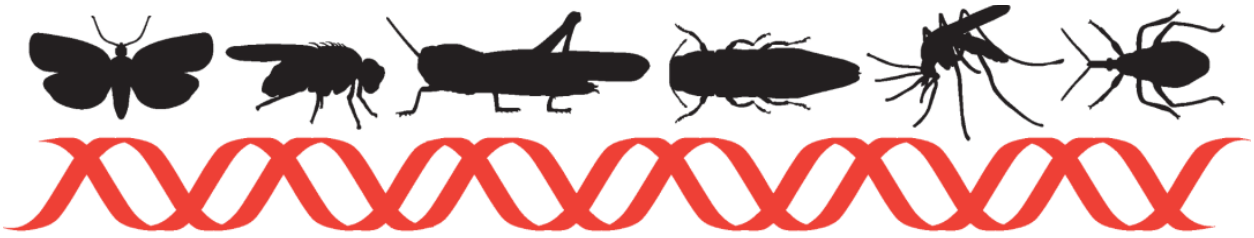

INSECT BIOTECH CONFERENCE

Annual Scientific Meeting

June 5-7, 2019

Schedule and Contributed Abstracts

Hilton Garden Inn, Niagara-on-the-Lake, ON

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Insect Biotech Conference – 2019

Conference Schedule

Wednesday Evening – June 5

6:00 pm	**Registration:** Outside Niagara Gardenview Room
7:00 pm	**Plenary Talk:** Gardenview Room
	BEE GENOMICS AND HEALTH. ***[Page 10]*** Amro Zayed, Department of Biology, York University, Toronto, ON, Canada.
7:45 – 9:00 pm	**Reception**: Niagara Gardenview Room (Pizza, Veggies and Beverages)

Thursday, June 6

7:45 – 9:00 am **Breakfast:** Niagara Gardenview Room

9:00 am **Opening Remarks:** Niagara Gardenview Room: **Andrew Donini**

Session Chair: **Ian Orchard** (Niagara Gardenview Room)

9:10 am **NEGATIVE SELECTION IN SOCIAL INSECTS.** ***[Page 11]***
[1]Imrit, M.I., [2]Harpur, B.A., [1]Dogantzis, K. and [1]Zayed, A.
[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Department of Entomology, Purdue University, West Lafayette, IN, USA.

9:30 am **IMPROVING GENOMIC TOOLS FOR IDENTIFYING AND TRACKING INVASIVE AFRICANIZED HONEY BEES.** ***[Page 12]***
Dogantzis, K., Patel, H., Tiwari, T., Rose, S., Conflitti, I., Dey, A. and Zayed, A.
Department of Biology, York University, Toronto, ON, Canada.

9:50 am **NICOTINE METABOLISM IN *TRICHOPLUSIA NI*: A TOXICOGENOMIC APPROACH.** ***[Page 13]***
Hassanpour, N., Saremba, B.N. and Rheault, M.R.
Department of Biology, University of British Columbia - Okanagan, Kelowna, BC, Canada.

10:10 am **THE EFFECTS OF THE ENTOMOPATHOGENIC FUNGUS, *C. NEOFORMANS*, ON INTEGRATED SYSTEMS OF THE MEALWORM.** ***[Page 14]***
Jarvie, S., Van Mierlo, V., Samarasinghe, H., Xu, J. and da Silva, R.
Department of Biology, McMaster University, Hamilton, ON, Canada.

10:30 – 10:50 am **Coffee Break**: Niagara Gardenview Room

Session Chair: **Sima Jonusaite** (Niagara Gardenview Room)

10:50 am **STOP THE CROP: TOWARDS ELUCIDATING THE MODE AND MECHANISM OF ACTION OF A NATURAL PRODUCT INSECTICIDE.** ***[Page 15]***
Piermarini, P.M., Kalsi, M. and Rakatondraibe, H.L.
Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH, USA.

11:10 am **THE ROLE OF RH AND AMT PROTEINS IN AMMONIA EXCRETION DURING BLOOD MEAL DIGESTION IN THE ADULT FEMALE *AEDES AEGYPTI* MOSQUITO.** ***[Page 16]***
Durant, A.C. and Donini, A.
Department of Biology, York University, Toronto, ON, Canada.

11:30 am **GLYCOPROTEIN HORMONE SIGNALING AND ITS INVOLVEMENT IN MOSQUITO (*AEDES AEGYPTI*) SPERMATOGENESIS.** ***[Page 17]***
Rocco, D., and Paluzzi, J-P.V.
Department of Biology, York University, Toronto, ON, Canada.

11:50 am **ANTI-DIURETIC HORMONE ACTIVITY AND SIGNALING CASCADE OF CAPA NEUROPEPTIDE IN MOSQUITO, *AEDES AEGYPTI.*** ***[Page 18]***
Sajadi, F. and Paluzzi, J-P.V.
Department of Biology, York University, Toronto, ON, Canada.

12:10 – 1:20 pm **Lunch Break**: Niagara Gardenview Room

Session Chair: **Andrea Durant** (Niagara Gardenview Room)

1:20 pm **THE ROLE OF SIFAMIDE AS A NEUROMODULATOR AND NEUROHORMONE IN THE BLOOD-GORGING INSECT *RHODNIUS PROLIXUS.*** ***[Page 19]***
Ayub, M., Lange, A.B. and Orchard, I.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

1:40 pm **THE INTERACTION OF KININ AND CAPA ANALOGS IN *RHODNIUS PROLIXUS.*** ***[Page 20]***
[1]Sangha, V., [2]Nachman, R.J., [1]Orchard, I. and [1]Lange, A.B.,
[1]Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.
[2]United States Department of Agriculture, College Station, TX, USA.

2:00 pm **EXAMINING EFFECTS OF OCTOPAMINE AS COTRANSMITTER IN *DROSOPHILA.*** ***[Page 21]***
Kornel, A.L. and Mercier, J.A.
Department of Biology, Brock University, St. Catharines, ON, Canada.

2:20 pm **THE INVOLVEMENT OF NUTRITIONAL SIGNALING IN REPRODUCTIVE SUCCESS OF *RHODNIUS PROLIXUS*, A VECTOR OF CHAGAS' DISEASE.** ***[Page 22]***
Leyria, J., Orchard, I. and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

2:40 – 3:00 pm **Coffee Break**: Niagara Gardenview Room

Session Chair: **Mark Rheault** (Niagara Gardenview Room)

3:00 pm **VOLTAGE GATED CALCIUM CHANNELS IN *TRICHOPLAX ADHAERENS*, AN ANIMAL WITH NO NEURONS OR MUSCLE.** ***[Page 23]***
Gauberg, J., Abdallah, S. and Senatore, A.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

3:20 pm **STRESS RESPONSE OF NINE ATP-DEPENDENT HEAT SHOCK PROTEINS IN THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (L.).** ***[Page 24]***
[1]Quan, G., [2]Duan, S.J., [1]Fick, W. and [1]Ladd, T.
[1]Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada.
[2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada.

SHORT EXPOSURES (5 MINUTE TALK and QUESTIONS)

3:40 pm **HETEROLOGOUS EXPRESSION OF *AEDES AEGYPTI* CATION CHLORIDE COTRANSPORTER 2 (AECCC2) IN *XENOPUS* OOCYTES.** ***[Page 25]***
[1]Kalsi, M., [2]Gillen, C. and [1]Piermarini, P.M.
[1]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH, USA.
[2]Department of Biology, Kenyon College, Gambier, OH, USA.

3:50 pm **THE EFFECTS OF ENVIRONMENTAL SALINITY ON AQUAPORIN EXPRESSION IN THE GASTRIC CAECA AND MALPIGHIAN TUBULES OF LARVAL *AEDES AEGYPTI*.** ***[Page 26]***
Grieco, E., Misyura, L., Jass, A. and Donini, A.
Department of Biology, York University, Toronto, ON, Canada.

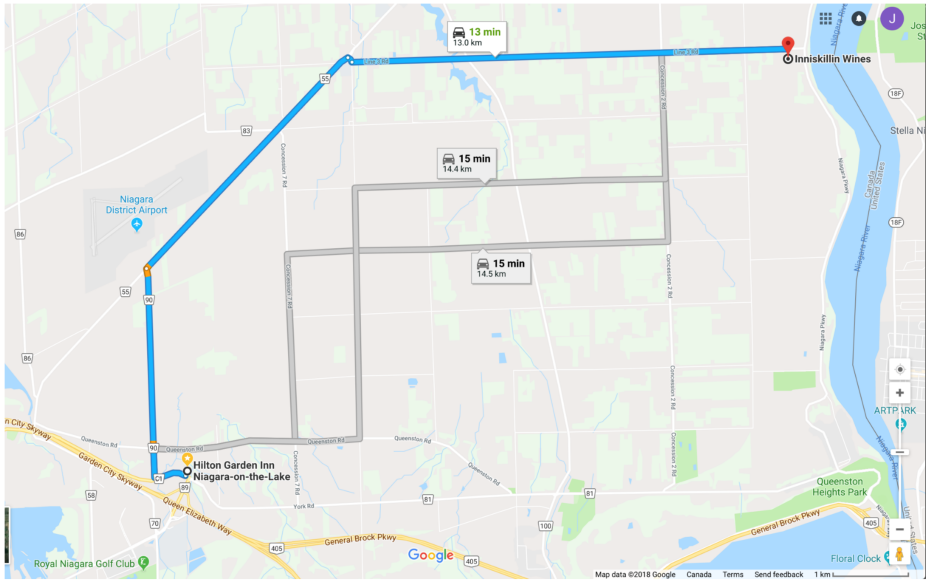
4:00pm **ACUTE EXPOSURE TO ACID/BASE ALTERS ION TRANSPORT ACROSS THE ANAL PAPILLAE OF *AEDES AEGYPTI* MOSQUITO LARVAE.** ***[Page 27]***
Jass, A., and Donini, A.
Department of Biology, York University, Toronto, ON, Canada.

4:10PM **End of Session**

6:00 to 10:00pm **Inniskillin Winery: Piazza Party**
Inniskillin Niagara Estate, 1499 Line #3, Niagara-on-the-Lake, Ontario, L0S 1JO

Gather at the Piazza for welcome reception. Throughout the evening there will be a number of wine and culinary stations and guests are free to enjoy, mingle as well as interact with Chefs at the stations and demonstration kitchen. Guests may also tour the winery at their leisure with guided tours offered throughout the evening.

Map and Directions for Banquet at Inniskillin Niagara Estate:



Estimated travel time ~13 min (13 km) via the following driving directions:

Hilton Garden Inn Niagara-on-the-Lake
500 York Rd, Niagara-on-the-Lake, ON L0S 1J0

- Head northwest on Niagara Regional Rd 81
  400 m
- Turn right onto Airport Rd/Regional Rd 90
  2.8 km
- Turn right onto Niagara Stone Rd/Regional Rd 55
  3.9 km
- At the roundabout, take the 1st exit onto Concession 6 Rd
  87 m
- Turn left onto Line 3 Rd
  5.8 km

Inniskillin Wines
1499 Line 3, Niagara-on-the-Lake, ON L0S 1J0

Friday, June 7

7:45 – 9:10 am **Breakfast:** Niagara Gardenview Room

Session Chair: **David Rocco** (Niagara Gardenview Room)

9:10 am **MALPIGHIAN TUBULES OF CATERPILLARS: RNASEQ AND PHYSIOLOGY APPROACHES TO REVEALING NOVEL REGIONAL FUNCTIONAL DIVERSITY.** ***[Page 28]***
Kolosov, D. and O'Donnell, M.J.
Department of Biology, McMaster University, Hamilton, ON, Canada.

9:30 am **HELICOKININ REGULATES ION TRANSPORT, WATER PERMEABILITY AND SEPTATE JUNCTION PERMEABILITY IN MALPIGHIAN TUBULES OF CATERPILLARS.** ***[Page 29]***
O'Donnell, M.J. and Kolosov, D.
Department of Biology, McMaster University, Hamilton, ON, Canada.

9:50 am **THE ROLE OF THE SEPTATE JUNCTION PROTEIN MESH IN *DROSOPHILA* MALPIGHIAN TUBULE.** ***[Page 30]***
Jonusaite, S. and Rodan, A.
Department of Internal Medicine, University of Utah, Salt Lake City, UT, USA.

10:10 am **PYROKININ RECEPTOR CHARACTERIZATION AND EXPRESSION IN ADULT *AEDES AEGYPTI* MOSQUITO.** ***[Page 31]***
Lajevardi, A. and Paluzzi, J-P.V.
Department of Biology, York University, Toronto, ON, Canada.

10:30 – 10:50 am **Coffee Break**: Niagara Gardenview Room

Session Chair: **Angela Lange** (Niagara Gardenview Room)

SHORT EXPOSURES (5 MINUTE TALK and QUESTIONS)

10:50 am **GENE EXPRESSION PROFILES IN HONEYBEE BRAINS USING THE FOOD SEARCH BOX PROTOCOL.** ***[Page 32]***
Morrison, B. and Zayed, A.
Department of Biology, York University, Toronto, ON, Canada.

11:00 am **IDENTIFYING THE GENETIC MARKERS FOR PATHOGEN LOADS IN THE HONEY BEE (*APIS MELLIFERA*).** ***[Page 33]***
[1]Tiwari, T., [1]Kent, C., [1]Rose, S., [1]Patel, H., [2]Dey, A., [1]Conflitti, I., Zayed, A. and [3]BeeOMICs consortium.
[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Provincial Health Services Authority, Vancouver, B.C., Canada.
[3]BeeOMICS consortium, Canada.

11:10 am **EXAMINING EFFECTS OF DROMYOSUPPRESSIN ON *DROSOPHILA* MUSCLES.** ***[Page 34]***
Wasilewicz, L.J. and Mercier, J.A.
Department of Biology, Brock University, St. Catharines, ON, Canada.

11:20 - 12:00 noon **Check out of hotel rooms**

12:00 – 1:10 pm **Lunch Break**: Niagara Gardenview Room

Session Chair: **Dennis Kolosov** (Niagara Gardenview Room)

1:10 pm **MULTIPLE FUNCTIONS OF ION TRANSPORT BY THE NUCHAL ORGAN IN EMBRYOS AND NEONATES OF *DAPHNIA MAGNA*.**
[Page 35]
Morris, C. and O'Donnell, M.J.
Department of Biology, McMaster University, Hamilton, ON, Canada.

1:30 pm **MAYFLY NYMPHS EXPOSED TO WATER CONTAMINATED WITH SUGAR BEET DE-ICING LIQUID EXPERIENCE OSMOREGULATORY ALTERATIONS.**
[Page 36]
Cuciureanu, A., Nowghani, F., Kelly, S.P. and Donini, A.
Department of Biology, York University, Toronto, ON, Canada.

1:50 pm **THE EFFECTS OF ENVIRONMENTAL SALINITY AND DIURETIC FACTORS ON THE EXCRETORY SYSTEM OF MAYFLY NYMPHS, *HEXAGENIA RIGIDA*.**
[Page 37]
[1]Nowghani, F., [2]Watson-Leung, T., [1]Donini, A. and [1]Kelly, S.P.
[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Aquatic Toxicology Unit, Ontario Ministry of the Environment, Conservation and Parks, Etobicoke, ON, Canada.

2:10 pm **Closing Remarks (Jean-Paul Paluzzi and Andrew Donini)**

BEE GENOMICS AND HEALTH

Zayed, A.

The honey bee, *Apis mellifera*, is an ecologically and economically important species that provides pollination services to natural and agricultural systems. The publication of the honey bee's genome in 2006 led to a major intensification of research on this emerging model species. Over the past five years, our group has sequenced the genomes of over 2,000 honey colonies and individuals. I will summarize our efforts to use this data to understand the bee's remarkable evolutionary history and to develop biotech tools to improve the health of failing colonies.

NEGATIVE SELECTION IN SOCIAL INSECTS

[1]Imrit, M.I., [2]Harpur, B.A., [1]Dogantzis, K. and [1]Zayed, A.

[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Department of Entomology, Purdue University, West Lafayette, IN, USA.

Eusociality, characterized in part by cooperative brood care, and reproductive division of labor, evolved independently several times in insects. The evolution of eusociality has been hypothesized to lead to differences in the extent of both positive and negative selection. While population genomics studies of eusocial insects have
so far focused on positive selection, there has been no study of the extent of negative selection in social insects, and its relationship to the evolution of caste-biased genes. To address this knowledge gap, my research will estimate the extent of negative selection in honey bees, bumble bees, and wasps, through analysis of published population genomic datasets. My study will compare the relationship between the strength of negative selection and caste-specific patterns of gene expression, and examine if the strength of negative selection correlates with the level of social complexity in this species triad.

IMPROVING GENOMIC TOOLS FOR IDENTIFYING AND TRACKING INVASIVE AFRICANIZED HONEY BEES

Dogantzis, K., Patel, H., Tiwari, T., Rose, S., Conflitti, I., Dey, A. and Zayed, A.

Department of Biology, York University, Toronto, ON, Canada.

The honey bee, *Apis mellifera*, is an ecologically and economically important species contributing to pollination services worldwide. Consequently, it is essential that potential threats to honey bee populations are identified and mitigated to prevent losses to the beekeeping industry. Africanized honey bees (AHB) are a hybrid population composed of European and African ancestry and are considered undesirable for beekeeping due to their aggressive defensive behaviour. Given the large-scale trade and movement of honey bees, there is a concern that AHBs will spread from South America and the southern United States to the rest of North America, Australia, New Zealand, and Hawaii. Developing an accurate and cost effective assay to detect AHB is an important first step towards restricting the accidental importation of AHBs. Here, we used an extensive population genomic dataset composed of individuals from all known evolutionary lineages to assess the genomic composition of Apis mellifera populations, and patterns of genetic admixture in North and South American commercial honey bee colonies. Our genomic dataset includes over 150 newly sequenced individuals from at least 14 subspecies encompassing the known distribution of Apis mellifera's native range. We used this data set to develop a SNP assay that shows high accuracy in assigning bees of unknown genetics as either African or non-African. Our SNP assay has been validated on over 2000 individuals from commercial colonies located in Canada, Texas, and Australia, as well as feral colonies from Texas, and Brazil.

NICOTINE METABOLISM IN *TRICHOPLUSIA NI*: A TOXICOGENOMIC APPROACH

Hassanpour, N., Saremba, B.N. and Rheault, M.R.

Department of Biology, University of British Columbia - Okanagan, Kelowna, BC, Canada.

The cabbage looper, *Trichoplusia ni* (T. ni) is a generalist insect (Lepidoptera: Noctuidae) that is an agricultural pest of crucifers and other crops of economic importance. The most common insecticides currently in use around the world are neonicotinoids, which are chemically similar to nicotine. Enriched metabolic detoxification of xenobiotics like nicotine have been linked to the overexpression of cytochrome P450s (CYPs) in insects. Studies in our lab have shown that the Malpighian (renal) tubules of T. ni actively excrete and detoxify nicotine into the three major metabolites; cotinine, continine-N-oxide, and nicotine-N-oxide. High-throughput transcriptome sequencing methods were used to analyze the expression profile of genes in the midgut and Malpighian tubules of both control and nicotine exposed T. ni. Sequencing identified 21,509 genes, including 577 novel genes. In nicotine exposed insects there were 929 and 721 genes showing increased expression in the midgut and Malpighian tubules, respectively. Conversely, there were 1179 and 585 genes showing decreased expression in the midgut and Malpighian tubules, respectively. Of the genes that displayed up-regulation, 15 genes in the Malpighian tubules and 9 genes in the midgut were identified to be CYPs, which are of particular interest in studies of insecticide resistance. Additional analysis of differential gene expression between isolated midgut and Malpighian tubule tissues under control and nicotine exposed conditions will be reported. This work contributes to a more thorough understanding of nicotine metabolism in general agricultural crop pests.

THE EFFECTS OF THE ENTOMOPATHOGENIC FUNGUS, *C. NEOFORMANS*, ON INTEGRATED SYSTEMS OF THE MEALWORM

Jarvie, S., Van Mierlo, V., Samarasinghe, H., Xu, J. and da Silva, R.

Department of Biology, McMaster University, Hamilton, ON, Canada.

The yellow mealworm *Tenebrio molitor*, is a pest that feeds on a wide variety of stored grains. Much like other insects, *T. molitor* possess a robust innate immune system, where pathogen detection can activate a variety of cell-mediated and humoral immune responses. One such immune response involves the production of melanin, a pigmented tyrosine-derived polymer, used to coat and neutralize pathogens. *Cryptococcus neoformans* is a pathogenic encapsulated yeast with a wide host range. *C. neoformans* produces its own fungal melanin in its cell wall, in varying amounts, to protect against host immune responses. This study investigated the effect of melanin-producing and melanin-deficient strains of *C. neoformans* on integrated cardiovascular and immune responses of *T. molitor*. We have found that, when compared to insects infected with melanin-producing fungal strains, *T. molitor* infected with melanin-deficient *C. neoformans* have a higher mortality rate, as well as lowered metabolic rate. A reduction in heart rate was also observed, but will require further experimentation to validate. The information obtained from this study sheds light on the fact that host microbe interactions are indeed complex, and that this can perhaps be taken advantage of towards the development of possible pest-management strategies.

STOP THE CROP: TOWARDS ELUCIDATING THE MODE AND MECHANISM OF ACTION OF A NATURAL PRODUCT INSECTICIDE

Piermarini, P.M., Kalsi, M. and Rakatondraibe, H.L.

Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH, USA.

We recently discovered that cinnamodial (CDIAL), a drimane sesquiterpene dialdehyde from a Madagascan medicinal plant (*Cinnamosma fragrans*), was insecticidal and repellent to mosquitoes. The mechanism of CDIAL's repellent action was mediated via its activation of transient receptor potential A1 (TRPA1) channels, but the mode and mechanism of insecticidal activity remain to be determined. To generate insights into the mode of CDIAL's insecticidal activity, we tested its effects in vitro on the spontaneous peristaltic contractions of the ventral diverticulum (crop) in adult female mosquitoes (*Aedes aegypti*). Remarkably, CDIAL completely inhibited the contractile activity of the crop. Other natural and semi-synthetic drimane sesquiterpenes with reported insecticidal activity showed similar inhibition of the crop as CDIAL, whereas those with nominal insecticidal activity did not inhibit crop contractions. To develop a hypothesis for the mechanism by which CDIAL inhibited crop activity, we screened a panel of pharmacological modulators of neuromuscular function against the crop in vitro. Our results suggest that the predominant inhibitory pathways in the crop involve the modulation of glutamate receptors and/or intracellular calcium homeostasis. Thus, we hypothesize CDIAL interacts with one or more of those mechanisms to disrupt contractile activity of the crop. In conclusion, we demonstrate a paralytic effect of CDIAL on a representative mosquito visceral muscle, which may explain its insecticidal activity against mosquitoes.

THE ROLE OF RH AND AMT PROTEINS IN AMMONIA EXCRETION DURING BLOOD MEAL DIGESTION IN THE ADULT FEMALE *AEDES AEGYPTI* MOSQUITO.

Durant, A.C. and Donini, A.

Department of Biology, York University, Toronto, ON, Canada.

In the present study, we explored the function of ammonia (NH3 and NH4+) transporters during blood meal digestion in mosquitoes. Adult females of the disease vector mosquito, *Aedes aegypti*, imbibe a large protein rich blood meal for egg development and will excrete unutilized digested protein as nitrogenous wastes. Terrestrial animals typically excrete uric acid and/or urea because ammonia is toxic at low concentrations; however, *A. aegypti* excrete ammonia as their primary nitrogenous waste. The organs and molecular mechanisms involved in ammonia excretion in the adult mosquito are not fully understood. A. aegypti possess at least four specific ammonia transporters; two Rhesus glycoproteins (Rh proteins), AeRh50-1 and AeRh50-2, which are vertebrate homologs and two ammonium transporters (Amts), AeAmt1 and AeAmt2 which are plant, fungal, and bacterial homologs. Using ion-selective micro-electrodes (ISMEs) and the Ramsay assay for analysis of Malpighian tubule (MT) secretions, we show that hemolymph NH4+ levels and NH4+ transport rates by the MT are significantly elevated up to 48 hr post blood meal. We also show that the Malpighian tubules, hindgut, and fat body contain high levels of Rh and Amt transcripts using qPCR. Immunolocalization of the Amt and Rh proteins reveals high expression in the fat body, hindgut and reproductive organs relative to other organs of blood fed female *A. aegypti*. Preliminary results using RNA interference (RNAi) suggests an important role of the Rh and Amt proteins in facilitating ammonia transport during blood meal digestion of adult mosquitoes.

GLYCOPROTEIN HORMONE SIGNALING AND ITS INVOLVEMENT IN MOSQUITO (*AEDES AEGYPTI*) SPERMATOGENESIS.

Rocco, D., and Paluzzi, J-P.V.

Department of Biology, York University, Toronto, ON, Canada.

GPA2/GPB5 and its receptor (LGR1) represent an ancient glycoprotein hormone-signaling system identified in the genomes of both vertebrates and invertebrates, however its function remains elusive. To determine the function of GPA2/GPB5 in the mosquito *Aedes aegypti*, we aimed to characterize the expression profile of GPA2/GPB5 and LGR1 in the adult stage, elucidate downstream signaling pathways and utilize reverse genetics (i.e. RNA interference). Immunohistochemical, RT-qPCR and in situ hybridization data have revealed GPA2 and GPB5 subunit expression co-localize in bilateral pairs of neuroendocrine cells situated within the first five abdominal ganglia of adult mosquitoes. To study dimerization patterns of the *A. aegypti* GPA2 and GPB5 hormone subunits, immunoblotting experiments were performed using protein collected from HEK293T cells transiently expressing *A. aegypti* GPA2/GPB5. Moreover, the functionality of individual subunits and GPA2/GPB5 in LGR1 activation using bioluminescent reporter assays was examined. Subcellular immunolocalization analyses of LGR1 throughout spermatogenesis in adult testes determined LGR1 localized to the plasma membrane of spermatids and is associated with the centriole adjunct, which is a region responsible for coordinating the proper development of flagella. LGR1 knockdown using RNAi resulted in significant spermatozoa defects, such as shortened flagella, in adult males. Moreover, LGR1 knockdown mosquitoes possessed an average ~60% less sperm and were less fertile than controls. Taken together, this data supports the notion that GPA2/GPB5 and LGR1 signaling regulates male reproductive biology, a significantly understudied research area in mosquitoes and other insects.

ANTI-DIURETIC HORMONE ACTIVITY AND SIGNALING CASCADE OF CAPA NEUROPEPTIDE IN MOSQUITO, *AEDES AEGYPTI*

Sajadi, F. and Paluzzi, J-P.V.

Department of Biology, York University, Toronto, ON, Canada.

Female *Aedes aegypti* mosquitoes face the challenge of excess water and ion intake after a blood meal. To cope with this, blood-feeding insects have a highly active excretory system that includes the Malpighian tubules (MTs), which are under rigorous control by neuroendocrine factors to regulate transepithelial movement of ions and osmotically-obliged water. While CAPA exhibits variable response in insect species, the role and signaling pathway of these peptides remains unclear in adult *Aedes* mosquitoes. Given that CAPA receptor transcript was localized to the principal cells of the MTs, we sought to examine the effects of a mosquito CAPA peptide family member, AedaeCAPA-1, on adult female MTs stimulated with various diuretic factors. AedaeCAPA-1 was found to inhibit secretion of MTs stimulated by select diuretic factors, 5-HT and DH31. Additionally, although AedaeCAPA-1 elicits anti-diuretic activity, it does not influence the relative proportions of cations transported by adult MTs, thus maintaining the kaliuretic activity of 5-HT and the natriuretic activity of DH31. Effects of second messenger cGMP tested on adult MTs revealed that both 5-HT and DH31–stimulated secretion is strongly inhibited by cGMP, similar to effects seen with AedaeCAPA-1. Furthermore, pharmacological inhibition of PKG/NOS signaling abolishes the anti-diuretic activity of AedaeCAPA-1, which collectively confirms the role of cGMP/PKG/NOS in the CAPA signaling pathway. Notably, the inhibitory effect of CAPA was also abolished through knocking down the receptor, verifying its role in anti-diuresis. Further understanding of the role of each specific hormone family, including both diuretic and anti-diuretic factors, will help resolve this complex regulatory network.

THE ROLE OF SIFAMIDE AS A NEUROMODULATOR AND NEUROHORMONE IN THE BLOOD-GORGING INSECT *RHODNIUS PROLIXUS.*

Ayub, M., Lange, A.B. and Orchard, I.

Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

SIFamides are a family of highly conserved neuropeptides among arthropods, expressed mainly in four medial neurons in the pars intercerebralis of the brain. In *Rhodnius prolixus*, we have previously shown processes projecting into the corpus cardiacum and along the dorsal vessel, indicating for the first time in insects, that SIFamide may be a neurohormone. In this study, we explore the function of SIFamide in *R. prolixus* (Rhopr-SIFa), specifically in relation to feeding. *R. prolixus* is a blood-gorging insect and a vector for human Chagas disease. Consumption of a blood meal triggers important developmental events for this hemimetabolous hemipteran. Immunohistochemistry of the CNS showed diminished SIFamide-like staining in the neurons in the brain two hours following feeding, and restocking of those cells 24 hours later, suggesting Rhopr-SIFa is involved in feeding. The results of temporal qPCR analysis were consistent with the immunohistochemical findings, showing an increase in Rhopr-SIFa transcript expression in the brain two hours after feeding, suggesting restocking in the medial neurons following release of the peptide during feeding. We also observed enhanced feeding (increased size of meal) in insects injected with Rhopr-SIFa, in comparison to those injected with saline. In addition, insects with RNAi mediated knockdown of Rhopr-SIFa transcript consumed a significantly smaller blood meal relative to controls. This data suggests that the four SIFamidergic neurons and associated arborizations may play an important function in the neuronal circuitry controlling *R. prolixus* feeding, as a central and peripheral neuromodulator/neurohormone.

THE INTERACTION OF KININ AND CAPA ANALOGS IN *RHODNIUS PROLIXUS*

[1]Sangha, V., [2]Nachman, R.J., [1]Orchard, I. and [1]Lange, A.B.

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The kinin and CAPA family of neuropeptides are responsible for a variety of physiological processes in insects. In *Rhodnius prolixus*, Rhopr-kinins stimulate hindgut contractions, and RhoprCAPA-2 is an anti-diuretic hormone. The effects of these neuropeptides are mediated by G protein-coupled receptors, with intracellular calcium and cGMP possibly acting as secondary messengers. In *R. prolixus*, the CAPA transcript encodes for 3 peptides: RhoprCAPA-1, RhoprCAPA-2, RhoprCAPA-pk1. A CAPA receptor that binds RhoprCAPA-2 and, in a minor way, RhoprCAPA-pk1, and a pyrokinin receptor that binds RhoprCAPA-pk1, are expressed in the hindgut of *R. prolixus*. Neuropeptide analogs for Rhopr-kinin 2 and RhoprCAPA-2 have been synthesized, with changes to their amino acid sequences, and the analogs still possess the ability to bind to their specified GPCRs in other insects. Here we show that these kinin and CAPA analogs elicit more potent effects on hindgut contractions of *R. prolixus* compared to the native neuropeptides and are implicated in altering the feeding behaviour and rate of diuresis of the insect. In order to elucidate the role of the kinin signaling system in feeding and diuresis related behaviours, the Rhopr-kinin receptor has been targeted using RNA interference (RNAi). RNAi mediated silencing of the kinin receptor resulted in decreased effects of Rhopr-kinin 2 and its analog on the hindgut.

EXAMINING EFFECTS OF OCTOPAMINE AS COTRANSMITTER IN *DROSOPHILA*

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Drosophila larvae serve as a model system for studying modulation of synapses. Motor neurons innervating the body wall muscles release glutamate (L-Glu) as the "classical" neurotransmitter to depolarize the muscle fibers and initiate contraction. The motor neurons also contain octopamine (OA) and several neuropeptides that are thought to act as cotransmitters. To examine a role for OA as a cotransmitter, muscle contractions were evoked by stimulating the motor neurons, and OA was applied in the bath. At high (50 Hz) and low (5 Hz) stimulus frequencies, 10^{-6} M OA enhanced the muscle contractions but 10^{-8} M OA did not. The effect of 10^{-6} M OA was blocked by the simultaneous application of 10^{-5} M phentolamine, an OA antagonist. At an intermediate stimulus frequency, 32 Hz, both high and low concentrations of OA enhanced the nerve-evoked contractions. At this stimulus frequency, however, the enhancement by 10^{-6} M OA was not blocked by phentolamine at 10^{-5} or 10^{-4} M concentrations. These results suggest some frequency-dependence of octopamine's modulatory effects. To investigate whether OA is released as a cotransmitter, we examined effects of phentolamine by itself on nerve-evoked contractions. Phentolamine (10^{-5} M) did not alter nerve-evoked contractions, which does not support the hypothesis that OA is released by nerve stimulation in our trials. We are presently investigating whether or not OA can act postsyanptically by modulating glutamate-evoked contractions.

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THE INVOLVEMENT OF NUTRITIONAL SIGNALING IN REPRODUCTIVE SUCCESS OF *RHODNIUS PROLIXUS*, A VECTOR OF CHAGAS' DISEASE

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Nutrients are critical for successful insect reproduction. In many insects, yolk deposition (called vitellogenesis) is initiated by the uptake of proteins, lipids and amino acids (AAs). In this sense, the amino acid/target of rapamycin (AA/TOR) and insulin pathways have vital roles as nutritional sensors, affecting reproductive tissues and controlling the biosynthesis of hormones. Over the past century, *Rhodnius prolixus*, a primary vector of the etiological agent of Chagas´ disease, has been the subject of intense investigations, which have contributed to our understanding of important aspects of metabolism and physiology in insects. The aim of the current work was to study events involved in egg formation, focusing on AA/TOR and insulin pathways. Using *R. prolixus* as a model, we have performed experiments on non-vitellogenic (unfed condition) and vitellogenic (fed condition) females. By qPCR, transcriptome (RNA seq) and Western Blot of the central nervous system (CNS), fat bodies and ovaries, we have evidence that the genes involved with nutritional signaling are expressed in both, non-vitellogenic and vitellogenic tissues. However, after the blood meal, an up-regulation post-translational of AA/TOR and insulin pathways is observed. By immunofluorescence, we have identified cells in the CNS with positive signal to insulin and have shown that the insulin receptor (InR) in oocytes is differentially expressed according to the stage of development. Using RNA interference for the InR and mTOR, we tested the involvement of the nutritional signaling in synthesis of yolk precursor proteins and oocyte development. As reproduction is an event responsible for propagation of insect populations, this work is important to lead the development of innovative biocontrol methods.

VOLTAGE GATED CALCIUM CHANNELS IN *TRICHOPLAX ADHAERENS*, AN ANIMAL WITH NO NEURONS OR MUSCLE.

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In excitable cells, voltage-gated calcium (CaV) channels are necessary for rapidly converting electrical signals into cytoplasmic calcium signals. Most animals have three types of voltage gated calcium (CaV) channels, CaV1, CaV2, CaV3, which overlap in function but also exhibit unique specializations that are highly conserved among phyla. For example, CaV1 channels are expressed in muscle where they drive muscle contraction, while CaV2 channels are expressed in nerve terminals where they drive exocytosis of synaptic vesicles. Although these functions are conserved across many animal phyla, the evolution of this functional divergence is unclear. To gain a better understanding of the divergence of these channels, we are studying the most early-diverging animal to possess all three types of CaV channels, *Trichoplax adhaerens*. Remarkably, *Trichoplax* lacks neurons and muscle and yet demonstrates complex and coordinated motile behaviour, including feeding, chemotaxis, and phototaxis. *Trichoplax* has six functionally distinct cell types, including contractile and neuroendocrine-like cells. We are exploring whether *Trichoplax* CaV1 and CaV2 channels are expressed in these cells, and whether they provide them with “muscle-like” and “neuron-like” qualities. This study examines the biophysical properties of *Trichoplax* CaV1 and CaV2 channels as well as CaV channel localization. Our work will provide important insights into the evolution of Cav1 and Cav2 channel biophysical properties, cellular localization, and physiological functions.

STRESS RESPONSE OF NINE ATP-DEPENDENT HEAT SHOCK PROTEINS IN THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (L.)

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Most heat shock proteins (HSPs) function as molecular chaperons that help organisms to deal with stress of both an internal and external nature. Based on their molecular weights, insect HSPs have been classified into four families: HSP90, HSP70, HSP60, and small heat shock proteins (sHSPs). Members of the HSP90, HSP70 and HSP60 have been highly conserved during evolution and are all ATP dependent. In contrast, the sHSPs are less conserved and are ATP independent. Many insect HSPs are up-regulated by diverse stresses, including heat, cold, starvation, anoxia, infection, ultraviolet light and numerous chemicals. They have been linked with insect stress tolerance, and it has been suggested that they have important roles affecting pest insect population size and geographical distribution. Previously, we identified 14 sHSP genes from the spruce budworm, a major forest pest in North America, and found that individual sHSPs have different expression patterns during different diapause phases. In this paper, we will present the identification and characterization of nine ATP-dependent HSPs, as well as their expression profiles under normal and stressful conditions including heat, cold, starvation, and virus infection. These results suggest that the ATP-dependent HSPs have important roles under normal and stressful conditions in the spruce budworm.

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HETEROLOGOUS EXPRESSION OF *AEDES AEGYPTI* CATION CHLORIDE COTRANSPORTER 2 (AECCC2) IN *XENOPUS* OOCYTES

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The functional properties of insect cation chloride cotransporter (CCCs) have not been extensively characterized. The goal of the current study was to heterologously express *Aedes aegypti* CCC2 (aeCCC2) in *Xenopus* oocytes and characterize its transport properties for the first time. Western blotting confirmed heterologous expression of aeCCC2 immunoreactivity in oocytes injected with aeCCC2 cRNA (aeCCC2 oocytes). The aeCCC2 oocytes were characterized by a greater uptake of Li+ (a tracer for Na+), but not Rb+ (a tracer for K+), compared to water-injected (control) oocytes. Surprisingly, the Li+ uptake was independent of extracellular chloride and insensitive to common inhibitors of CCCs (e.g., bumetanide) or Na+-transporters/channels (e.g., amiloride). Two-electrode voltage clamping (TEVC) of aeCCC2 oocytes revealed an enhanced conductance for Na+ and Li+, but not K+, compared to control oocytes. The functional properties of aeCCC2 and its physiological role in mosquitoes remains to be determined.

THE EFFECTS OF ENVIRONMENTAL SALINITY ON AQUAPORIN EXPRESSION IN THE GASTRIC CAECA AND MALPIGHIAN TUBULES OF LARVAL *AEDES AEGYPTI*

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The larvae of the disease vector *Aedes aegypti* can survive in aquatic habitats of varying salinity, from hypo-osmotic freshwater (FW) to brackish-water (BW) that is iso-osmotic to the larval haemolymph. These environments pose different osmoregulatory challenges. Previous studies have shown increased levels of haemolymph Na+ ions when larvae are exposed to BW and this study shows that larvae reared in ion-poor water (IPW) have lower K+ and Na+ levels in the haemolymph compared to larvae reared in FW. The gastric caeca (GC) and Malpighian tubules (MTs) are osmoregulatory organs in which ion transport and transporter expression has been studied; however, comparatively little is known about water transport. Aquaporins (AQPs) are water and/or solute channels which mediate water flux across cell membranes. This study examined the expression of AQP1 and AQP5 in the GC and MTs of larvae reared in different salinities using immunohistochemistry and Western blots. Water-specific AQP1 was found in the apical membranes of both GC and MTs; intensity and protein expression were not affected by salinity. AQP5 is a water/solute channel and was localized in the GC basolateral membrane; and previously in the MT principal cells. Exposure to BW resulted in increased staining intensity but a decreasing trend in AQP5 protein abundance in the GC. Using RNAi we show that whole organism knockdown of AQP5 affects larval survival in different salinities; we previously reported that larval survival decreased in FW and now show that survival also decreases in BW but not in IPW after treatment with AQP5 dsRNA. Our results suggest that aquaporins in the GC and MTs play an important role in osmoregulation and contribute to the ability of larval mosquitoes to tolerate water with different salt levels.

ACUTE EXPOSURE TO ACID/BASE ALTERS ION TRANSPORT ACROSS THE ANAL PAPILLAE OF *AEDES AEGYPTI* MOSQUITO LARVAE

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Aedes aegypti mosquito larvae are tolerant of a wide pH range, contributing to their ability to inhabit harsh aquatic environments, such as acidic drain water and raw sewage. We predicted that this capacity involved the use of the anal papillae, ionoregulatory organs that have previously been shown to be acid-base permeable. Although ion movements across the papillae under neutral conditions have been well characterized, little is known about how these organs respond to altered pH. In this study, we used the scanning ion-selective electrode technique (SIET) to demonstrate reversal of H+, Na+, and NH4+ transport directions upon acute exposure to acidic treatment. In contrast, short exposure to base increased and decreased NH4+ and H+ efflux, respectively, while having no impact on Na+ transport. Furthermore, we found that the low pH component of high external ammonia induces NH4+ influx. We speculate that these effects may be caused in part by sodium-hydrogen exchangers (NHEs). Counter to their proposed function, our findings suggest that the anal papillae may promote ion dysregulation in environmental extremes.

MALPIGHIAN TUBULES OF CATERPILLARS: RNASEQ AND PHYSIOLOGY APPROACHES TO REVEALING NOVEL REGIONAL FUNCTIONAL DIVERSITY

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Malpighian tubules of most insects demonstrate proximodistal heterogeneity in function. Such heterogeneity is typically confined to ion/fluid secretion in the distal portion and ion/fluid reabsorption in the proximal portion. By contrast, MTs of larval Lepidoptera (caterpillars of butterflies and moths), are comprised of five regions that differ in their association with the gut, their structure and their ion/fluid transport function. Recent studies have also shown that several regions can rapidly and reversibly switch between ion secretion and reabsorption. The current study employed RNAseq, pharmacology and electrophysiology to characterize four distinct regions of the MT in larval *Trichoplusia ni*. Luminal microelectrode measurements indicate that fluid changes in [K+], [Na+] and pH as it passes through different regions of the tubule. Additionally, the regions examined differ in gene ontology term enrichment of expressed transcripts, and also demonstrate robust differences in expression of ion transporters and endocrine ligand receptors. Lastly, the study provides evidence for direct involvement of voltage-gated and ligand-gated ion channels in epithelial ion transport of insect Malpighian tubule.

HELICOKININ REGULATES ION TRANSPORT, WATER PERMEABILITY AND SEPTATE JUNCTION PERMEABILITY IN MALPIGHIAN TUBULES OF CATERPILLARS

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Malpighian tubules (MTs) of insects rely on active ion transport to drive fluid secretion into their lumen osmotically. Additionally, changes in the paracellular junction permeability may play an important role in regulating the transport of ions and organic solutes into the tubule. Thus, a secreting tubule will rely on a combination of sustained active ion transport, water permeability, and paracellular junctional permeability. MTs of lepidopteran larvae consist of four distinct functional regions that differ in the mechanisms of ion and water transport as well as their endocrine control. Several recent studies in our lab demonstrated that one of these regions, the distal ileac plexus (DIP), is capable of rapidly and reversibly switching between ion secretion and reabsorption. This is thought to take place to facilitate ion retention, haemolymph expansion and to maintain MT function in the face of changing dietary ion availability. The switchover from ion secretion to ion reabsorption is accompanied by a reduction in water and paracellular junction permeability to avoid harmful back-flux of water and organics. We have to date demonstrated that the neuropeptide Helicokinin reduces K+ secretion, water permeability and paracellular septate junction permeability. All of these parameters are reduced in the DIP of larvae undergoing ion transport reversal. We thus conclude that HK plays an important role in facilitating the rapid reversible switch between ion secretion and ion reabsorption in the DIP of lepidopteran larvae.

THE ROLE OF THE SEPTATE JUNCTION PROTEIN MESH IN *DROSOPHILA* MALPIGHIAN TUBULE

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Septate junctions (SJs) are specialized occluding cell-cell junctions that form paracellular diffusion barriers in the epithelia of invertebrates. In arthropods, two types of SJs, i.e. pleated SJs (pSJs) and smooth SJs (sSJs), are present and in *Drosophila*, a number of pSJ-associated proteins have been identified and shown to be involved in epithelial barrier function. However, proteins of sSJs have received considerably less attention and many questions about their role in maintaining epithelial integrity and regulating paracellular solute transport remain open. Here, we studied integral sSJ protein Mesh in adult fly Malpighian tubules, which rely on regulated transepithelial ion and water transport to maintain internal homeostasis. To examine Mesh function in the tubule epithelium, the GAL4-UAS system and a temperature sensitive GAL4 repressor, tub-GAL80ts20, were used to achieve tubule principal cell specific mesh knockdown throughout development or during adulthood. We found that developmental mesh knockdown in the tubule principal cells was associated with early lethality in adult flies and bloated abdomen phenotype. The tubules of these flies revealed defects in epithelial architecture, SJ organization and complete abolishment of transepithelial fluid secretion and K+ flux. Furthermore, tubules subjected to principal cell mesh knockdown in adulthood had reduced transepithelial fluid and ion transport in conjunction with reduced paracellular permeability, despite intact SJ architecture. These data suggest that sSJ protein Mesh is essential for the development and maintenance of a functional *Drosophila* Malpighian tubule epithelium and support the notion that integral SJ proteins play an important role in insect ionoregulatory epithelia and homeostasis.

PYROKININ RECEPTOR CHARACTERIZATION AND EXPRESSION IN ADULT *AEDES AEGYPTI* MOSQUITO

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Pyrokinin neuropeptides, characterized by a conserved FxPRLamide C-terminal motif, have been identified as myotropic and pheromonotropic in some insects, but their functions remain unclear in blood-feeding arthropods. Herein, we have functionally deorphanized two pyrokinin receptors (PK1-R and PK2-R) identified in the yellow fever mosquito, *Aedes aegypti*, by profiling their selective and dose-dependent activation in response to pyrokinins and related peptides. We have also examined receptor transcript expression in adult mosquito organs, which showed enrichment of PK1-R in the rectum, and PK2-R in the ileum and reproductive organs. Immunohistochemical mapping in female adult mosquitoes revealed pyrokinin-like immunostaining in nerve projections innervating the rectum and terminating in close association to the rectal pads, which are structures proposed to be involved in ion transport. Coupling this to PK1-R enrichment in the rectum at the transcript level, we further examined prospective physiological roles of its confirmed ligand (AedaePK1) utilizing in vitro bioassays. Interestingly, AedaePK1 did not influence myotropic or ionomodulatory (Na+) activity in isolated recta. As a result, ongoing studies are examining the effects of receptor knockdown on diuresis, excretion, and reproductive biology, processes that heavily rely on organs where these receptors are enriched, in an attempt to better understand the functional role of pyrokinin signaling in adult mosquitoes.

GENE EXPRESSION PROFILES IN HONEYBEE BRAINS USING THE FOOD SEARCH BOX PROTOCOL

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Honeybees are central place foragers. Part of what allows the bees to improve their foraging skills is their ability to learn where food patches are relative to the hive, avoid predation risk and navigate new locations. The molecular mechanisms underlying this learning ability have been investigated by using Proboscis Extension Reflex (PER) and free-flight learning protocols. Examples of genes which are up-regulated in response to visual and olfactory learning in honeybees include Early Growth Response protein 1 (EGR1), cAMP response element binding protein (CREB), dopamine receptor 1 (dop1) and dopamine receptor 2 (dop2). However, very little attention has been given to changes in gene expression during spatial learning, a key feature of navigation in honeybees. Here, we utilize a newly developed learning protocol called the Food Search Box (FBS) paradigm to study spatial learning in the honeybee. By conducting time-course experiments in the context of FBS, we are investigating how gene expression changes over time in response to spatial learning.

IDENTIFYING THE GENETIC MARKERS FOR PATHOGEN LOADS IN THE HONEY BEE (*APIS MELLIFERA*)

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The honey bee, *Apis mellifera* is a model organism for sociogenomics and is one of the most important managed pollinators. As such, recent threats to honey bee health are particularly alarming. The social honey bees live in highly crowded nests providing favorable conditions for the spread of infectious diseases. But honey bees have several social and individuals mechanisms for protecting themselves against disease. The BeeOMICS consortium has sequenced the genomes of approximately 1,000 honey bee colonies in Canada, which were evaluated for a number of traits, including the abundance of several pathogens within each colony. I plan to carry out genome-wide association studies (GWAS) on colony pathogen loads to gain a deeper insight of the genetics of immunity in honey bees. This research will set the groundwork for breeding disease resistant honey bees using marker assisted selection.

EXAMINING EFFECTS OF DROMYOSUPPRESSIN ON *DROSOPHILA* MUSCLES

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In *Drosophila melanogaster*, FMRFamide-like peptides comprise a number of important modulators involved with muscular contractions. FMRFamide-like peptides have been examined in other insects such as locust or cockroach, through which some of the functional roles of these peptides have been determined. Of particular interest is myosuppressin family (-HVFLRFamide N-terminal sequence), that inhibit muscular contractions (i.e. heart, crop, oviduct) via activation of specific myosuppressin receptors. Previoius work reported that nerve-evoked contractions of 3rd instar larval body-wall muscles are not altered by Dromyosuppressin (TDVDHVDFLRFamide). We find that a 1 min exposure to 10 nM – 1 μM Dromyosuppressin decreases the amplitude of body-wall muscle contractions subsequently induced by 300 mM KCl. We will determine whether this effect is mediated through activation of one or both of the *Drosophila* myosuppressin receptors, DmsR-1 and DmsR-2. We will also examine the time course of inhibition by Dromyosuppressin and the threshold of inhibition, and we will determine whether muscle contractions induced by L-glutamate are also modulated by prior Dromyosuppressin exposure.

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MULTIPLE FUNCTIONS OF ION TRANSPORT BY THE NUCHAL ORGAN IN EMBRYOS AND NEONATES OF *DAPHNIA MAGNA*

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The nuchal organ, also referred to as the dorsal organ or neck organ, is a dorsal structure located posteriorly to the eye, between the bases of the second antenna of embryonic and neonate branchiopod crustaceans such as the 'water flea', *Daphnia magna*. The ultrastructure of the nuchal organ is similar to ion-transporting tissues in other crustaceans, including extensive amplification of apical and basal plasma membranes through microvilli and infoldings and abundant mitochondria, but direct evidence for ion transport is lacking. We have used the Scanning Ion-selective Electrode Technique to measure transport of Na^+, K^+, H^+, Cl^-, NH_4^+ and Ca^{2+} across the nuchal organ. Influx of Na^+ and efflux of H^+ and NH_4^+ were measured across the nuchal organ of both embryos and neonates. We propose that the efflux of K^+ and Cl^- across the nuchal organ in embryos is related to the expansion of the haemocoel and release of intracellular solutes into the extracellular space during development. K^+ is taken up later during development, coincident with re-expansion of the intracellular compartment through development of gills and other organs. Ca^{2+} influx in neonates is presumably related to calcification of the exoskeleton. Our data thus support roles of the nuchal organ in ionoregulation, pH regulation, nitrogenous waste excretion, and calcification.

MAYFLY NYMPHS EXPOSED TO WATER CONTAMINATED WITH SUGAR BEET DE-ICING LIQUID EXPERIENCE OSMOREGULATORY ALTERATIONS

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Mayfly nymphs are established bio indicators of freshwater (FW) quality. This study examined the ion/osmoregulatory physiology of *Hexagenia limbata* nymphs following exposure to beet juice de-icing (BJD) liquid, an alternative to rock salt. The LC50 of BJD liquid was determined to be 4%, therefore a sub-lethal dose of 3.2% was selected for a 7 day exposure and following this ion (K^+, Na^+, Cl^-, NH_4^+ and H^+) concentrations in nymph hemolymph were examined as well as ion fluxes across tracheal gills and rectum. The presence of 3.2% BJD liquid in FW distinctly elevated environmental Na^+, Cl^-, K^+, and NH_4^+ levels as well as pH, but all remained well below their individual LC50. In the hemolymph, Na^+, Cl^- and pH were higher in 3.2% BJD-exposed nymphs compared to FW nymphs, but other ions did not significantly differ. Na^+ was absorbed at the rectum regardless of treatment, while the gills in nymphs exposed to 3.2% BJD excreted Na^+, which was in contrast to the Na^+ uptake observed across FW nymph gills. Observations of systemic perturbations in Na^+, Cl^- and pH balance indicate that these mayfly nymphs attempt to deal with 3.2% BJD liquid exposure, in part, by excreting sodium via the gills.

THE EFFECTS OF ENVIRONMENTAL SALINITY AND DIURETIC FACTORS ON THE EXCRETORY SYSTEM OF MAYFLY NYMPHS, *HEXAGENIA RIGIDA*

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Salt contamination of freshwater (FW) from human activities (e.g. mining, agriculture and winter de-icing) is a global concern. FW dwelling mayfly nymphs appear to be particularly sensitive to salt contamination but very little is known about their ion/osmoregulatory response to this environmental insult. This study found that exposing nymphs of the mayfly (*Hexagenia rigida*) to salt contaminated water (SCW; 7 days in FW containing 7.25 g/L NaCl) resulted in a significant decrease in K+ reabsorption at the Malpighian tubules (MTs) and the hindgut (HG), organs which comprise the excretory system. To isolate the function of the MTs, Ramsay secretion assays were utilized to measure fluid secretion rates of MTs from FW and SCW nymphs in response to various diuretic factors. Neither the FW nor SCW MTs secreted fluid unless stimulated. cAMP resulted in elevated secretion rates in both FW and SCW tubules, cGMP completely inhibited fluid secretion in the SCW tubules while thapsigargen promoted fluid secretion in SCW tubules. Interestingly, in SCW a significant decline and a trend towards decline in Na+-K+-ATPase and V-type H+-ATPase activities in the MT and HG respectively, may suggest the role of cGMP in response to increased salinity. This study provides a first look at how salt contamination of FW can impact the normal transport processes of a mayfly nymph excretory system.