antimicrobial peptides (AMPs), whose antibacterial properties have been extensively studied. However, anti- fungal peptide's activity has not attracted a lot of attention so far, especially the *in vivo* activity of such peptides. The mealworm beetle, *Tenebrio molitor*, expresses an antifungal peptide, the tenecin3, which is a glycine-rich peptide, closely related to plant defensins and drosomycin, with no antibacterial activity and of unknown mode of action. Tenecin 3 has been proven to have *in vitro* antifungal activity against *Candida albicans*, but its activity against entomopathogenic fungi has never been studied. We used a gene knock-down approach to study the effect of tenecin 3 on the survival of *T. molitor* after an infection by two different strains of *B. bassiana* and one strain of *B. pseudobassiana*. Interestingly, the mortality pattern varied among the three strains whether tenecin3 was down-regulated or not.

CONTRIBUTED PAPERS — Wednesday, 13:30-15:30 - ***Vouvay***

Virus 5 - *Ikbal Agah Ince & Sassan Asgari*

Contributed paper. Wednesday, 13:30, **152-STU**

Interactions between the salivary gland hypertrophy virus and its host immune system

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Tsetse flies (Diptera; Glossinidae) are naturally infected by *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV; *Hytrosaviridae*); a large dsDNA virus specifically pathogenic to *Glossina* spp. GpSGHV infections are largely asymptomatic in most of the tsetse species. In *G. pallidipes* asymptomatic infection can convert to symptomatic infection that is characterized by overt salivary gland hyperplasia (SGH). This syndrome also leads to reproductive dysfunction of infected flies. We hypothesised that GpSGHV infection is maintained at low levels by dsRNA-mediated gene silencing, such that only few viral genes are expressed during asymptomatic infections. To test this hypothesis, we first investigated whether host-mediated dsRNA mechanisms are involved in asymptomatic virus infection by comparative analyses of *Argonaute* (*Ago*) and *Dicer* (*Dcr*) gene expression levels in asymptomatic and symptomatic *G. pallidipes*. We found that both *Ago* and *Dcr* were significantly up-regulated in symptomatic compared to asymptomatic flies. Furthermore, short RNA sequence analyses indicated that more small RNAs (19 miRNAs) were produced during symptomatic infections compared to asymptomatic infections (8 miRNAs). When mapped onto the host (*Glossina*) genome, the miRNAs in the asymptomatic flies mapped onto several genes with a putative relation to regulation of transcription, translation, macroautophagy, immunity, apoptosis and tumour suppression. In symptomatic flies, majority of miRNAs mapped to metabolic-related genes and a few to transcription genes. We recently set up knock-down bioassays to investigate the involvement of the miRNA targeted genes in regulating GpSGHV infection in *G. pallidipes* flies.

Contributed paper. Wednesday, 13:45, **153-STU**

Host range of Glossina pallidipes salivary gland hypertrophy virus (GpSGHV)

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The salivary gland hypertrophy virus (SGHV) is a dsDNA virus (family: *Hytrosaviridae*), and It has been reported in many species of tsetse fly (Diptera: Glossinidae). Generally, the virus infection is asymptomatic but in certain tsetse species i.e. *Glossina pallidipes* (Gp), the virus infection can convert to symptomatic and cause the salivary gland hypertrophied (SGH) symptoms. The high prevalence of SGH in tsetse colony is associated with a reduction of flies fecundity and fertility, which may cause colony collapse. To understand the molecular mechanism controlling the development of SGH in Gp and its rare presence/absence in other tsetse species, we attempted to analyse the host range of the GpSGHV in other tsetse species. The GpSGHV virus collected from SGH of Gp and injected into tsetse adults and 3rd instar larvae of Gp, *G. fuscipes* (Gf) *G. brevibalpis* (Gb), *G. p. gambiensis* (Gpg), *G. m. morsitans* (Gmm) and *G. m. centralis* (Gmc) Virus quantification at different times post injection indicated an increase of virus titre in the adults of all injected species except Gb. Dissection of both injected flies and F1 generation showed no development of SGH except *G. pallidipes* F1 generation (46%). Dissection of the flies 10 days post-emergence from injected larvae indicated the presence of SGH in Gp (67 %), Gf (26 %), Gpg (18 %), Gmm (9%), Gmc (6 %) and Gb (0 %). The hypertrophied salivary glands observed in the heterologous species were smaller than SGH normally found in Gp. These results indicate that (i) the GpSGHV can replicate in other tsetse species and (ii) the development of SGH requires a component from immature stages.

Contributed paper. Wednesday, 14:00, **154**

Highjack of intracellular signalling pathways and robust immune responses explain the hytrosavirus-induced differential pathologies in two *Glossina* model species

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Glossina pallidipes salivary gland hypertrophy virus (GpSGHV; *Hytrosaviridae*) is exclusively pathogenic to tsetse flies, vectors of African trypanosomoses. GpSGHV infection is largely latent, but can switch to a symptomatic infection state leading to salivary gland hyperplasia (SGH) and reproductive dysfunction. Of all tsetse species, *G. pallidipes* is the most susceptible to overt SGH symptoms. Whilst in naturally infected *G. pallidipes* SGH occurrence is the exception rather than the rule, SGH is only apparent in the F1 progenies of artificially infected *G. pallidipes*

mothers. Hypothetically, specific host-virus interactions account for the differential GpSGHV pathobiologies observed in different tsetse species and colonies. To test this hypothesis, we used mass spectrometry to investigate GpSGHV-induced protein expression modulations in the salivary gland (SG) proteomes of F1 progenies of two *Glossina* model species, *G. pallidipes* (SGH-susceptible) and *G. morsitans* (SGH-refractory). We identified 540 host proteins, of which 23 and 9 proteins were significantly up and down-regulated, respectively, in *G. pallidipes* compared to *G. morsitans*. We also detected 58 and 5 GpSGHV proteins in *G. pallidipes* and *G. morsitans*, respectively. Whilst *G. pallidipes* had significantly high GpSGHV titres, viral titres in the *G. morsitans* were insignificant, confirming that *G. morsitans* is largely SGH-refractory as compared to *G. pallidipes*. Finally we will discuss how GpSGHV seizes cohorts of intracellular signaling pathways to induce overt SGH in *G. pallidipes*, how robust immune responses block SGH expression in *G. morsitans*, and potential applications of our findings in management of viral infection is insect mass rearing facilities.

Contributed paper. Wednesday, 14:15, **155**

The salivary gland proteome of *Glossina m. morsitans*, parasitized with *Trypanosoma b. brucei*

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Trypanosoma brucei spp, causative agent of African trypanosomiasis, completes metacyclo- genesis (development of mammalian-infective trypomastigotes) in the salivary glands (SGs) of its tsetse vector. Since metacyclic trypomastigotes are largely uncultivable, information on the molecular processes that underpin SG metacyclogenesis is scanty. To bridge this knowledge gap, we employed LC-MS/MS to investigate protein expression modulations in SGs of *T. b. brucei*- infected and uninfected *Glossina m. morsitans*. We identified 361 (host) and 158 (parasite) proteins. Compared to uninfected SGs, the repertoire of the parasitized SG proteome contained proteins that were up-regulated (n = 276), down-regulated (n = 81) or un-modulated (n = 4). Whilst 11.5% (n = 32) of the 276 host proteins were significantly up-regulated, only one of the 81 proteins was significantly down-regulated. Despite high abundance, proteins associated with blood feeding process were down-regulated in parasitized SGs, probably to reduce feeding performance and thus promote vector competence (via increase of biting frequency). Amongst the differentially modulated host proteins in parasitized SGs were also proteins associated with translatome regulation (protein translation, stabilization and degradation), immunity, homeostasis and cytoskeletal traffic. Notable proteins specific to metacyclic trypomastigotes included GPI- anchored surface glycoproteins kinetoplastid calpain, peroxiredoxin AhpC-type, *Trypanosoma* RHS multigene, membrane transporters and molecular chaperone protein families. These data will be discussed in view of strategy development to combat African trypanosomiasis via enhancement of tsetse *Trypanosoma*-refractoriness.

Contributed paper. Wednesday, 14:30, **156**

Characterization of Bustos virus, a new member of the Negevirus group isolated from a *Mansonia* mosquito in the Philippines

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Mosquitoes are known to be important vectors for arthropod-borne viruses (arboviruses), which cause public health issues. In surveillance of mosquito-borne arboviruses, we isolated two distinct viruses from mosquitoes collected in Bustos Bulacan province, Phillipines in 2009, where dengue fever was prevalent. These viruses show rapid replication and strong cytopathic effects in mosquito C6/36 cells. Whole-genome analysis of these viruses demonstrated that both viruses belong to the Negevirus group. One of the viruses, from *Culex vishunui* mosquitoes, is a new strain of Negev virus. The other virus, from a *Mansonia sp*. mosquito, is a new Negevirus designated Bustos virus. Gene expression analysis of the Bustos virus revealed that infected cells contain viral subgenomic RNAs that probably encode proteins from open reading frame (ORF)2 or ORF3. In Bustos virus-infected C6/36 cells, the ORF2 and ORF3 products were distributed in cytoplasm, whereas the ORF1 products formed foci nearby perinuclear region. Purified Bustos virus particles contained at least three proteins, and the major component is encoded by ORF3 and the minor component is encoded by ORF2. Bustos virus did not show infectivity to mammalian BHK-21 cells, suggesting an insect-specific virus.

Contributed paper. Wednesday, 14:45, **157**

RNA activation in mosquito cells and its suppression by the dengue virus NS5 protein

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RNA activation (RNAa) is one of the emerging research areas in molecular biology, which involves small RNAs inducing gene expression by targeting the promoter. Thus far, RNAa has only been found in mammals, including humans, and *Caenorhabditis elegans*, but not in insects. Furthermore, there is no report about the effect of pathogen infection on RNAa. In this study, we employed dsRNA targeting the OpIE2 promoter with the *GFP* gene as the reporter, and checked its effect on GFP expression. In addition to that, the effect of dsRNA to the promoter on GFP expression was evaluated upon dengue virus infection. Our results clearly showed that dsRNA targeting the TATA box of the promoter could induce GFP expression in mosquito cells. In addition, dengue virus, in particular its non-structural protein 5 (NS5) could inhibit the RNA activation. The outcome of this research opens new avenues for RNAa-related research into insect's biology and its potential role in host-pathogen interactions.