in a karst cave of Tuscany (Central Italy). Molecular and morphological analyses were performed. Total DNA was extracted from individual nematodes and the mitochondrial COI, the ITS containing region and the 18S rRNA gene were amplified and sequenced. BLAST search at NCBI discriminate this new taxon, similar to other *Oscheius*. This species belongs to Dolichura group. Cuticle is finely annulated, stoma is short and cheilorhabdion is simple, not well cuticularized. Female body is almost straight upon fixation, the reproductive system is didelphic and tail is short, conoid with pointed tip. Males are rare and similar to female in general morphology except for smaller size. Male body is straight when heat-killed, testis is single, ventral reflexed. They show peloderan bursa, tail short rounded and spicules slender and small. Infective Juveniles are slender with elongate tail and have stoma morphology similar to adult. The nematodes were cultured in Petri dishes on several substrates: Nutrient Agar, *Escherichia coli*, *Botritis cinerea*, meat baby food, without satisfactory results. Only Petri dishes method with *G. mellonella* larvae produced IJs, suggesting the entomopathogenicity of this new taxon.

Contributed paper. Thursday, 9:30. **213**

Genetic improvement of the entomopathogenic nematode *Heterorhabditis bacteriophora*

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Abstract-The entomopathogenic nematode *Heterorhabditis bacteriophora* has been genetically improved in beneficial traits, like heat and desiccation tolerance, by cross breeding and genetic selection. For instance, a final overall increase in mean heat tolerance of 5.5°C was achieved with *Heterorhabditis bacteriophora* by cross breeding the most tolerant five strains and then selecting for heat resistance. Success of breeding programmes largely depends on the heritability of the investigated traits. Advances in enhancement of desiccation and heat tolerance often have been lost again during mass production. For heterorhabditid nematodes methods have now been developed to stabilize the traits by selection of tolerant inbred lines. This technique provides a pathway to genetic improvement of commercial strains which will maintain the improved characters also during in vitro mass production. The methodology to produce stable inbred lines for steinernematids needs further investigation, as these nematodes are amphimictic and production of inbred lines is much more laborious. The reproduction potential in liquid culture was also successfully increased. Future targets for genetic improvement are prolongation of shelf life and field persistence and enhancement of virulence.

Contributed paper. Thursday, 9:45. **214-STU**

Perspectives of new nematode formulation technology for biological control to pest insects in Georgia

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In the result of route investigations the soil samples for searching of entomopathogenic nematodes (EPNs) have been collected in several agrocenosis of different regions of Georgia. Samplings of testing material were done by using of recent methods in insect nematology (Stock & Goodrich-Blair, 2012). According to preliminary data some active strains of *Steinernema* sp. have been obtained. EPNs extract efficiency was established on laboratory culture of *Galleria mellonella*. Further research directions for the identification of local strains (under the Project CRDF/DTRA/GRDF #GMG-01/13) have been conducted at the University of Arizona, laboratory of Entomology by two different ways: morphological and molecular diagnostic methods. It was established that four local EPNs isolates belong to the genus *Steinernema*. Furthermore partial sequencing of the ITS rDNA gene revealed they are closely related to the species *Steinernema feltiae*. This conventionally called - *"*Georgian strain", considered as a raw material will be base for local production of bioformulation -*"Geo-nema"*. Provided technological product - environmentally safe nematode insecticide will be used for biological control to the pest insects of agricultural crops and ornamental plants. The researches will be continued under the projects CRDF/STEP and SRNSF/STCU financial support. The usage of nematode insecticide will take an important place in IPM (integrated pest management) system for agricultural crop protection in Georgia.

CONTRIBUTED PAPERS Thursday, 8:00-10:00

Viruses 6

Contributed paper. Thursday, 8:00. **215**

Interactions between salivary gland hypertrophy virus and tsetse microbiota

Güler Demirbas Uzel [1], Vangelis Doudoumis[2], Antonios Augustinos[1], Gisele Ouedroogo[1], Andrew Parker[1], Drion Boucias[3], Kostas Bourtzis[1], Adly Abd-Alla[1],
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Many species of tsetse flies are infected by a hytrosavirus that causes Salivary Gland Hypertrophy (SGH) syndrome. Flies with SGH have a reduced fecundity and fertility. Due to the deleterious impact of the salivary gland hypertrophy virus (SGHV) on *Glossina pallidipes* colonies, several approaches have been investigated to develop a virus management strategy including the exploitation of endogenous microbiota. Tsetse flies harbor three symbiotic bacteria *(Wigglesworthia glossinidia, Sodalis glossinidius* and *Wolbachia*) in addition to trypanosome, the causative agent of sleeping sickness disease in human and nagana in livestock. The interaction of the tsetse microbiota (gut bacteria and symbionts) with the SGHV and / or trypanosome is largely unexplored. In the present study, we show that ampicillin treatment of *G. pallidipes* impedes the transgeneration transmission of the SGHV suggesting the involvement of tsetse microbiota in the virus transmission. Quantitative-PCR analysis of the levels of SGHV and *Wolbachia* in wild tsetse flies (mainly *G. morsitans morsitans* and *G. austeni*) clearly indicated a negative interaction between SGHV and *Wolbachia*: flies heavily infected with *Wolbachia* presented significantly low viral titers. In addition, injection of GpSGHV into different *Wolbachia*-infected *Glossina* species did not result to the transgeneration transmission of SGHV as normally occurs in *G. pallidipes* colony, which is free of *Wolbachia*. Taken together, these data

suggest that *Wolbachia* may interfere with the establishment and transmission of this important DNA virus (SGHV), which represents a major hurdle for the application of SIT strategies for the control of tsetse flies and trypanosomosis in sub-Saharan Africa.

Contributed paper. Thursday, 8:15. **216-STU**

Mechanisms of tree-top disease induced by the specialist baculovirus SeMNPV

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Many parasites alter host behavior to enhance their transmission or survival. An intriguing example is the altered behavior of insect larvae infected by a baculovirus, e.g. their movement to elevated positions. This phenomenon (tree top disease or Wipfelkrankheit) is already known for over a century. However, the underlying mechanisms leading to this behavioral adaptation are still largely enigmatic. Here we studied tree-top disease induced by the baculovirus *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) in *S. exigua* larvae. We show that infected *S. exigua* caterpillars all climb to elevated positions prior to death. Furthermore, we investigate the role of the ecdysteroid UDP-glucosyl transferase (*egt*) gene from SeMNPV in tree-top disease. This gene is known to be important in tree-top disease in another baculovirus-host system, although the mechanism by which it exerts this effect is unknown. We hypothesize that the SeMNPV *egt* gene may directly trigger tree-top disease or induce this phenomenon indirectly by prolonging the larval time to death.

Contributed paper. Thursday, 8:30. **217**

Temporal proteomics to study virus infection and function in the host cell

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Invertebrate iridescent virus 6 (IIV-6) is a nucleocytoplasmic virus with a 212 kb-long linear double-stranded DNA genome that encodes 215 putative open reading frames. The IIV-6 virion proteome consists of at least 54 virally-encoded proteins. One of our previous findings showed that most of these proteins are encoded by genes from the early transcriptional class. This indicates that these structural proteins may not only function in the formation of the virion, but also in the initial stage of viral infection. In the current study, we followed the protein expression profile of IIV-6 over time in Drosophila S2 cells by label-free quantitation using nanoLC-FTMS. A total of 95 viral encoded proteins were detected in infected cells, of which 37 are virion proteins. The expressed IIV-6 virion proteins could be categorized into three main clusters based on their expression profiles. These clusters were: 1) proteins with stably low or 2) exponentially increased expression levels during infection, and 3) proteins that were initially highly abundant, and then showed slightly reduced levels after 48 hours (h) post infection (p.i.). The study supported that temporal expression patterns did not share direct correlation with protein expression classes phenomena, suggesting that both proteomic and transcriptomic approaches will be required to obtain a detailed understanding of the viral expressomics (infectome). Here, we provide novel information on the kinetics of virion and infected cell-specific protein levels that assists in understanding gene regulation in this lesser known DNA virus model.

Contributed paper. Thursday, 8:45. **218**

Characterization of an atypical fast-killing ascovirus: *Spodoptera frigiperda* ascovirus 1d (SfAV-1d)

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Ascoviruses (AVs) are large double-stranded DNA viruses that attack lepidopterans, mainly noctuid larvae. One of the unusual features of AVs is their mode of transmission via parasitoid wasps. AVs are poorly infectious *per os* compared to other insect viruses such as baculoviruses and cypoviruses. Additionally, AV infection results in production of a characteristic milky-white hemolymph due to accumulation of virion-containing vesicles produced by a modified apoptotic response in infected cells. Virtually all ascoviruses cause a chronic disease wherein larvae survive for as long as 28 days after infection, which enables an extended period of transmission among larvae by wasps. Here, we report characterization of *Spodoptera frugiperda* ascovirus 1d (SfAV-1d) isolated from a *S. frugiperda* larva. SfAV-1d killed *S. litura* 4th instar larvae within 3 days when compared to another AV (SfAV-N), which took as long as 23 days to kill larvae. Larvae infected with SfAV-1d contained the characteristic white hemolymph. Electron microscopy revealed that both SfAV-1d and SfAV-N infected the fat body but not the tracheal matrix or other tissues. Interestingly, despite the difference in the rate at which SfAV-1d and SfAV-N killed larvae, there was no apparent difference in the kinetics of viral DNA replication. The primary difference between these two isolates was that SfAV-1d formed and accumulated virion-containing vesicles in the hemolymph much more rapidly than SfAV-N. Our future studies will focus on characterizing the genetic differences between these viruses to identify determinants that influence their pathobiology, particularly as it relates to rate of kill.

Contributed paper. Thursday, 9:00. **219-STU**

Two nucleopolyhedroviruses isolated from the genus *Adoxophyes* inhibit juvenile hormone (JH) esterase activity but not JH epoxide hydrolase activity

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Insect metamorphosis is predominantly regulated by two hormones, juvenile hormone (JH) and ecdysone. During the final instar, a dramatic decrease in JH titer is required for the induction of pupation. JH is metabolized by two enzymes, JH

esterase (JHE) and JH epoxide hydrolase (JHEH). *Adoxophyes honmai* (Lepidoptera: Tortricidae) is susceptible to two nucleopolyhedroviruses (NPVs), *A. honmai NPV* (AdhoNPV) and *A. orana NPV* (AdorNPV), which are genetically closely related but differ in killing speed. AdhoNPV kills the host only in the final instar, whereas AdorNPV kills more quickly (5 to 8 days). When 4th instars of *A. honmai* are orally inoculated at >LC_{95} (1.0 x 10^8 OBs/ml), AdhoNPV and AdorNPV prevent pupation and kill the host in 10 and 8 days, respectively. In contrast, mock-inoculated larvae pupate in 7 days. Baculoviruses are known to prevent pupation through endocrynological regulation. Here, we monitored both JHE and JHEH activities in AdhoNPV-, AdorNPV-, and mock-infected larva of *A.honmai*. Mock-infected larvae showed increased JHE activity in the hemolymph and fat body during the final instar, with the highest activity found on the 3rd day of the 5th instar. Both AdhoNPV- and AdorNPV-infected larvae did not show JHE activation. On the other hand, JHEH activity in fat body was constant and no differences were found between treatments. Our data suggest that AdhoNPV and AdorNPV prevent pupation by specifically down-regulating JHE but have no effect on JHEH activity. Our data also suggest that JH titers remain relatively high during the final instar of baculovirus infection.

Contributed paper. Thursday, 9:15. **220**

Mechanism underlying virus-induced hyperactive behavior: Substrate identification of the baculovirus protein tyrosine phosphatase

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Many parasites alter the behavior of their host to maximize their transmission and survival. However, the underlying mechanisms are largely unknown. Baculoviruses manipulate the behavior of their caterpillar hosts by inducing hyperactivity and climbing behavior. Previous work demonstrated that a protein tyrosine phosphatase (PTP) encoded by the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) was involved in the induction of hyperactive behavior in *Spodoptera exigua* larvae. This finding prompted us to investigate which viral and/or host proteins interact with the baculovirus PTP enzyme and might be involved in altered host behavior. Using affinity-tag purification of a substrate-trapping mutant of AcMNPV PTP incubated with extracts of infected cells followed by proteomic analysis of the trapped protein, we identified six viral and six host proteins that co-purified with PTP. Several of these proteins are known to be important in cellular signaling and in behavior in other insects/organisms, and are therefore potentially involved in PTP-mediated hyperactivity of infected larvae. For one of these identified host proteins, the 14-3-3 ζ protein, RNA expression levels were significantly higher for AcMNPV wild type-infected larvae as compared to AcMNPV Δ*ptp*-infected larvae, indicating that *14-3-3 ζ* expression levels are dependent on the presence of the baculovirus *ptp* gene. The 14-3-3 ζ protein is known to be important for the synthesis of serotonin and dopamine, which are neurotransmitters that play important roles in many behavioral pathways. It is hypothesized that baculovirus *ptp* targets 14-3-3 ζ at both the RNA and protein level, which consequently leads to baculovirus-induced hyperactivity.

Contributed paper. Thursday, 9:30. **221-STU**

The genome of a baculovirus isolated from *Lonomia obliqua* (Lepidoptera:Saturniidae) reveals a new transcription terminator factor possible acquired from the host

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Lonomia obliqua (Lepidoptera: Saturniidae) is a poisonous larvae of medical importance due to the severity of accidents occurred in Brazil caused by the contact of these larvae with the human skin. The possibility of controlling these populations is being evaluated by using pathogens such as a nucleopolyhedrovirus isolated from *L. obliqua*. In this work, we have sequenced the genome of the baculovirus *Loob*MNPV and analyzed its genomic composition and evolutionary history. The genome is 120.022 bp long, comprising 135 putative ORFs. Furthermore, in an evolutionary context, based on analysis that include the core gene from 93 sequenced baculovirus , *Loob*MNPV fell into a basal position related to the *Alphabaculovirus* group I (lepidopteran-infective NPV). Interestingly, one ORF showed significant identity (*e-value* equals to $3e10^{-11}$) to a eukaryotic transcription terminator factor (TTF2) from the lepidoptera *Danaus plexippus* (GenBank: EHJ68439.1). On the other hand, when restricting this search only to baculoviruses, this ORF also demonstrated identity (*e-value* of $1e10^{-6}$) to the Global Transactivator (GTA) gene from *Antheraea pernyi* nucleopolyhedrovirus (Genbank:YP_611073.1). Phylogenetic analysis were performed with the TTF2 gene from various organisms, as well as with the GTA from baculovirus. These results indicated two hypothesis: (i) this gene may have been independently acquired from the host through horizontal transfer, acting as an inhibitor of the host´s transcriptional machinery in order to benefit viral translation; (ii) or it is a divergent variation of the GTA gene that has undergone positive selection.

Contributed paper. Thursday, 9:45. **222**

The essential baculovirus protein VP1054 is a hijacked cellular PURα,
a nucleic-acid-binding protein specific for GGN repeats

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The baculovirus VP1054 protein is a structural component of both budded virus (BV) and occlusion-derived virus (ODV), but its exact role in virion morphogenesis is poorly defined. We reveal sequence and functional similarity between the baculovirus protein VP1054 and the cellular *pu*rine-*r*ich element binding protein PUR-alpha (PURα). The data strongly suggest that gene transfer has occurred from a host to an ancestral baculovirus. Deletion of the AcMNPV *vp1054* gene completely prevented viral cell-to-cell spread. Electron microscopy data showed that assembly of progeny nucleocapsids was dramatically reduced in the absence of VP1054. More precisely, VP1054 is required for proper viral DNA encapsidation, as deduced from the formation of numerous electron-lucent capsid-like tubules. Complementary searching identified the presence of genetic elements composed of repeated GGN trinucleotide motifs in baculovirus

genomes, the target sequence for PURα proteins. Interestingly, these GGN-rich sequences are disproportionally distributed in baculoviral genomes and mostly occurred in proximity to the polyhedrin gene. At the same time they encode crucial proline-rich domains in *p78/83*, an essential gene adjacent to the *polyhedrin* gene in the AcMNPV genome. We further demonstrate that the VP1054 protein specifically recognizes GGN-repeats and are currently analyzing the significance of these GGN motifs for DNA packaging. Together, whilst some viruses like human immunodeficiency virus 1 (HIV-1) and human JC virus (JCV) utilize host PURα protein, baculoviruses encode the PURα-like protein VP1054, which is crucial for viral progeny production.

SYMPOSIUM (Special) Thursday, 8:00-10:00

DFG Priority Program Host Parasite Coevolution

Symposium. Thursday, 8:00 **223**

Escaping parasite manipulation: Apoptosis and host-parasite co-evolution in *Apis mellifera*

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Programmed cell death (apoptosis) does not only play an important role in the development of multicellular organisms, but also in the protection against pathogens. Nevertheless, numerous intracellular pathogens have evolved diverse strategies to interfere with and overcome the apoptotic machinery of their hosts. Yet, little is known about the actual mechanisms and how hosts might counter act. We here study the interaction of the intestinal microsporidian parasite *Nosema ceranae* in a susceptible and tolerant honeybee host under laboratory controlled conditions, to understand the importance of apoptosis in this case of host-parasite co-evolution. We visualize apoptotic processes in the gut epithelium using TUNEL assays; relate this to the expression levels of key genes in the apoptotic cascade over the course of the infection, and consequences for metabolic energetics affecting honeybee performance.

Symposium. Thursday, 8:15 **224**

Overcoming external immunity: An increase in virulence as a result of host-parasite coevolution in *Beauveria bassiana*

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An increase in virulence is a trait often observed as a result of host-parasite coevolution. Specific immune responses overcome in order to achieve increased virulence can, however, be difficult to elucidate. We carried out a coevolution experiment with the red flour beetle, *Tribolium castaneum*, and the general entomopathogenic fungus, *Beauveria bassiana*. After just seven host generations of evolution we saw a substantial increase in virulence in all evolved isolates of *B. bassiana*. Furthermore, we were able to show that this increase in virulence was a result of the *B. bassiana* isolates evolving resistance to the external immune defences of the *T. castaneum* beetles, who are able to secrete antimicrobial compounds into their environment. This is a rare example of a virulence increase seen as a result of a coevolution experiment where the exact barrier of host immune defence that the parasite has gained resistance to in order to achieve the increase in virulence has been described.

Symposium. Thursday, 8:30 **225**

Rapid adaptation of *Bacillus thuringiensis* to its nematode host *Caneorhabditis elegans*

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Antagonistic interactions between host and pathogen can produce very high selection intensities. They are often one of the main driving forces during evolution, especially if the interactions persist across time. We specifically assessed the evolutionary impact of these interactions for the pathogen, using evolution experiments with the Gram positive biocontrol agent *Bacillus thuringiensis* and one of its animal hosts *Caenorhabditis elegans*. Our results demonstrate that differences in the experienced selection conditions during the evolution experiment favour distinct characteristics across the pathogen life cycle: (i) pathogen adaptation to a co-evolving host associates with high virulence; (ii) pathogen adaptation to a non-changing host increases infection load; whereas (iii) adaptation without host favours environmental persistence. Concomitant genomic changes in the pathogen were observed at two levels: (i) the different evolution conditions caused clonal selection of distinct, broad-scale genotypes, while (ii) one of these with high virulence showed additional nucleotide changes, including copy number variations of nematocidal toxin genes. Based on one of the most comprehensive data sets collected for an experimentally evolved pathogen, we conclude that sustained coevolution is distinct from other types of selective constraints in shaping pathogen genome and life-history characteristics. Surprisingly, our findings also suggest that sustained virulence, as desired for pest control, may be contingent on the unwanted co-adaptation of the target host.