London, ON, N5V 4T3, Canada; ³Pacific Agri-Food Research Centre, AAFC, Summerland, BC, V0H 1Z0, Canada
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Infection of an insect by a baculovirus occurs in two distinct phases, an initial infection of host midgut by occlusion-derived virions (ODVs) and subsequent systemic infection of other tissues by budded virions (BV). A vast majority of investigations of the infection process have been restricted to cell culture studies using BV that emulate the systemic phase of infection. In the current study we investigate baculovirus gene expression in ODV infected midgut cells. We have focused on the critical first phase of in vivo infection by *Mamestra configurata* nucleopolyhedrovirus-A in *M. configurata* larvae, using qPCR and RNAseq mass sequencing strategies to examine virus gene expression in midgut cells. The earliest genes detected by each method had significant overlap and included known early baculovirus genes as well as genes unique to MacoNPV-A and genes of unknown function. The RNAseq datasets also revealed a large range of expression levels across most ORFs. These datasets provide a whole genome transcriptomic analysis of viral genes required for virus infection in vivo and will provide the basis for functionally analyzing specific genes that may be critical elements in baculovirus midgut infectivity and host range.

Contributed paper. Wednesday, 10:45 **155-STU****Genomic adaptation to different hosts – Impact of genetic diversity on viral fitness**

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Ecological and genomic adaptations underpin evolutionary processes. Nucleopolyhedroviruses, enclosing many virions in their occlusion bodies, evolve as populations of genomes, adapting to particular ecological niches. We previously showed that all the possible variation is present in a genome population of the size of baculoviruses. When adapting to a new niche, genome populations should differentiate. We conducted experimental evolution on AcMNPV wild type population by passaging 10 times through 4 different host species, in 10 replicates. We then characterised the genetic make up the original and evolved baculovirus populations by ultra-deep Illumina sequencing and their phenotypes by virulence bioassays. We were able to compare virulence components (time, dose and yield) to population diversity. Our experiment allowed us to follow the evolution of a population of genome and its phenotype in different environments to link

fitness with genomic changes.

From all the evolved populations, different profiles emerge, with different relations between intra-population variation and fitness. Actually, it seems that all the species that have evolved on a host show a reduction of intra-population variation while increasing fitness on this host. But when looking at the generalist potential of the population, a lower diversity doesn't always bring a lower fitness. Of course, there are variations in these results that seem to be modulated with the primary fitness of the virus to the infected host; spectacular fitness increase can emerge when infecting a very resistant host. These results give new indications in the evolution of the relation between fitness and genetic diversity.

Contributed paper. Wednesday, 11:00 **156-STU****Transcriptomic analysis of a host-parasitoid interaction between a Hymenoptera *Cotesia congregata*, a Lepidoptera *Manduca sexta* and a Polydnviridae**

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Cotesia congregata develops as a gregarious endoparasitoid into larvae of the tobacco hornworm *Manduca sexta*. The parasitoid wasp has evolved virulence strategies using an obligatory viral symbiont from the Polydnvirus (PDV) family named *Cotesia congregata bracovirus* (CcBV). CcBV particles are produced by specialized cells of the wasp ovaries and are injected along with the eggs into the host body and act by manipulating host immune defenses, and development, thereby enabling wasps to survive in a potentially harmful environment.

In the caterpillar host, the expression of only a few selected candidate virulence genes had been studied, and so far we lacked a global vision of viral and host gene expression.

To identify viral and host gene regulation during parasitism we performed a large-scale transcriptomic analysis by 454 sequencing of two distinct immune tissues (fat body and hemocytes) of the host *M. sexta* isolated in four experimental contexts: (i) non-treated *M. sexta*; (ii) parasitism of *M. sexta* by *C. congregata*; (iii) immune stimulation of *M. sexta* by heat-killed bacteria; (iv) parasitism of *M. sexta* by *C. congregata* followed by bacterial challenge. Following this analysis, we were able to identify 76 CcBV genes and 1993 *M. sexta* genes expressed 24hrs after parasitism.

The data obtained allows us to draw for the first time a functional map of the CcBV genome, and to visualize at a global level *M. sexta* genes that are regulated during parasitism. This type of analysis will help us to highlight viral virulence genes that play an essential role in the host-parasitoid interaction.

Contributed paper. Wednesday, 11:15 **157****Expressed viral ORF and new virus discovery from high throughput transcriptomes of non-model animal**Diane Bigot¹, Marion Ballenghien², Vincent Cahais², Nicolas Galtier², Elisabeth Herniou¹, Philippe Gayral¹¹Institut de Recherches sur la Biologie de l'Insecte, CNRS UMR 7261, Université François-Rabelais, 37200 Tours, France.²Université Montpellier 2, CNRS UMR 5554, Institut des Sciences de l'Evolution de Montpellier, Place E. Bataillon, 34095 Montpellier, France

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High-throughput sequencing allows quantifying the viral biodiversity by studying the diversity of endogenous viral ORF and discovering new virus pathogens of impacting host species. A total of 114 non-model wild animal species from 33 taxonomic groups (i.e. 441 individual transcriptomes) were screened. Virus detection pipeline started with de-novo assembly of Illumina reads and prediction of 17 million ORF. Protein annotation was performed by a sequence homology search. Taxonomic assignment of each ORF was finally achieved using the NCBI taxonomy database.

Viral ORF from 8 species of termites, mosquito, ants, crustacean and marine annelid were analyzed thus far. We detected 146 viral ORF, i.e. 10 viral ORF per host species, mostly related to dsDNA viruses. Genomic analysis showed that their (G+C) content was at intermediate level between those from host genes and from exogenous viruses, suggesting a genuine and recent viral origin. Viral ORF were shorter than their exogenous counterparts but still expressed: their function might have thus been retained. This result illustrated potential cases of viral gene domestications by the host's genomes.

A dozen of complete viral genomes were identified thus far; mostly RNA viruses. Molecular phylogenies allowed assessing the taxonomic position of the viruses. *Lake sinai virus-like* (LSV; unclassified ssRNA virus) were discovered in ants and solitary bees. LSV was recently discovered in honey-bees associated with colony collapse disorder. LSV-like discovery in non-*Apis* insects suggests that hymenopterans could act as a viral reservoir toward domesticated bees. This work illustrated the great potential of our method for high-throughput virus discovery.

Contributed paper. Wednesday, 11:30 **158**

Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons

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Horizontal transfer (HT) of DNA is an important factor shaping eukaryote evolution. Although several hundreds of eukaryote-to-eukaryote HTs of transposable elements (TEs) have been reported, the vectors underlying these transfers remain elusive. Here, we show that multiple copies of two TEs from the cabbage looper (*Trichoplusia ni*) transposed in vivo into genomes of the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) during caterpillar infection. We further demonstrate that both TEs underwent recent HT between several sympatric moth species (*T. ni*, *Manduca sexta*, *Helicoverpa* spp.) showing different degrees of susceptibility to AcMNPV. Based on two independent population genomics data sets (reaching a total coverage >330,000X), we report a frequency of one moth TE in 8,500 AcMNPV genomes. Together, our results provide strong support for the role of viruses as vectors of TE HT between animals, and they call for a systematic evaluation of the frequency and impact of virus-mediated HT on the evolution of host genomes.

Contributed paper. Wednesday, 11:45 **159**

Genomic analysis of five *Lymantria dispar* multiple nucleopolyhedrovirus isolates and biological activity against different host strains of *Lymantria dispar*

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To evaluate genetic diversity of *Lymantria dispar* multiple nucleopolyhedrovirus (LdMNPV) at the genomic level, five isolates of LdMNPV from North America, Europe, and Asia were selected for complete genome sequence determination. These isolates consist of LdMNPV-2161 from Korea; LdMNPV-3029, a sample of the product Virin-Ensh, from Russia; LdMNPV-3041 from Japan; LdMNPV-3054 from Spain, and LdMNPV-Ab-a624, a plaque isolate from a sample collected in Massachusetts, USA. The genome sequences of these isolates were co-linear with the genome sequence of the reference isolate LdMNPV 5-6, derived from the Gypchek product. LdMNPV 5-6 ORFs Id31, Id66, and Id133 were not found in the other five isolates, while all other ORFs annotated for isolate 5-6 were present in at least one other isolate. The greatest degree of sequence divergence among the isolates was observed among the *bro* genes, especially in the two clusters of *bro* genes between *chitinase* (Id70) and Id76 and between Id111 and *dutpase* (Id116). A 2-nt deletion in the enhancin gene *vef-2* (Id160) of LdMNPV-Ab-a624 resulted in a frameshift and truncation of the *vef-2* ORF, while a deletion in LdMNPV-3041 entirely removed *vef-1* (Id65). Bioassays against the New Jersey Standard Strain of *L. dispar* did not indicate any reduced pathogenicity due to mutation or deletion of *vef* genes in either isolate 3041 or Ab-a624. In bioassays against *L. dispar* from Japan, Russia, Europe, and North America, isolates 2161, 3029, and 3041 exhibited a greater degree of pathogenicity against neonate larvae than a sample of Gypchek at the lower dose range.

Contributed paper. Wednesday, 12:00 **160**

Phylogenomics reveals ecological factors that lead to speciation in *Baculoviridae*

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The study of drivers of species diversification is complex due to the entanglement of numerous ecological factors defining ecological niches. By their nature, viruses provide a confined system of interactions to study diversification from micro to macroevolution. Virus ecological niches are clearly defined by their hosts, which consequently should primarily drive virus evolution. Baculoviruses (BVs) have been well studied, showing the peculiarity of BV life cycle with the dissemination of viral particles on insect host plants. This strongly suggests that host plants may play an important role in their evolution. Here we study phylogenetic patterns of host use in the large radiation of BVs that attack the insect order Lepidoptera (moths and butterflies). We generated a phylogeny for ~500

BV isolates using four core genes (*polh*, *lef-8*, *lef-9*, *pif-2*) from which we delimited virus species, to obtain a comprehensive timed molecular BV species phylogeny. We then used a combination of phylogenetic (BV and insects) and ecological (host range of BVs, host plant range of lepidopteran hosts) data to address the following hypothesis: BVs are host specialists and show high levels of phylogenetic conservatism, BVs have the same ages as their lepidopteran hosts and the host plants of the insects drive also the evolution of BVs. We found that in general, hosts primarily induced BV species speciation over a short timeframe. But on a larger evolutionary scale, the insect-host co-evolutionary relationship signal is confused. Surprisingly we revealed that insect host plant specificity contributed largely to BV evolutionary history.

CONTRIBUTED PAPERS Wednesday, 10:30-12:15

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Contributed paper. Wednesday, 10:30 **162**

An entomopathogenic strain of *Beauveria bassiana* against *Frankliniella occidentalis* with no detrimental effect on the predatory mite *Neoseiulus barkeri*

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Among 28 isolates of *Beauveria bassiana* tested for virulence against *F. occidentalis* in laboratory bioassays, we found strain SZ-26 as the most potent, causing 96% mortality in adults at 1×10^7 mL⁻¹ conidia after 4 days. The effect of the strain SZ-26 on survival, longevity and fecundity of the predatory mite *Neoseiulus (Amblyseius) barkeri* Hughes were studied under laboratory conditions. The bioassay results showed that the corrected mortalities were less than 4 and 8% at 10 days following inoculation of the adult and the larvae of the predator, respectively, with 1×10^7 conidia mL⁻¹ of SZ-26. Furthermore, no fungal hyphae were found in dead predators. The oviposition and postoviposition durations, longevity, and fecundity displayed no significant differences after inoculation with SZ-26 using first-instar larvae of *F. occidentalis* as prey in comparison with untreated predator. In contrast, the preoviposition durations were significantly longer. Observations with a scanning electron microscope, revealed that many conidia were attached to the cuticles of *F. occidentalis* at 2 h after treatment with germ tubes oriented toward cuticle at 24 h, penetration of the insect cuticle at 36 h, and finally, fungal colonization of the whole insect body at 60 h. In contrast, we never observed penetration of the predator's cuticle and conidia were shed gradually from the body, further demonstrating that *B. bassiana* strain SZ-26 show high toxicity against *F. occidentalis* but no pathogenicity to predatory mite.

Contributed paper. Wednesday, 10:45 **163-STU**

Interactions between the insect pathogenic fungus *Metarhizium*, the wheat pathogen *Fusarium culmorum* and the mycoparasitic fungus *Clonostachys rosea*

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The current study was conducted to determine if wheat seeds co-inoculated with the insect-pathogenic fungus *Metarhizium* (three species) and the mycoparasitic fungus *Clonostachys rosea* are protected from both insect pests and plant pathogens. The experiment was done in two parts: First, a co-infection bioassay was performed to determine if the virulence of *Metarhizium* was affected by the presence of other fungi by co-treating *Tenebrio molitor* larvae with combinations of *Metarhizium*, *C. rosea*, and the wheat pathogen *Fusarium culmorum*. Second, wheat seeds were co-inoculated with the both beneficial fungi and compared to single inoculations of the effects on *F. culmorum* when allowed to grow for two weeks under controlled laboratory conditions. The resulting root systems were then placed with *T. molitor* larvae which were evaluated daily for mortality. Pathogenicity to insect persisted in all treatments, but *Metarhizium* virulence was affected by co-treatments with other fungi. Root-infection by *F. culmorum* was not reduced directly by the presence of *Metarhizium* while *C. rosea* reduced *F. culmorum* infection and this effect was not diminished in combination with *Metarhizium*. The results of this study suggest that combination of beneficial fungi may effectively protect roots from both pathogens and insects pests..

Contributed paper. Wednesday, 11:00 **164**

Diversity, ecology and virulence of entomopathogenic fungi isolates naturally infecting the red palm weevil *Rhynchophorus ferrugineus* (Olivier) in the Mediterranean Basin

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The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), and the moth *Paysandisia archon* (Burmeister) (Lepidoptera: Castniidae) are considered nowadays the most important palm pest worldwide. Current tactics commonly used to manage the weevil are based on chemical control, although the use of these compounds is hampered by several environmental concerns. In recent years, the *R. ferrugineus* (Rf) microbial control potential of entomopathogenic fungi (EPF) has been highlighted. In this work, several strains of EPF have been isolated from diverse naturally infected specimens of both species, found in different countries through the Mediterranean Basin. Firstly, the usefulness of the elongation factor 1-alpha (EF1-α) region, the nuclear intergenic region BLOC and inter simple sequence repeat (ISSR) or microsatellite markers were assessed as *R. ferrugineus* EPFs diagnostic tool, alone or in combination, and relationships among the Mediterranean *Beauveria* and *Metarhizium* isolates obtained from the red palm weevil were inferred.

Secondly, the effect of diverse environmental parameters such as temperature, humidity and UV-B radiation were assessed on germination and colony growth of these EPFs strains as function of their genealogy and geographic origin.

Finally, virulence of selected isolates was tested against both Rf larvae and adults.

Our results show a distribution pattern of *Beauveria bassiana* through the Mediterranean Basin, possibly associated with the host insect dispersion, with the same genetic group presented throughout the European distribution area of phytophagous. Furthermore, several differences were observed between the different genetic groups found, regarding the different factors analyzed: temperature, humidity, UV-B radiation and virulence.