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VIRUSES

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Mamestra configurata nucleopolyhedrovirus-A transcriptome from infected host midgut

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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 passing 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 Polydnaviridae

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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 Polydnaviridae (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.

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Expressed viral ORF and new virus discovery from high throughput transcriptomes of non-model animal

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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 sequenced. 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 sini 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.

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

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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 ld111 and dupase (id116). A 2-n deletion in the enhancer 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.
An entomopathogenic strain of Beauveria bassiana against Frankliniella occidentalis with no detrimental effect on the predatory mite Neoseiulus barcki

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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 1×10⁷ 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 1×10⁷ 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.

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 (R) 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.

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.