INSECT BIOTECH CONFERENCE

Annual Scientific Meeting

June 6-8, 2018

Schedule and Contributed Abstracts

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

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Insect Biotech Conference – 2018

Conference Schedule

Wednesday Evening – June 6

6:00 pm	**Registration:** Outside Niagara Gardenview Room
7:00 pm	**Plenary Talk:** Gardenview Room **CHARACTERIZING MOLECULAR DIFFERENCES THAT LEAD TO SEASONAL CHANGES IN MOSQUITO PHYSIOLOGY.** ***[Page 1]*** Megan E. Meuti, Department of Entomology, The Ohio State University, Columbus, Ohio, USA
7:45 – 9:00 pm	**Reception**: Niagara Gardenview Room (Pizzeria and Beverages)

Thursday, June 7

7:30 – 8:50 am **Breakfast:** Niagara Gardenview Room

8:55 am **Opening Remarks:** Niagara Gardenview Room: **Jean-Paul Paluzzi**

Session Chair: **Jean-Paul Paluzzi** (Niagara Gardenview Room)

9:00 am **EXPRESSION OF AMMONIA TRANSPORTERS IN THE OSMOREGULATORY ORGANS OF AEDES AEGYPTI LARVAE IN FRESHWATER AND HIGH AMMONIA.** ***[Page 2]***
Durant, A. and Donini, A.
Department of Biology, York University, Toronto, ON, Canada.

9:20 am **THE GASTRIC CAECUM OF LARVAL AEDES AEGYPTI: STIMULATION AND INHIBITION OF EPITHELIAL ION TRANSPORT.** ***[Page 3]***
D'Silva, N.M. and O'Donnell, M.J.
Department of Biology, McMaster University, Hamilton, ON, Canada

9:40 am **EXPRESSION PATTERN AND SEQUENCE ANALYSIS OF AEDES AEGYPTI SODIUM-DEPENDENT CATION-CHLORIDE COTRANSPORTERS.** ***[Page 4]***
[1]Gillen, C., and [2]Piermarini, P.M.
[1]Kenyon College, Gambier, OH, USA and [2]Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH, USA

10:00 am **ORCHESTRATION OF DROSOPHILA POST-FEEDING PHYSIOLOGY AND BEHAVIOR BY THE NEUROPEPTIDE LEUCOKININ.** ***[Page 5]***
[1]Zandawala, M. , [2]Yurgel, M., [1]Liao, S., [2]Keene, A.C. and [1]Nässel, D.R.
[1]Department of Zoology, Stockholm University, Stockholm, Sweden
[2]Department of Biological Sciences, Florida Atlantic University, Jupiter, FL, USA

10:20 – 10:40 am **Coffee Break**: Niagara Gardenview Room

Session Chair: **Andrew Donini** (Niagara Gardenview Room)

10:40 am **INVESTIGATING THE IMPORTANCE OF TWO ZINC FINGER DOMAINS IN THE AUTOGRAPHA CALIFORNICA NUCLEOPOLYHEDROVIRUS ME53 PROTEIN.** ***[Page 6]***
Ralph, R. and Krell, P.J.
Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada.

11:00 am **INTERACTION BETWEEN VP80 AND ME53 FROM AUTOGRAPHA CALIFORNICA NUCLEOPOLYHEDROVIRUS.** ***[Page 7]***
Özşahin, E. and Krell, P.J.
Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada.

11:20am **INTERACTIONS BETWEEN THE INSECT IMMUNE SYSTEM AND THEIR PATHOGENS.** ***[PAGE 8]***
Mierlo, V. V., Radauskas, V.J., Tahir, I. Samarasinghe, H., Xu, J. and da Silva, R.
Department of Biology, McMaster University, Hamilton, ON, Canada

11:40 am **DIRECT EFFECT OF DEFENSIVE PHYTOHORMONES ON CATERPILLAR HERBIVORES.** ***[Page 9]***
Su, H., Smith, R. and Bede, J.
Department of Plant Biology, McGill University, Ste-Anne-de-Bellevue, QC, Canada

12:00 – 1:10 pm **Lunch Break**: Niagara Gardenview Room (Executive Deli)

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

1:10 pm **ANALYSIS OF WALKING BEHAVIOUR AT YOUNG AGES IN LONG-LIVED FLIES.** ***[Page 10]***
Xiao, C., Chippindale, A. and Robertson, R.M.
Department of Biology, Queen's University, Kingston, ON, Canada

1:30 pm **CHARACTERIZATION OF CAV1 VOLTAGE-GATED CALCIUM CHANNELS IN THE BASAL ANIMAL TRICHOPLAX ADHAERENS.** ***[Page 11]***
Gauberg, J. and Senatore, A.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

1:50pm **A PARADIGM TO STUDY MODULATION OF MUSCLE CONTRACTION IN DROSOPHILA.** ***[Page 12]***
Jung, J. Kornel, A. and Mercier, A.J.
Department of Biological Sciences, Brock University, St. Catharines, ON, Canada.

2:10 pm **IDENTIFICATION AND CHARACTERIZATION OF DENSE CORE VESICLE TRAFFICKING AND FUSION AT THE DROSOPHILA NMJ.** ***[Page 13]***
[1,2]Ormerod, K.G. and [1,2]Littleton, J.T.
[1]Massachusetts Institute of Technology, Picower Institute for Learning and Memory, Cambridge, MA, USA.
[2]Massachusetts Institute of Technology, Department of Biology, Cambridge, MA, USA.

2:30 pm **EXAMINING PROCTOLIN AND OCTOPAMINE AS CO-TRANSMITTERS IN DROSOPHILA.** ***[Page 14]***
Kornel, A.L., Jung, J.H., Aksamit, S. and Mercier, A.J.
Department of Biology, Brock University, St. Catharines, ON, Canada.

2:40 pm **EXAMINING NEUROPEPTIDE FUNCTION IN DROSOPHILA MELANOGASTER USING THE CRISPR/CAS9 SYSTEM.** ***[Page 15]***
Aksamit, S., Mansour, H., Li, F., Necakov, A. and Mercier, A.J.
Department of Biology, Brock University, St. Catharines, ON, Canada.

2:50 – 3:10 pm **Coffee Break**: Niagara Gardenview Room

Session Chair: **Joffre Mercier** (Niagara Gardenview Room)

3:10 pm **PHYSIOLOGICAL EFFECTS OF STRUCTURAL ANALOGS OF KININS, TACHYKININS, AND CAPA IN RHODNIUS PROLIXUS.** ***[Page 16]***
Sangha, V., Lange, A.B. and Orchard, I.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

3:30 pm **EXAMINING RECEPTOR LOCALIZATION AND PHYSIOLOGICAL FUNCTION OF A PYROKININ NEUROPEPTIDE IN AEDES AEGYPTI.** ***[Page 17]***
[1]Lajevardi, A., [2]Brown, M.R. and [1]Paluzzi, J.P.
[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Department of Entomology, University of Georgia, Athens, GA, USA.

3:50pm **THE ROLE OF SULFAKININS ON FEEDING IN RHODNIUS PROLIXUS.** ***[Page 18]***
Al-Alkawi, H., Lange, A.B. and Orchard, I.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

4:10pm **ELUCIDATING THE ROLE OF GPA2/GPB5 AND ITS RECEPTOR (LGR1) IN MOSQUITO SPERMATOGENESIS.** ***[Page 19]***
Rocco, D., Correia, D. and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada.

4:30pm **OCTOPAMINE AND TYRAMINE MODULATE REPRODUCTIVE PROCESSES IN RHODNIUS PROLIXUS.** ***[Page 20]***
Hana, S. and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

4:40pm **DOES RAPID COLD HARDENING MODULATE K+ HOMEOSTASIS IN THE DROSOPHILA MELANOGASTER BRAIN VIA AN OCTOPAMINERGIC SIGNALLING PATHWAY?** ***[Page 21]***
Srithiphaphirom, P. and Robertson, R.M.
Department of Biology, Queen's University, Kingston, ON, Canada

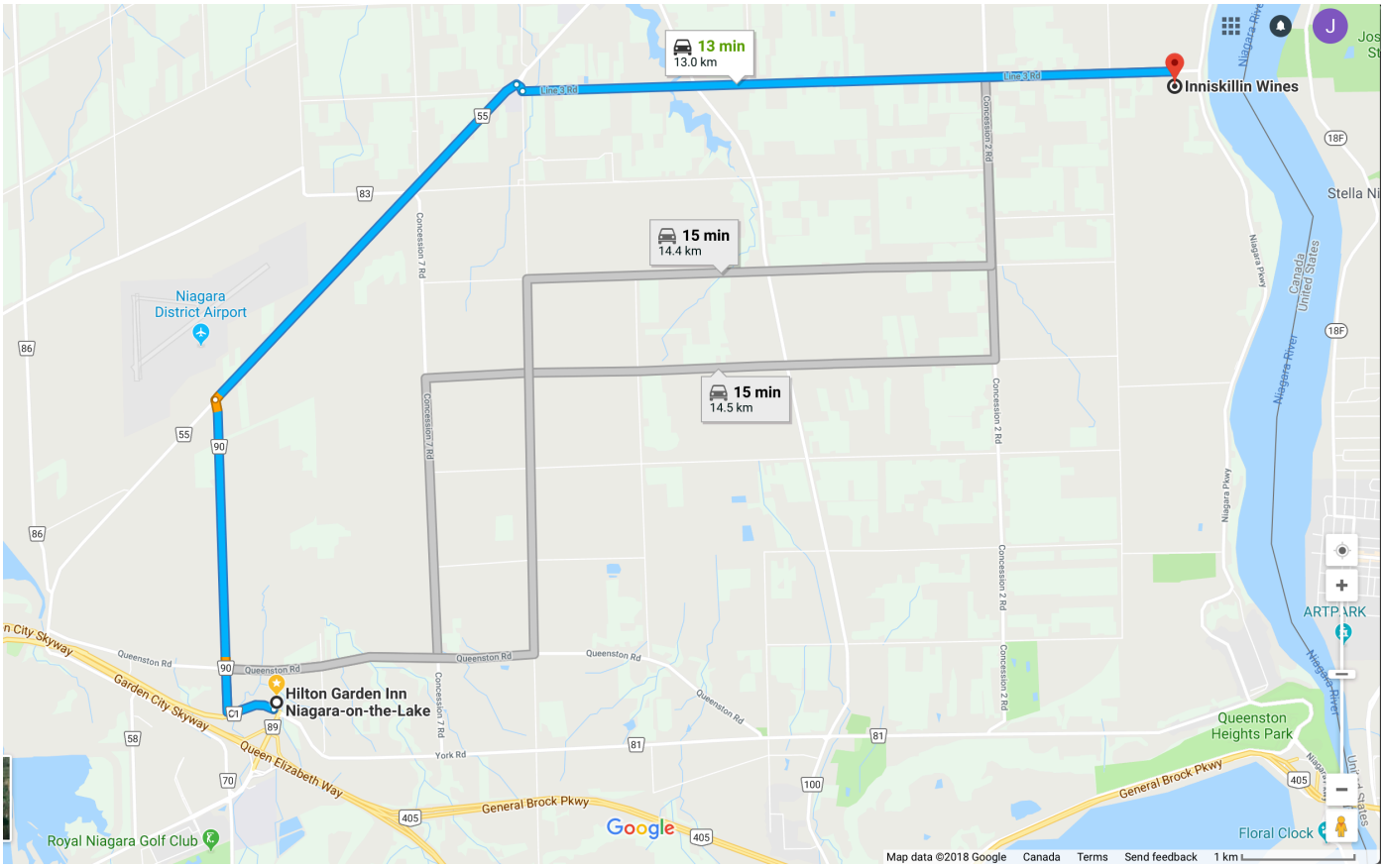
4:50pm **INVESTIGATING THE ROLE OF SIFAMIDE IN FEEDING BEHAVIOR OF THE BLOOD-SUCKING BUG, RHODNIUS PROLIXUS.** ***[Page 22]***
Ayub, M., Lange, A.B. and Orchard, I.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

5:00pm **End of Session**

6:30 pm **Winery Tour**
Inniskillin Niagara Estate, 1499 Line #3, Niagara-on-the-Lake, Ontario, L0S 1JO

7:00 pm **Banquet Dinner in Founders' Hall**
Inniskillin Niagara Estate, 1499 Line #3, Niagara-on-the-Lake, Ontario, L0S 1JO

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 8

7:30 – 8:50 am **Breakfast:** Niagara Gardenview Room

Session Chair: **Cam Donly** (Niagara Gardenview Room)

9:00 am **PANDORA'S BOX: RNASEQ APPROACH TO IDENTIFYING ION TRANSPORT MECHANISMS IN THE DISTAL ILEAC PLEXUS OF TRICHOPLUSIA NI.** ***[Page 23]***
Kolosov, D. and O'Donnell, M.J.
Department of Biology, McMaster University, Hamilton, ON, Canada

9:20 am **NOVEL CHLORIDE TRANSPORT MECHANISMS IN THE MALPIGHIAN TUBULES OF THE LARVAL TRICHOPLUSIA NI.** ***[Page 24]***
O'Donnell, M.J. and Kolosov, D.
Department of Biology, McMaster University, Hamilton, ON, Canada

9:40 am **IMPACT OF SALINATION ON OSMOREGULATION AND TRACHEAL GILL FUNCTION IN MAYFLY (HEXAGENIA RIGIDA) NYMPHS.** ***[Page 25]***
[1]Nowghani, F., [1]Chen, C.C., [2]Watson-Leung, T., [1]Donini, A. and [1]Kelly, S.P.
[1]Department of Biology, York University, Toronto, ON, Canada
[2]Ministry of the Environment and Climate Change, Etobicoke, ON, Canada

10:00 am **SALINITY RESPONSIVENESS OF AQUAPORINS IN OSMOREGULATORY ORGANS OF LARVAL AEDES AEGYPTI.** ***[Page 26]***
Misyura, L. and Donini, A.
Department of Biology, York University, Toronto, ON, Canada.

10:20 – 10:40 am **Coffee Break**: Niagara Gardenview Room

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

10:40 am **IMPACT OF SUGAR BEET DE-ICING LIQUID ON SALT AND WATER BALANCE IN MAYFLY NYMPH, HEXAGENIA LIMBATA.** ***[Page 27]***
Cuciureanu, L.A., Nowghani, F., Donini, A. and Kelly, S.P.
Department of Biology, York University, Toronto, ON, Canada.

10:50 am **NUTRITIONAL QUALITY EFFECTS CATERPILLAR ABILITY TO COPE WITH PLANT SPECIALIZED METABOLITES.** ***[Page 28]***
Demers, E., Ji, J. and Bede, J.C.
Department of Plant Biology, McGill University, Ste-Anne-de-Bellevue, QC, Canada

11:00 am **NEGATIVE SELECTION IN SOCIAL INSECTS.** ***[Page 29]***
Imrit, M.A., Dogantzis, K.A. and Zayed, A.
Department of Biology, York University, Toronto, ON, Canada.

11:10 am **CHARACTERIZING THE EFFECTS OF HUNTINGTON POLYQ-EXPANSION ON AXONAL TRAFFICKING AND NEURONAL DYSFUNCTION IN DROSOPHILA.** ***[Page 30]***

[1,2]Lin, A., [1,2]Ormerod, K.G. and [1,2]Littleton, J.T.
[1]Massachusetts Institute of Technology, Picower Institute for Learning and Memory, Cambridge, MA, USA.
[2]Massachusetts Institute of Technology, Department of Biology, Cambridge, MA, USA.

11:20 - 11:50 am **Check out of hotel rooms**

11:50 am – 1:00 pm **Lunch Break**: Niagara Gardenview Room (Picnic Lunch Buffet)

Session Chair: **Heath MacMillan** (Niagara Gardenview Room)

1:00 pm **EXPRESSION PROFILES OF FOURTEEN SMALL HEAT SHOCK PROTEIN TRANSCRIPTS DURING LARVAL DIAPAUSE AND UNDER THERMAL STRESS IN THE SPRUCE BUDWORM** ***[Page 31]***

[1]Quan, G., [2]Duan, 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

1:20 pm **RNA INTERFERENCE OF MANDUCA SEXTA USING CHLOROPLAST-ENCODED LONG DSRNA.** ***[Page 32]***

[1,2]Burke, W.G., [2]Kaplanoglu and [2]Donly, C.
[1]Department of Biology, University of Western Ontario, London, ON, Canada
[2]London Research & Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada

1:40 pm **A PEEK INSIDE THE NOSE OF THE EMERALD ASH BORER: FUNCTIONAL CHARACTERIZATION OF THE ANTENALLY-EXPRESSED CHEMOSENSORY PROTEIN 5.** ***[Page 33]***

[1]Doucet, D., [2]Zhou, Z., [1]Pavlik, L., [1]Duan, J., [1]Bowman, S. [1]Wen, F., [1]Quan, G., [3]Krell, P. and [4]Seguin, A.
[1]Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, ON, Canada
[2]Department of Plant Protection, College of Forestry, Henan University of Science & Technology, Luoyang, Henan, P.R. China.
[3]Department of Cellular and Molecular Biology, University of Guelph, Guelph, ON, Canada
[4]Laurentian Forestry Centre, Natural Resources Canada, Québec City, QC, Canada

2:00 pm **EFFECTS OF RNA INTERFERENCE ON GREENHOUSE WHITEFLY (TRIALEURODES VAPORARIORUM).** ***[Page 34]***

[1,2]Donly, C., [1]Kaplanoglu, E., [2]Ludba, K.K., [3]Kolotilin, I., [1,2]Menassa, R. and [1,2]Scott, I.M.
[1]London Research & Development Centre, AAFC, London, ON Canada
[2]Department of Biology, University of Western Ontario, London, ON Canada
[3]Scattered Gold Biotechnology, London, ON Canada

2:20 – 2:40pm **Coffee Break**: Niagara Gardenview Room

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

2:40 pm **INVESTIGATING COLD-INDUCED CHANGE IN PARACELLULAR BARRIER PERMIABILITY IN THE GUT EPITHELIA OF LOCUSTA MIGRATORIA.** ***[Page 35]***
Brzezinski, K. and MacMillan, H.A.
Department of Biology, Carleton University, Ottawa, ON, Canada

3:00 pm **INVESTIGATING THE ROLE OF ANTI-DIURETIC HORMONE, CAPA, AND THE SIGNALLING CASCADE INVOLVED IN THE FEMALE MOSQUITO, AEDES AEGYPTI.** ***[Page 36]***
Sajadi, F. and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada.

3:20 pm **THE COLD TOLERANCE OF THE ARBOVIRAL DISEASE VECTOR, AEDES AEGYPTI, IS THERMALLY PLASTIC AND SEX-DEPENDENT.** ***[Page 37]***
[1]Yerushalmi, G., [2]MacMillan, H.A. and [1]Donini, A.
[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Department of Biology, Carleton University, Ottawa, ON, Canada.

3:40 pm **ANTI-DIURETIC ACTIVITY OF A CAPA NEUROPEPTIDE CAN COMPROMISE DROSOPHILA CHILL TOLERANCE.** ***[Page 38]***
[1,2]MacMillan, H.A., [1]Nazal, B., [1]Wali, S., [1]Yerushalmi, G., [1]Misyura, L., [1]Donini, A and [1]Paluzzi, J.P.
[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Department of Biology, Carleton University, Ottawa, ON, Canada.

4:00 pm **Closing Remarks and End of Conference:** Jean-Paul Paluzzi and Andrew Donini

CHARACTERIZING MOLECULAR DIFFERENCES THAT LEAD TO SEASONAL CHANGES IN MOSQUITO PHYSIOLOGY

Meuti, M.E

Department of Entomology; The Ohio State University; Columbus, Ohio, USA

Females of the Northern house mosquito, *Culex pipiens*, are the major vectors of West Nile Virus and overwinter as adults in a reproductive diapause. Diapausing females fail to take a blood meal and keep sperm viable until they terminate diapause ~3-6 months later. The hormonal pathways regulating diapause in females of *Cx. pipiens* are well-characterized: JH is suppressed thereby preventing ovarian maturation, and the Forkhead Transcription Factor is upregulated, leading to fat hypertrophy. The short daylengths of late summer and early fall are the cues that females of *Cx. pipiens* use to anticipate the coming winter. However, how daylength is measured and whether the circadian clock is involved is unknown. Additionally, no one has yet investigated whether male mosquitoes, that do not enter diapause, alter their accessory gland proteins in response to short daylengths to inhibit female blood-feeding and promote sperm storage. My lab's work suggests that the circadian clock is initiates the female diapause response and that male mosquitoes of *Cx. pipiens* differentially regulate their accessory gland proteins in response to photoperiod. Specifically, suppressing core clock genes caused short-day reared females to avert diapause while knocking down a clock-associated gene caused long-day reared females to enter a diapause-like state. Additionally, several genes that promote host-seeking, blood meal-processing and egg maturation are down-regulated in short-day reared males of *Cx. pipiens*, while genes involved in protecting sperm and enhancing immune responses are upregulated. Together, these results suggest that the circadian clock is involved in measuring daylength in females of *Cx. pipiens* and that short photoperiods induce changes in mosquito reproductive physiology.

EXPRESSION OF AMMONIA TRANSPORTERS IN THE OSMOREGULATORY ORGANS OF *AEDES AEGYPI* LARVAE IN FRESHWATER AND HIGH AMMONIA

Durant, A. and Donini, A.

Department of Biology, York University, Toronto, Ontario, Canada

Ammonia (NH3/NH4+) is an important nutrient for microorganisms and plants but is toxic to animal tissues at relatively low levels. Nearly all living cells produce ammonia (NH3/NH4+), the nitrogenous waste product of amino acid metabolism. Additionally, aquatic animals can be exposed to elevated levels of ammonia from industrial, agricultural and sewage effluent. The freshwater-dwelling larvae of the disease vector mosquito, *Aedes aegypi*, inhabit ammonia rich sewage demonstrating remarkable tolerance to ammonia. Important ammonia-excreting organs of these larvae, the four anal papillae, extend from the terminal segment of the body and are in contact with the surrounding environment. Within the anal papillae, we have previously characterized two Rh proteins, AeRh50-1 and AeRh50-2, and two Amt/Mep proteins, AeAmt1 and AeAmt2, which all function in facilitating ammonia excretion and regulating hemolymph ammonia levels. Besides the anal papillae, there is reason to believe that other organs are involved in ammonia excretion and regulation. This study examined the immunolocalization of AeAmts and AeRh50s in the gastrointestinal tract and Malpighian tubules of larvae. Based on the results from these studies functional assays were conducted on the Malpighian tubules. We then investigated the changes in ammonia transporter protein abundance and immunolocalization in response to high environmental ammonia, with significant differences observed in the hindgut and Malpighian tubules. We suggest that in addition to the anal papillae, other organs such as the Malpighian tubules may play important roles in ammonia excretion and hemolymph ammonia regulation as is seen in other insects.

THE GASTRIC CAECUM OF LARVAL *AEDES AEGYPI*: STIMULATION AND INHIBITION OF EPITHELIAL ION TRANSPORT

D'Silva, N.M. and O'Donnell, M.J.

Department of Biology, McMaster University, ON Canada

There is a striking regionalization of vacuolar-type H+-ATPase (VA) and Na+/K+-ATPase (NKA) along the gastric caeca of the *Aedes aegypi* larva. VA is expressed along the apical membrane throughout the length of the caeca, with expression on both the apical and basal membrane along the distal third. NKA is expressed only on the basal membrane of the proximal two-thirds of the caeca. Based on previous morphological studies, we propose that the VA-rich cells correspond to the ion-transporting cells of the distal gastric caecum and the NKA-rich cells correspond to the digestive cells of the proximal gastric caecum. We provide the first measurements of H+, K+, and Na+ fluxes across the distal and proximal gastric caecum, and have shown that fluxes differ in the two regions, consistent with previously reported regionalization of ion transporters. We also provide the first measurements of the effect of 5-hydroxytryptamine (5-HT) and leucokinin (LK) on transepithelial potential (TEP), luminal ion concentrations and electrochemical potentials, as well as basolateral membrane potential and H+, Na+ and K+ fluxes. In the caecum of unstimulated larvae, ion fluxes decline rapidly, as do the basolateral membrane potential and the transepithelial potential. Stimulation with 5-HT restored both the TEP and active accumulation of H+, K+ and Na+ in the lumen. Additionally, 5-HT restored H+, K+ and Na+ fluxes across the distal caecum, but had no effect on ion fluxes across the proximal caecum. 5-HT restores the basolateral membrane potential in cells of the distal, but not proximal caecum, whereas, LK restores the basolateral membrane potential in cells of the proximal, but not distal caecum. Additionally, we also provide pharmacological evidence for ion transporters in the distal caecum.

EXPRESSION PATTERN AND SEQUENCE ANALYSIS OF *AEDES AEGYPI* SODIUM-DEPENDENT CATION-CHLORIDE COTRANSPORTERS

[1]Gillen, C.M., and [2]Piermarini, P.M.

[1]Kenyon College, Gambier, OH;
[2]Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH

The yellow fever mosquito *Aedes aegypi* has three genes with sequence similarity to the vertebrate sodium-dependent cation-chloride cotransporters (NaCCCs). One gene, aeNKCC1, is an ortholog of *Drosophila melanogaster* ncc69, a classic loop-diuretic sensitive Na-K-Cl cotransporter that contributes to ion secretion by the renal tubule. The other two genes, aeCCC2 and aeCCC3, group with a clade of transporters that have not been functionally characterized. The aeCCC2 and aeCCC3 paralogs probably arose from a tandem gene duplication at the base of the mosquito lineage. They are consecutive in the genome, have similar exon structures, and have orthologs in all mosquito genomes that we have evaluated. Analysis of functionally important residues in predicted transmembrane domain three suggests that aeCCC2 and aeCCC3 may have different ion and inhibitor binding properties than vertebrate Na-K-Cl cotransporters and *Drosophila* ncc69. The aeNKCC1 sequence has a C-terminal dileucine motif that matches a basolateral targeting motif in mammalian NKCC1, whereas aeCCC2 and aeCCC3 lack a corresponding dileucine motif. Consensus phosphorylation sites for Ste20-related proline alanine-rich kinase (SPAK) are present on aeNKCC1, aeCCC3, and one splice variant of aeCCC. A second splice variant of aeCCC2 lacks a SPAK site and has fewer consensus protein kinase A sites. In qPCR experiments, aeCCC3 was 100-fold more abundant in larvae than in adults. In larval tissues, aeCCC2 was 2-fold more abundant in Malpighian tubules compared to anal papillae. In contrast, aeCCC3 was nearly 100-fold more abundant in larval anal papillae compared to Malpighian tubules, suggesting a role in absorption.

ORCHESTRATION OF *DROSOPHILA* POST-FEEDING PHYSIOLOGY AND BEHAVIOR BY THE NEUROPEPTIDE LEUCOKININ

Zandawala, M., Yurgel, M., Liao, S., Keene, A.C. and Nässel, D.R.

[1]Department of Zoology, Stockholm University, Stockholm, Sweden
[2]Department of Biological Sciences, Florida Atlantic University, Jupiter, FL, USA

Behavior and physiology is orchestrated by neuropeptides acting as neuromodulators and/or circulating hormones. A central question is how these neuropeptides function to coordinate complex and competing behaviors. The neuropeptide leucokinin modulates diverse behaviors and physiological functions including circadian rhythms, water balance, feeding, and sleep, but the mechanism underling this complex behavioral interaction remains poorly understood. Here, we delineate the leucokinin circuitry governing homeostatically regulated behaviors and physiological functions that are critical for survival. We found that impaired LK signaling affects diverse, but coordinated behavioral and physiological processes, including regulation of stress, water homeostasis, locomotor activity, and metabolic rate. We show that the calcium activity of abdominal ganglia LK neurons increases specifically following water consumption, but not under other conditions suggesting that these neurons regulate water homeostasis and its associated physiology. In order to identify targets of LK peptide, we mapped the distribution of *Lkr*. The *Lkr* distribution in peripheral tissues, including the gut, renal tubules and sensory structures, correlate well with the mutant phenotypes. In addition, the expression of *Lkr* in insulin-producing cells (IPCs), indicates a link between LK and insulin-signaling. In line with this, the expression of two insulin-like peptides (DILPs) in IPCs increased in *Lk* and *Lkr* mutants and targeted knockdown of *Lkr* in IPCs affects stress response. Taken together, our data suggest that the LK neurons orchestrate the establishment of post-prandial homeostasis by regulating distinct physiological processes and behaviors such as diuresis, organismal activity and insulin-signaling.

INVESTIGATING THE IMPORTANCE OF TWO ZINC FINGER DOMAINS IN THE *AUTOGRAPHA CALIFORNICA* NUCLEOPOLYHEDROVIRUS ME53 PROTEIN

Ralph, R. and Krell, P.J.

Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario Canada

Baculoviruses are large, double-stranded DNA viruses that infect insects of the lepidopteran order. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), named for the Alfalfa looper moth host, infects several lepidopteran species and is commonly used for recombinant protein production. The AcMNPV early/late gene me53 is required for efficient budded virus production and is conserved in all alpha and betabaculoviruses. ME53 is a 449-amino acid protein that contains a nuclear translocation sequence and two highly conserved, putative, C4 zinc finger domains. C4 zinc fingers are implicated in numerous processes including DNA binding, protein-protein, and protein-RNA interactions that can be predicted by their secondary structure. Confirmation of zinc binding and secondary structure determination by circular dichroism can predict the functional role of these conserved regions. Partial deletion of the N-terminal zinc finger (ZnF-N), through an infectious bacmid intermediate, results in a 99.9% reduction of BV production at seven days post-transfection while deletion of the C-terminal zinc finger (ZnF-C) reduces BV production by 47%. Interestingly, ZnF-C deletion results in an early delay of BV production from 12 to 18 hours post transfection (hpt) correlating to ME53's cytoplasmic localization. Cytoplasmic functions at early times post-transfection may include translational regulation, like inhibition of monosome disassociation which occurs during proteotoxic stress. Nuclear translocation of ME53 is critical for optimal budded virus production suggesting that ME53 may also play a role in transcription, mRNA processing, or DNA packaging and transport. The importance of these putative domains for ME53 function is unknown and is the focus of this study.

INTERACTION BETWEEN VP80 AND ME53 FROM AUTOGRAPHA CALIFORNICA NUCLEOPOLYHEDROVIRUS

Özşahin, E. and Krell, P.J.

Department of Molecular and Cellular Biology, University of Guelph, Ontario, Canada

ME53 and VP80 are conserved structural proteins incorporated into the nucleocapsid of both budded and occlusion-derived viruses of alphabaculoviruses. ME53 is expressed at both early and late times post-infection while VP80 is expressed at late times. ME53 localizes to the cytoplasm at early times post-infection and forms foci on the cytoplasmic membrane which may represent budded zones of the virus. Despite the absence of a recognizable nuclear localization signal, ME53 also translocates to the nucleus which is dependent on virus infection and localizes mainly in the ring zone where occlusion-derived viruses form. VP80 is expressed at late times post infection and is localized to the nucleus via its nuclear translocation sequence. Our research aimed to identify ME53 interacting proteins using yeast two-hybrid prey libraries constructed from virus-infected or uninfected Sf9 cells which showed thatME53 interacts strongly with VP80. The interaction between VP80 and ME53 was further confirmed by bimolecular fluorescence complementation. VP80 and ME53 knockout viruses were constructed to understand their co-localizations and their effects on localizations. Deletion of ME53 reduces virus production by 99% causing a restriction of cell-to-cell virus spread though lack of ME53 does not affect viral DNA synthesis and occlusion body formation. VP80 is essential for nucleocapsid formation, suggesting that localization of ME53 on the cellular membrane and interaction with VP80 may enable the attachment of nucleocapsids to the cellular membrane allowing for nucleocapsid budding. The interaction between VP80 and ME53 may also involve embedding of occlusion-derived viruses into polyhedra or facilitate nucleocapsid transport from the nucleus to the cellular membrane.

INTERACTIONS BETWEEN THE INSECT IMMUNE SYSTEM AND THEIR PATHOGENS

Mierlo, V.V., Radauskas, V., Tahir, I., Samarasinghe, H., Xu, J. and da Silva, R.

Department of Biology, McMaster University, Hamilton, Ontario, Canada

The yellow meal worm *Tenebrio molitor* is a cosmopolitan postharvest pest insect that has significant negative impacts on both agriculture and human health across the world. As successful pests, these and other insects are faced with many biotic challenges, which they overcome with the help of an immune system. Immune system components include protein-associated molecular pattern (PAMP)-activated macromolecule dependant humoral whose cell wall is composed partially of melanin. Different strains of *C. neoformans* vary in the exact compositions of responses, and a hemocyte-dependant cell-mediated response. A key contribution to the humoral response is melanization, a process by which invading pathogens are encapsulated in melanin, a pigmented tyrosine derived polymer, and rendered inert. *Cryptococcus neoformans* is an encapsulated yeast and an entomopathogenic fungus the melanin in their cell wall, and their virulence has been shown to vary along with this characteristic. In this study, we have characterized the cellular composition (hemocytes) of the *T. molitor* immune system and have elucidated the possible interaction that may take place between these immune cells and different strains of *C. neoformans*. Four hemocyte classes were identified within *T. molitor* and were characterized as prohemocytes, granulocytes, adipohemocytes, and plasmatocytes. Survival assays have revealed that the *C. neoformans* strain with the least amount of melanin within its cell wall is the most virulent strain to *T. molitor*. We hypothesize that the high virulence of low melanin producing strains of *C. neoformans* may be attributed to a combination of potential cytotoxic effects caused by an exaggerated host immune response and immunosuppressive effects caused by fungal factors.

DIRECT EFFECT OF DEFENSIVE PHYTOHORMONES ON CATERPILLAR HERBIVORES

Su, H., Smith, R. and Bede, J.

Department of Plant Biology, McGill University, Ste-Anne-de-Bellevue, QC, Canada

In response to chewing herbivory, plants mount a set of defense responses co-ordinated through the important oxylipin phytohormone jasmonic acid (JA). Often the rapid JA burst in response to insect damage results in an increase in noxious plant specialized metabolites that have an adverse effect against the herbivore. However, little is known about the direct effect of JA directly on the caterpillar. In this study, the effects of phytohormones 12-oxo-phytodienoic acid and JA and their biosynthetic precursors on the development, pupal weight and mortality of caterpillars of the generalist *Spodoptera exigua* and the facultative specialist *Trichoplusia ni* were investigated.

ANALYSIS OF WALKING BEHAVIOUR AT YOUNG AGES IN LONG-LIVED FLIES

Xiao, C., Chippindale, A. and Robertson, R.M.

Department of Biology, Queen's University, Kingston, ON, Canada

Experimental evolution of *Drosophila melanogaster* has been used in the study of senescence for nearly four decades. Increased longevity readily evolves through experimental selection of progeny from old parents. Research into senescence has focused on life history traits such as development time, age-specific fecundity, and longevity, but has scarcely considered behaviour. Using techniques of fly tracking and behavioral computation, we examined the locomotor behaviour of adult flies with two long-term selected treatments. Long-lived flies walked persistently throughout the observation time at relatively stable speeds compared with controls. Long-lived females performed short pauses intermittently whereas controls paused more often with varying durations. These results suggest that at young ages long-lived flies have different locomotor behaviour that might be associated with reproductive success later in life.

CHARACTERIZATION OF CAV1 VOLTAGE-GATED CALCIUM CHANNELS IN THE BASAL ANIMAL *TRICHOPLAX ADHAERENS*

Gauberg, J. and Senatore, A.

Department of Biology, University of Toronto Mississauga, Ontario, Canada

Voltage-gated calcium (CaV) channels are essential for translating electrical potentials into cytoplasmic calcium signals. Most animals have three types of CaV channels (CaV1, CaV2, CaV3), each with specialized cellular functions and localizations. CaV1 channels, which are the focus of this study, are primarily expressed in muscle of most animals and are involved in excitation-contraction coupling. To date, how the specialized association of CaV1 channels and muscle evolved is presently unknown. To gain a better understanding of the evolution of muscle-associated CaV1, a basal metazoan, *Trichoplax adhaerens*, was examined. *Trichoplax* is the most basal animal known to possess homologues for each of the three CaV channel types. *Trichoplax* possesses only six cell types and lacks true neuronal and muscle tissues. Instead, it possesses (1) gland cells that are "neuron-like" in their gene expression, and (2) contractile "muscle-like" fiber cells. Remarkably, despite its cellular simplicity and lack of synapses, *Trichoplax* demonstrates coordinated feeding behaviour: it ceases moving upon detection of food, "churns" a part of its body to aid with digestion, and recommences moving following digestion. Here, muscle-like fiber cells are hypothesized to mediate a churning motion during feeding, which is driven by CaV1 channels. To begin understanding the function of CaV1 channels in *Trichoplax* and how they relate to CaV1 channels in other animals, its biophysical properties must be determined. Therefore, this study employs heterologous expression of *Trichoplax* CaV1 channels in HEK293T cells coupled with the whole-cell patch clamping technique to investigate the biophysical properties of *Trichoplax* CaV1 channels.

A PARADIGM TO STUDY MODULATION OF MUSCLE CONTRACTION IN *DROSOPHILA*

Jung, J., Kornel, A. and Mercier, A.J.

Department of Biological Sciences, Brock University, St. Catharines, ON, Canada

Proctolin is a neurohormone and a co-transmitter at body wall muscles of *Drosophila* larvae. Proctolin increases the amplitude of contractions evoked by stimulating the motorneurons; the threshold and EC50 for this effect are reported to decrease as the stimulus frequency and number of stimuli increase. We examined whether this increase in effectiveness of proctolin is due to increased release of glutamate (Glu), which would increase intracellular Ca2+ that, in turn, might alter second messenger signaling. We designed a protocol to simulate Glu release by applying Glu directly to body wall muscles, avoiding synaptic release of co-transmitters that might contribute to enhancement of contraction. Applying 2-30 mM Glu in the bath for 1 min elicited contractions of similar amplitude to those elicited by nerve stimulation with 500 ms trains and 25-75 Hz within each train. Applying Glu twice in a given preparation with a 5 min wash in between yielded two contractions of similar amplitude. Co-application of proctolin with the 2nd Glu treatment increased contraction amplitude. Increasing Glu concentration, however, did not reduce the threshold or EC50 for proctolin's ability to enhance muscle contraction. In trials where Glu was applied only once, the These results suggest that the change in effectiveness of proctolin previously reported for nerve-evoked contractions does not depend on increasing the amount of Glu released. This experimental paradigm appears to be useful for studying co-transmission. Future work will examine the possibility that release of co-transmitters alters the effectiveness of proctolin at enhancing nerve-evoked contractions.

IDENTIFICATION AND CHARACTERIZATION OF DENSE CORE VESICLE TRAFFICKING AND FUSION AT THE *DROSOPHILA* NMJ

[1,2]Ormerod K.G, and [1,2]Littleton J.T

[1]Massachusetts Institute of Technology, Picower Institute for Learning and Memory, Cambridge, MA, U.S.A.
[2]Massachusetts Institute of Technology, Department of Biology , Cambridge, MA, U.S.A

Regulated secretion in neurons occurs via two main classes of neurosecretory vesicles, i) synaptic vesicles (SVs) and, ii) dense core vesicles (DCVs). The fusion of SVs is a rapid and heavily regulated process coordinated by synaptic proteins that modulate docking, priming, and fusion. SVs, responsible for rapid communication involving the release of neurotransmitter molecules, are well characterized, and the molecular machinery underlying their fusion is fairly well understood. DCVs are responsible for the transport, storage, and release of proteins and neuropeptides at multiple cellular locations, and are known to be involved in a multitude of biological processes including synaptogenesis, synaptic transmission, synaptic plasticity, and others. Unlike SVs, DCVs require a much larger stimulus (30+ Hz stimulation) in order to trigger exocytosis and are therefore likely differentially regulated compared to SVs. Using tagged versions of DCV cargo and transmembrane components, we are beginning to investigate how DCVs are trafficked to the neuromuscular junction (NMJ) in *Drosophila*. Once at the NMJ, we are characterizing the synaptic machinery that mediates trafficking and fusion of DCVs. We are employing quantal resolution imaging of vesicle fusion at individual actives zones to determine how DCV containing neuromodulators regulate synaptic communication.

EXAMINING PROCTOLIN AND OCTOPAMINE AS CO-TRANSMITTERS IN *DROSOPHILA*

Kornel A.L., Jung, J., Aksamit, S. and Mercier, A.J.

Department of Biology, Brock University, St. Catharines, Ontario, Canada

Drosophila 3rd instar larvae serve as a model system for studying modulation of synaptic transmission. Motor neurons innervating the body wall muscles release glutamate (L-Glu) as the primary neurotransmitter to depolarize the muscle fibers and initiate contraction. The motor neurons also contain substances, including proctolin, octopamine (OA), pituitary adenylate cyclase activating peptide (PACAP), insulin-like peptides and leucokinin, that are thought to act as co-transmitters. Proctolin and OA are known to increase the amplitude of nerve-evoked contractions when they are applied in the bathing solution. Although OA release has been detected and quantified recently with amperometric methods, detection of proctolin release is problematic. We wish to provide indirect evidence that proctolin and OA are released when the motor axons are stimulated electrically and that these substances contribute to contraction. To do this we will combine a genetic approach to knock down transmitter expression with electrophysiolgical methods to elicit and measure muscle contractions. We will use fly lines that conditionally express RNAi to knockdown expression of: (a) the proctolin pre-prohormone and (b) the enzymes tyrosine decarboxylase (TDC) and tyramine-B-hydroxylase (TBH) that synthesize tyramine and OA, respectively. RNAi lines will be crossed with appropriate Gal4-expressing lines to generate progeny with reduced expression. We predict that lower expression of the putative co-transmitters will result in lower contraction amplitude, particularly at higher stimulus frequencies, compared to control lines that do not express RNAi in neurons. If successful this approach could be expanded to investigate other putative co-transmitters.

EXAMINING NEUROPEPTIDE FUNCTION IN *DROSOPHILA MELANOGASTER* USING THE CRISPR/CAS9 SYSTEM

Aksamit, S., Mansour, H., Li, F., Necakov, A. and Mercier, A.J.

Department of Biology, Brock University, St. Catharines, Ontario, Canada

Chemical signalling in the nervous system occurs at chemical synapses, where nerve terminals release "classical transmitters" that act on ion channels that alter the membrane potential of the postsynaptic cell. At many chemical synapses the nerve terminals also release co-transmitters, which can modulate the effect of classical transmitters downstream of release from the presynaptic terminal. Proctolin is a neuropeptide that acts as a co-transmitter and neurohormone in arthropods. Proctolin is known to induce contractions of *Drosophila* muscle fibers and to enhance nerve-evoke contractions of these fibres. Currently, there is only one known proctolin receptor in *Drosophila*. The EC50 concentrations for proctolin to induce contraction and enhance nerve-evoked contractions, however, differ by about 1000 fold, suggesting low affinity and high affinity effects mediated by different receptors. The CRISPR/Cas9 system is being used to generate fly mutants lacking the proctolin receptor. We generated short guide RNA (sgRNA) to target sites within the proctolin receptor gene, and we tested the sgRNA on an in-vitro template to examine its specificity for proper cleavage of the target sites. Proper cleavage was observed on a smaller DNA substrate and on a bacmid containing the proctolin receptor gene. Further experiments include use of CRISPR/Cas9 system in-vivo in *Drosophila* to produce mutants lacking the receptor. These mutants will be used to determine whether the only known proctolin receptor mediates both proctolin's ability to increase evoked contractions and its ability to induce contractions. These experiments will further studies of mechanisms of co-transmission.

PHYSIOLOGICAL EFFECTS OF STRUCTURAL ANALOGS OF KININS, TACHYKININS, AND CAPA IN *R. PROLIXUS*

Sangha, V., Lange, A. and Orchard, I.

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

The kinin, tachykinin, and CAPA family of neuropeptides are responsible for a variety of physiological processes in insects. In *R. prolixus*, Rhopr-kinins and Rhopr-tachykinins have been shown to stimulate hindgut contractions, and Rhopr-CAPA-a2 is an anti-diuretic peptide. 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-a1, RhoprCAPA-a2, RhoprCAPA-apk1. A CAPA receptor that binds RhoprCAPA-a2 and, in a minor way, RhoprCAPA-apk1, and a pyrokinin receptor that binds Rhopr-CAPA-apk1, are expressed on the hindgut of *R. prolixus*. Despite the presence of these receptors, there is no stimulation of hindgut contraction upon application of RhoprCAPA-a1, RhoprCAPA-a2 or RhoprCAPA-apk1. Application of a mixture of Rhopr-Kinin-2 and Rhopr-CAPA-a2 on the hindgut, however, results in a stronger contraction than that produced by Rhopr-Kinin-2 alone. Neuropeptide analogs of kinin, tachykinin and CAPA-a2 are currently being examined for their ability to modulate hindgut contractions. These neuropeptide analogs have been synthesized with changes in their amino acid sequences, yet still possess the ability to bind to their specified GPCRs in other insects.

EXAMINING THE RECEPTOR LOCALIZATION AND PHYSIOLOGICAL FUNCTION OF A PYROKININ NEUROPEPTIDE IN *AEDES AEGYPI* HINDGUT

[1]Lajevardi, A., [2]Brown, M.R. and [1]Paluzzi, J.P.

[1]Department of Biology, York University, Toronto, ON. Canada
[2]Deptartment of Entomology, University of Georgia, Athens, GA. USA

The mosquito, *Aedes aegypi*, is a chief vector responsible for transmitting pathogens causing dengue fever and Zika virus disease. Upon blood feeding, adult female mosquitoes face the challenge of excess fluid and ion uptake from their host. The Malpighian tubules and hindgut serve as the primary osmoregulatory organs to help eliminate this excess load and alleviate this insult to haemolymph homeostasis. The hindgut is composed of an anterior ileum and posterior rectum. Through the coordination of ion transport processes along with myotropic activity, the rectum serves as the final site for the reabsorption of ions, water and metabolites before excreting waste via rectal peristalsis. Pyrokinin-related peptides, characterized by a conserved FxPRLamide carboxyl terminus, have been shown to influence hindgut physiology in some insects, but their functions remain unclear in blood-feeding arthropods. Herein, we have examined the expression of two *A. aegypi* pyrokinin-related receptors within the alimentary canal (i.e. hindgut) and reproductive tissues. Immunohistochemical mapping in female adult mosquitoes revealed pyrokinin-1 receptor immunoreactivity within the rectum, specifically localized to the rectal pads. Based on the receptor expression profiles at the transcript and protein level, we examined prospective physiological roles of its proposed ligand. Interestingly, pyrokinin-1 neuropeptide did not influence myotropic or ionomodulatory activity in these regions. Ongoing studies are examining the association of these receptors with other structures of the rectal pads, including ion transporters and aquaporins, to better understand the role of pyrokinin signaling in the mosquito hindgut.

THE ROLE OF SULFAKININS ON FEEDING IN *RHODNIUS PROLIXUS*

Al-Alkawi, H., Lange, A.B, and Orchard, I.

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

Sulfakinins (SKs) are neuropeptides that have been shown to function as feeding satiety factors in insects. We confirmed the presence of two sulfakinins (Rhopr-SK-1 and Rhopr-SK-2) in *Rhodnius prolixus*. Reverse transcriptase quantitative PCR (RT-qPCR) demonstrated that the Rhopr-SK transcript is mainly expressed in the central nervous system (CNS) of fifth-instar *R. prolixus*. Fluorescent in situ hybridization showed transcript expression in the brain. Immunohistochemical staining of SK-like peptides was observed in the same neurons and in processes throughout the CNS, as well as in processes on the posterior midgut and anterior hindgut. Two seven-transmembrane Rhopr-SK G protein-coupled receptors (GPCRs) were cloned and characterized. A functional receptor assay that utilized Human Embryonic Kidney (HEK)- 293 cells stably expressing a modified cyclic nucleotide-gated (CNG) channel (HEK293/CNG) deorphaned the two SK receptors, which are sensitive to Rhopr-SK-1 and 2 in the pM range. RTqPCR of the receptors revealed that their highest expression is in the CNS, with further expression in peripheral tissues. Rhopr-SK-1 induced contractions of the hindgut in a dose-dependent manner. Injection of Rhopr-SK-1 decreased the weight of the blood meal consumed. In addition, Rhopr-SK-1 inhibits contractions of adult oviduct and ejaculatory ducts in a dose-dependent manner.

ELUCIDATING THE ROLE OF GPA2/GPB5 AND ITS RECEPTOR (LGR1) IN MOSQUITO SPERMATOGENESIS

Rocco, D., Correia, D. and Paluzzi, J.P.

Department of Biology, York University, Toronto, Ontario, Canada

GPA2/GPB5 and its receptor (LGR1) represent an ancient neuroendocrine glycoprotein hormone-signaling system found in both vertebrate and invertebrate genomes, however its function remains elusive. To elucidate the physiological role of GPA2/GPB5 in the mosquito *Aedes aegypi*, we aimed to characterize the expression profile of both GPA2/GPB5 and LGR1, as well as elucidate downstream signaling pathways. Immunohistochemical, RT-qPCR and in situ hybdrization techniques have revealed GPA2 and GPB5 subunit expression localizes to three bilateral pairs of neuroendocrine cells situated within the first five abdominal ganglia of adult mosquitoes. Using a heterologous system with mammalian cell lines that expresses *A. aegypi* GPA2/GPB5, immunoblot analyses on collected protein samples were used to test dimerization of the alpha and beta hormone subunits, and functionality of GPA2/GPB5 homo-/ heterodimers in LGR1 binding and activation using a cAMP luciferase assay. In adult mosquitoes, immunohistochemical analyses revealed LGR1 localizes to basolateral surfaces of epithelia associated with various regions of the gut, indicating a likely role in feeding-related processes and/or hydromineral balance for GPA2/GPB5. Interestingly, modest levels of LGR1 transcript and strong immunostaining was also identified in reproductive tissues. Regionalized LGR1 immunoreactivity was found within distinct developmental stages of sperm in the male testes as well as the follicular epithelium of the adult female ovaries in non-blood fed females. Using reverse genetic (i.e. RNA interference), we aim to determine the physiological role of GPA2/GPB5 in reproductive processes of adult mosquitoes.

OCTOPAMINE AND TYRAMINE MODULATE REPRODUCTIVE PROCESSES IN *RHODNIUS PROLIXUS*

Hana, S. and Lange, A.

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

Octopamine and tyramine belong to a class of neuroactive chemicals, biogenic amines, involved in diverse physiological processes in insects. We have shown previously that octopamine and less so tyramine modulate the myogenic contractions in the reproductive visceral muscles in the adult female *R. prolixus*. Two amine G-protein coupled receptors named Octopamine β 2-Receptor (Octβ2-R) and Tyramine 1 Receptor (Tyr1-R) were cloned, functionally characterized and found to be expressed throughout the reproductive system of *R. prolixus*. It is likely that octopamine and tyramine utilize these receptors to modify the contractility in the reproductive visceral muscles. Injection of octopamine and tyramine into fed and mated adult females led to an increase in egg production and egg laying. Previous research in *Drosophila melanogaster* and Nilaparvata lugens has shown the knockdown of Octβ2-R significantly disrupted the process of ovulation and egg laying. We plan to use RNA interference to knockdown Octβ2-R and Tyr1-R and investigate the role of these receptors in oogenesis, ovulation and other reproductive processes.

DOES RAPID COLD HARDENING MODULATE K+ HOMEOSTASIS IN THE *DROSOPHILA MELANOGASTER* BRAIN VIA AN OCTOPAMINERGIC SIGNALLING PATHWAY?

Srithiphaphirom, P. and Robertson, R.M.

Department of Biology, Queen's University, Kingston, Ontario, Canada

Temperature has profound effects on the neural function and behaviour of insects. When exposed to low temperature, chill-susceptible insects enter chill coma, a reversible state of neuromuscular paralysis. Despite the popularity of the study of the effects of low temperature on insects, we know little about the physiological mechanisms controlling the entry to, and recovery from, chill coma. Spreading depolarization (SD) is a phenomenon that causes a neural shutdown in the central nervous system (CNS) and it is associated with a loss of K+ homeostasis in the CNS. Using *Locusta migratoria*, we found that: (1) SD in the CNS causes a loss of coordinated movement immediately prior to chill coma; (2) rapid cold hardening (RCH) reduces the temperature that evokes neural shutdown; and (3) octopamine (OA) plays a role in regulating chill coma temperature and recovery time. Using *Drosophila melanogaster*, which we can genetically manipulate and which is thus better suited for the investigation of molecular mechanisms, we will investigate the effect of RCH on SD and the role of OA during chill coma. We predict that RCH modulates K+ regulation via an octopaminergic signalling pathway, which affects the threshold for SD during chilling and the entry to chill coma.

INVESTIGATING THE ROLE OF SIFAMIDE IN FEEDING BEHAVIOR OF THE BLOOD-SUCKING BUG, *RHODNIUS PROLIXUS*.

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

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

SIFamides (SIFa) are a family of neuropeptides that are highly conserved among arthropods. In insects, this peptide is mainly expressed in four medial interneurons in the pars intercerebralis of the brain, with projections into every ganglion. Although SIFamide has been shown to influence sexual behavior, feeding and sleep regulation in holometabolous insects such as *Drosophila melanogaster*, little is known about its role in hemimetabolous insects. In this study, we use immunohistochemistry to show characteristic expression of SIFamide in four cells of the pars intercerebralis in the hemimetabolous blood-sucking insect, *Rhodnius prolixus*. A novel discovery is that processes also project into the corpus cardiacum and along the dorsal vessel, indicating for the first time in insects, that SIFa can be a neurohormone. To understand the influence of SIFamide on insect physiology, we performed contraction assays on the dorsal vessel. Our results show a dose-dependent increase in heart beats/minute upon application of *R. prolixus* SIFamide (Rhopr-SIFa). In addition, we observed enhanced feeding behaviour in insects injected with Rhopr-SIFamide, in comparison to those injected with saline (Student's t-test, $p = 0.04$). This data suggests that the four SIFamidergic neurons and associated arborizations may play an important function in the neuronal circuitry controlling *R. prolixus* feeding behavior, as a central and peripheral neuromodulator/neurohormone.

PANDORA'S BOX: RNASEQ APPROACH TO IDENTIFYING ION TRANSPORT MECHANISMS IN THE DISTAL ILEAC PLEXUS OF *TRICHOPLUSIA NI*

Kolosov, D. and O'Donnell, M.J.

Department of Biology, McMaster University, Hamilton, Ontario, Canada

Excretion of metabolic wastes in insects is coupled to ion/fluid secretion by the Malpighian tubules (MTs). Larvae of lepidopterans demonstrate a complex and regionalized morphology of the MTs. Recent studies in our lab revealed several unusual aspects of ion transport in the MTs of a lepidopteran *Trichoplusia ni*. These include: cation reabsorption via secondary cells (SC), while most insects secrete ions via this cell type; coupling of SCs to neighbouring principal cells (PCs) via gap junctions to enable reabsorption; and a reversal from cation secretion to reabsorption by the PCs in the distal ileac plexus (DIP) region of the tubule in response to dietary ion loading. A recent attempt at identifying molecular mechanisms of ion transport in the DIP of *T. ni*. resulted in a model with roles for multiple ion transporters in the PCs. The current experimental RNAseq approach was aimed at detecting previously uncharacterised ion transporters and endocrine regulatory mechanisms in the DIP with the aim of painting a composite picture of ion transport and identifying putative regulatory mechanisms. Results indicated expression of 9103 transcripts in the DIP, including 4 ion-motive ATPases, 71 ion channels, 117 non-channel ion transporters, 4 aquaporins, 75 nutrient and xenobiotic transporters, 43 cell adhesion and junction components, and 116 receptors. Additionally, several voltage-gated ion channel and cell volume regulation-associated transporter transcripts were detected. The study provided insights into the transport of nutrients, xenobiotics, phosphate and inorganic ions. Many of the transcripts expressed by the DIP are indicative of the ability of this MT region to respond to, process (and sometimes produce) neuropeptides, steroid hormones and neurotransmitters.

NOVEL CHLORIDE TRANSPORT MECHANISMS IN THE MALPIGHIAN TUBULES OF THE LARVAL *TRICHOPLUSIA NI*

O'Donnell, M.J. and Kolosov, D.

Department of Biology, McMaster University, Hamilton, Ontario, Canada

Larvae of lepidopterans are specialized plant-feeders. Malpighian tubules (MTs) of lepidopteran larvae demonstrate a complex and regionalized morphology presumably aimed at recycling of ions and water expended during feeding. MTs of most insect groups consist of two secretory cell types: principal cells (PCs) and secondary cells (SCs). SCs of lepidopterans are found in the ileac plexus (IP) region. Previous studies reported that SCs of the larval *Trichoplusia ni* paradoxically reabsorb cations, and described gap junctional coupling that is thought to aid in this process. The current study demonstrates that all secondary cells in the IP of *T. ni* secrete Cl- while simultaneously absorbing Na+ and K+. PCs secrete Cl-only in the middle and proximal IP, while reabsorbing it in the rest of the tubule. Microelectrode impalement of the distal IP (DIP) lumen and Ramsay assay measurements on the fluid secreted by the whole IP indicate cumulative chloride secretion along the length of the IP. A recent RNAseq study revealed a glycine receptor (GlyR), a ligand-gated Cl- channel in the DIP of *T. ni*; the current study examined the role of GlyR in Cl- transport by the DIP. When examined in Gly-free (mannitol-balanced) saline, rundown in chloride and fluid secretion was evident relative to control tubules bathed in Gly-replete saline. Microelectrode impalement of the DIP lumen demonstrated an increase in the TEP following stimulation with 1 mM Gly. Thus, Gly may play an important role in regulating Cl- secretion and diuresis in lepidopteran larvae. We suggest that coordination of free amino acid hemolymph content and ion/fluid secretion provides a control mechanism linking the fed/unfed state of the animal with the excretory function of the MTs.

IMPACT OF SALINATION ON OSMOREGULATION AND TRACHEAL GILL FUNCTION IN MAYFLY (*HEXAGENIA RIGIDA*) NYMPHS

[1]Nowghani, F., [1]Chen, C.C., [2]Watson-Lewng, T., [1]Donini, A. and [1]Kelly, S.P.

[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Ministry of the Environment and Climate Change, Etobicoke, ON, Canada.

This study investigates the ionoregulatory strategies used by freshwater (FW) nymphs of the mayfly (*Hexagenia rigida*) in response to salt-contaminated water (SCW). *H. rigida* nymphs maintain hemolymph ion levels far in excess of their surroundings under FW conditions. This trend is sustained when nymphs are exposed to increased salinity in SCW. Furthermore, no change was detected in the body water content of FW versus SCW treated nymphs, suggesting that the nymph is able to maintain ionoregulatory processes in SCW. This adaptation was studied via the actions of the tracheal gills, which were found to be sites of Na+ absorption in FW and Na+ secretion in SCW conditions. Significant differences were also observed in Na+ transport spatially along individual tracheal gills within each treatment. Finally, to explore the dense population of NKA and/or VA immunoreactive cells (putative ionocytes), morphometric measurements of gill ionocytes reveal no significant difference in ionocyte distribution between the two salinity treatments. This data provides a new insight into how FW mayfly nymphs regulate salt and water balance in response to environmental stressors (NaCl contamination) using the tracheal gills.

SALINITY RESPONSIVENESS OF AQUAPORINS IN OSMOREGULATORY ORGANS OF LARVAL *AEDES AEGYPI*

Misyura, L. and Donini, A.

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

Aedes aegypi mosquitoes are arboviral vectors for debilitating human diseases including Zika, chikungunya, yellow fever, and dengue, that infect millions of people worldwide. Larval *A. aegypi* typically reside in freshwater environments where their hemolymph is hypertonic to the environment and as a result encounter passive water influx into the hemolymph along with passive ion loss via diffusion into the water. Salt contaminated freshwater from evaporation, irrigation run off, or estuaries are also commonly exploited by mosquito larvae, completing development in salinated water up to the equivalent of 30% seawater which is isosmotic to the larval hemolymph. Therefore, water is in equilibrium with larval hemolymph while homeostatic ion composition is actively regulated to maintain physiological function under these conditions. Water traverses the cellular and epithelial membranes through water channels termed aquaporins (AQPs) with 6 AQP genes identified in the *A. aegypi* genome. Given variations in the osmotic challenges faced by larvae in freshwater and salinated water, the localization and quantification of AQP expression in osmoregulatory organs would shed light on water regulation in *A. aegypi* larvae. This study assessed the expression of AQPs in osmoregulatory organs of *A. aegypi* larvae reared in either freshwater or salinated water. Based on mRNA and protein abundance, AQP1, AQP3, AQP4, AQP5, and AQP6 were salinity responsive in various osmoregulatory organs. The results provide a basis of understanding for the role of AQPs in the osmoregulatory processes of *A. aegypi* larvae which, through further studies may identify new targets for the development of novel mosquito control agents for larvae prior to their emergence into the disease vector adult life stage.

IMPACT OF SUGAR BEET DE-ICING LIQUID ON SALT AND WATER BALANCE IN MAYFLY NYMPH, *HEXAGENIA LIMBATA*

Cuciureanu, L.A., Nowghani, F., Donini, A. and Kelly, S.P.

Department of Biology, York University, Toronto, Ontario, Canada

Mayflies are benthic macroinvertebrates that are sensitive indicators of water pollution particularly to runoff contaminants such as salts and metals. This study investigated osmoregulatory changes in freshwater (FW) mayfly nymphs (*Hexagenia limbata*) following exposure to a de-sugarized beet juice molasses used as a liquid de-icer for roads. Knowing that beet juice contains high concentrations of K^+ which is an important ion in maintaining electrochemical gradients; excess runoff into FW may disrupt ionoregulatory mechanisms of mayfly nymphs, particularly since K^+ settles in the sediment where nymphs burrow. To consider the impact of beet de-icing liquid at the physiological level, a seven day chronic toxicity test was first carried out to establish LC_{50}. This was then used to determine a 7 day experimental exposure dose. Following exposure, measurements of hemolymph ions (K^+, Na^+, Cl^- and H^+) as well as Na^+-K^+-ATPase and V-type H^+-ATPase activity assays in tracheal gills, rectum and Malpighian Tubules are being used to evaluate perturbations. To our knowledge, this is the first study to explore the physiological effects of beet juice de-icer on FW organisms. This is an important initial step in identifying any potential risk to FW ecosystems that relate to the introduction of beet juice de-icing products as a substitute or additive to road salt.

NUTRITIONAL QUALITY EFFECTS CATERPILLAR ABILITY TO COPE WITH PLANT SPECIALIZED METABOLITES

Demers, E., Ji, J. and Bede, J.C.

Department of Plant Biology, McGill University, Ste-Anne-de-Bellevue, QC, Canada

When allowed to feed ad liberatum, caterpillars of the beet armyworm, *Spodoptera exigua*, self-select a slightly protein-biased diet with an optimal nutritional ratio of 22:20 protein to digestible carbohydrate. Many nutritional studies have focused on insect performance. Less is known about how dietary nutritional quality affects the caterpillars ability to cope with noxious host plant specialized metabolites. Caterpillar midgut-specific enzyme activity was influenced by plant diet. Future research will focus on how dietary nutritional quality affects caterpillar midgut enzyme activity.

NEGATIVE SELECTION IN SOCIAL INSECTS

Imrit, M.A., Dogantzis, K.A. and Zayed, A.

Department of Biology, York University, Toronto, Ontario

Eusociality, characterized in part by complex social behaviours, 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 gap in knowledge, 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.

CHARACTERIZING THE EFFECTS OF HUNTINGTIN POLYQ-EXPANSION ON AXONAL TRAFFICKING AND NEURONAL DYSFUNCTION IN *DROSOPHILA*

Lin, A., [1,2]Ormerod, K.G. and [1,2]Littleton, T.

[1]Massachusetts Institute of Technology, Picower Institute for Learning and Memory, Cambridge, MA, U.S.A.
[2]Massachusetts Institute of Technology, Department of Biology , Cambridge, MA, U.S.A

Huntington's Disease (HD) is a neurodegenerative genetic disorder caused by the expansion of a polyglutamine (polyQ) region of the Huntingtin (Htt) protein. The pathogenic version of Htt has been shown to disrupt a multitude of neuronal processes, including gene expression, proteasome and mitochondrial activity, and axonal trafficking. Previously our lab and others have observed an accumulation of large Htt-positive aggregates in the axons of motor-neurons innervating third-instar larvae. Here we take advantage of the molecular and genetic tools in *Drosophila* to study how polyQ expansions contributes to neuronal dysfunction. We previously created *Drosophila* transgenic strains expressing human Htt encoding pathogenic (Htt-Q15) and non-pathogenic (Htt-Q138) tagged with a fluorescent marker. We combined these lines with other fluorescently labelled axonal trafficking cargo like dense core vesicles, synaptic vesicles, and mitochondria in order to more extensively examine how peripheral accumulation of pathogenic Htt protein contributes to neuronal dysfunction and perturbations in axonal trafficking. By combining these approaches along with the RNA interference against known regulators of axonal trafficking we hope to identify the cellular machinery that contributes to this dysfunction.

EXPRESSION PROFILES OF FOURTEEN SMALL HEAT SHOCK PROTEIN TRANSCRIPTS DURING LARVAL DIAPAUSE AND UNDER THERMAL STRESS IN THE SPRUCE BUDWORM

[1]Quan, G., [2]Duan, 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

Diapause is an important strategy for certain insect species to survive unfavorable environmental conditions. Many studies have indicated that the increased expression of heat shock proteins during diapause increases the thermal tolerance of insects. Based on their molecular weights, insect HSPs have been classified into four families: HSP90, HSP70, HSP60 and small heat shock proteins (sHSPs). Phylogenetic and biological analyses suggest that most insect sHSP have evolved independently among different insect orders and it may be that species-specific sHSP greatly contribute to the adaptability of insects in diverse environments. In this study, we invested the transcript levels of 14 sHSP genes in the spruce budworm, a major pest of the boreal forest, during pre-diapause, diapause and post-diapause under laboratory condition and their responses to heat shock. We found that sHSP expression profiles could be classified into five patterns under laboratory condition. Pattern I were up-regulated during pre-diapause; pattern II were up-regulated only during the diapause stage; pattern III were constantly expressed throughout diapause; pattern IV were up-regulated in pre-diapause and diapause; pattern V were up-regulated during the post-diapause stage. After heat shock, five different expression patterns were observed. Pattern I responded weakly or not at all to heat shock throughout diapause; pattern II responded weakly during the main diapause stage; pattern III were up-regulated in post-diapause; pattern IV was strongest during diapause; pattern V was strongest only in early diapause. These complex expression profiles indicate that sHSP are involved in the diapause process and that they may have multiple and important roles in different phases of diapause.

RNA INTERFERENCE OF *MANDUCA SEXTA* USING CHLOROPLAST-ENCODED LONG DSRNA

[1,2]Burke, W.G., [2]Kaplanoglu, E. and [2]Donly, C.

[1]Department of Biology, The University of Western Ontario, London, ON N6A 3K7 Canada
[2]London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON N5V 4T3 Canada

RNA interference is a promising tool for the development of pest management strategies which target specific species of insect. However, orally administered double-stranded RNA is inefficient at knocking down gene expression in species within the order Lepidoptera, a clade which includes some of the most common agricultural pests. Transplastomic plants expressing dsRNA within their chloroplasts are a promising method of delivering dsRNA to the pest, as bio-encapsulation within the plastid protects the dsRNA from nucleases in the feeding insect's midgut and localization within the chloroplast prevents processing by the plant's innate RNAi pathway. We transformed the chloroplast genome of tobacco *Nicotiana tabacum* to express a 2222 base pair dsRNA with sequence complementarity to the vacuolar ATPase subunit A gene of tobacco hornworm *Manduca sexta*. Bioassays using *M. sexta* larvae are being performed to determine if knockdown of V-ATPase subunit A gene expression or lethality occur as a result of feeding on the transformed plants. Plants expressing insecticidal chloroplast dsRNA could be utilized as trap plants to be grown alongside agriculturally important crops in a greenhouse or field setting to lure and kill pest insects.

A PEEK INSIDE THE NOSE OF THE EMERALD ASH BORER: FUNCTIONAL CHARACTERIZATION OF THE ANTENALLY-EXPRESSED CHEMOSENSORY PROTEIN 5.

[1]Doucet, D., [2]Zhou, Z., [1]Pavlik, L., [1]Duan, J., [1]Bowman, S. [1]Wen, F., [1]Quan, G., [3]Krell, P. and [4]Seguin, A.

[1]Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, ON, Canada
[2]Department of Plant Protection, College of Forestry, Henan University of Science & Technology, Luoyang, Henan, P.R. China.
[3]Department of Cellular and Molecular Biology, University of Guelph, Guelph, ON, Canada
[4]Laurentian Forestry Centre, Natural Resources Canada, Québec City, QC, Canada

Chemosensory proteins (CSPs) are small, soluble proteins present in many species of arthropods. Some CSPs are strongly expressed in insect sensory organs and are therefore hypothesized to play major roles in olfaction, for instance for finding mates and host plants. Understanding the molecular underpinnings of olfaction can help us devise optimized survey and detection methods (e.g. pheromone-baited traps), especially for forest insects that are difficult to detect like the Emerald Ash Borer (*Agrilus planipennis*). Previous work has established that the transcripts of two CSP genes (AplaCSP4 and AplaCSP5) are expressed predominantly in adult antennae of the Emerald Ash Borer. To better understand the function of AplaCSP5, its ORF was cloned in a bacterial expression vector and produced in E. coli. A protein of the expected size (13.5 KDa) was purified from E. coli. We confirmed that recombinant AplaCSP5 binds the synthetic fluorescent probe N-phenylnaphthalen-1-amine (1-NPN) and could therefore be used in competitive binding assays with a panel of volatile compounds. We will report on the first wave of the binding assay results which focused on 6-carbon green leaf volatiles, such as (Z)-3-hexen-1-ol and the related ester and aldehyde compounds. The data accumulated so far point to the possible involvement of AplaCSP5 in green leaf volatile compound detection and/or processing.

EFFECTS OF RNA INTERFERENCE ON GREENHOUSE WHITEFLY (*TRIALEURODES VAPORARIORUM*)

[1,2]Donly, B.C., [1]Kaplanoglu, E., [2]Ludba, K.K., [3]Kolotilin, I., [1,2]Menassa, R., and [1,2]Scott, I.M.

[1]London Research & Development Centre, AAFC, London, ON Canada
[2]Department of Biology, University of Western Ontario, London, ON Canada
[3]Scattered Gold Biotechnology, London, ON Canada

RNA interference (RNAi) is a biological process by which many eukaryotic cells respond to detrimental genetic elements such as transposons and viruses. Through this process, host cells are able to silence the expression of genes for which RNA is detected, thus suppressing the propagation of the element. RNAi can also be deployed to silence genes in specific organisms by the application of double-stranded RNA (dsRNA) targeted to essential genetic loci. This approach has promise in agriculture as a means to specifically target pest insects by exposing them to dsRNA as part of crop protection strategies. We are investigating approaches for applying RNAi in the control of the agricultural pest, the greenhouse whitefly (*Trialeurodes vaporariorum*). Whitefly causes damage to crops by feeding on sap and by vectoring plant viruses. We first demonstrated that dsRNA targeting an essential gene in *T. vaporariorum* can kill the insects when delivered during normal adult feeding on leaflets supplied with the dsRNA through soaking. Our next goal was to determine whether dsRNA supplied by synthesis in the chloroplasts of the plant would have the same effect. Therefore, the plastids of tomato plants were transformed to produce the same dsRNA and feeding assays with whiteflies are now being conducted to measure gene silencing and mortality. This will reveal if a sucking insect pest such as whitefly is exposed to dsRNA present in plant plastids, and if so, whether this delivery strategy can effectively protect crop plants against this pest.

INVESTIGATING COLD-INDUCED CHANGE IN PARACELLULAR BARRIER PERMEABILITY IN THE GUT EPITHELIA *LOCUSTA MIGRATORIA*

Brzezinski, K. and MacMillan, H.A.

Department of Biology, Carleton University, Ottawa, ON, Canada

Chill-susceptible insects, like the migratory locust, often die when exposed to low temperatures from an accumulation of tissue damage that is unrelated to freezing (chilling injuries). Chilling injury is consistently associated with ion imbalance across the gut epithelia. It has been recently suggested that this imbalance is at least partly driven by cold-induced disruption of epithelial barrier function. Here, we aim to test this hypothesis in the migratory locust. To quantify chill tolerance, locusts were exposed to -2℃ for various durations, and monitored for chill coma recovery time and survival 24h post-cold exposure. Longer exposure times significantly increased recovery time, and caused injury and death. We tested for barrier failure by monitoring movement of an epithelial barrier marker (FITC-dextran) across the gut epithelia during exposure to -2℃. There was minimal marker movement across the epithelia in the cold, suggesting that locust gut barrier function is generally conserved during chilling. However, this finding may be a consequence of the large, polar, and uncharged nature of FITC-dextran, and small ions may yet leak in the cold. We therefore took a different approach, and used a temperature controlled Ussing chamber to investigate whether chilling disrupts the electrical resistance of the gut epithelia.

INVESTIGATING THE ROLE OF ANTI-DIURETIC HORMONE, CAPA, AND THE SIGNALING CASCADE INVOLVED IN THE FEMALE MOSQUITO, *AEDES AEGYPI*

Sajadi, F. and Paluzzi, J.P.

Department of Biology, York University, Toronto, Ontario, Canada

Female *Aedes aegypi* 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, including the Malpighian tubules (MTs), which is under rigorous control by neuroendocrine factors to regulate diuresis. Although both diuretic and anti-diuretic roles for CAPA peptides have been found in larval *A. aegypi*, its role and signaling pathway remains unclear in adults. In this experiment, the effects of AedaeCAPA-1 were measured on adult female MTs stimulated with various diuretic factors. AedaeCAPA-1 specifically inhibits secretion of MTs stimulated by diuretics, 5-HT and DH31. Furthermore, although AedaeCAPA-1 acts as an anti- diuretic, it does not influence the relative proportions of cations transported by adult MTs, thus maintaining the kaliuretic activity of 5-HT and natriuretic activity of DH31. Effects of the second messenger cGMP and PKG/NOS inhibitors were measured in adult MTs indicating 5-HT and DH31–stimulated secretion is strongly inhibited by cGMP, similar to effects seen with AedaeCAPA-1. Additionally, pharmacological inhibition of PKG/NOS signaling abolishes the anti-diuretic activity of AedaeCAPA-1, confirming the role of cGMP/PKG/NOS in the CAPA signaling pathway. Interestingly, although AedaeCAPA-1 has no inhibitory activity on kinin-stimulated fluid secretion, cGMP strongly inhibited fluid secretion by this diuretic hormone, which targets stellate cells specifically, suggesting that another anti-diuretic factor in addition to AedaeCAPA-1 exists and may utilize cGMP as a second messenger.

THE COLD TOLERANCE OF THE ARBOVIRAL DISEASE VECTOR, *AEDES AEGYPI*, IS THERMALLY PLASTIC AND SEX-DEPENDENT

[1]Yerushalmi, G.Y., [1,2]MacMillan, H. and [1]Donini, A.

[1]Department of Biology, York University, Toronto, Ontario, Canada (GYY and AD)
[2]Department of Biology, Carleton University, Ottawa, Ontario, Canada (HM)

The global distribution of the arboviral disease vector *Aedes aegypi* is largely restricted to tropical and subtropical habitats due to the suitability of their thermal conditions. In the context of climate change, there is a growing need to understand the cold tolerance of *A. aegypi*, as it may spread into temperate regions of the world. Despite this, little is known about the cold tolerance physiology of the adult *A. aegypi*. Here, we assess the impact of (1) environmental temperatures (cold acclimation), (2) sex, and (3) blood-feeding on the cold tolerance of *A. aegypi*. We show that cold acclimation (5-days at 15°C) decreases the temperature of chill coma onset (CTmin), speeds up chill coma recovery time (CCRT), and decreases the mean lethal temperature (LT50) by ~1.5°C. Female *A. aegypi* were more cold tolerant compared to their male counterparts in all three measured traits – albeit to a lesser degree than the effect of acclimation – and both exhibited thermal plasticity. Lastly, we show that blood-feeding 20-180 min prior to a cold exposure has no effect on chill coma recovery time following 6 h at 2°C. Our results demonstrate that despite their tropical habitat, adult *A. aegypi* retain thermal plasticity, that female *A. aegypi* are moderately more cold tolerant than males, and that blood-feeding has no impact on cold tolerance.

ANTI-DIURETIC ACTIVITY OF A CAPA NEUROPEPTIDE CAN COMPROMISE DROSOPHILA CHILL TOLERANCE.

[1,2]MacMillan, H.A., [1]Nazal, B., [1]Wali, S., [1]Yerushalmi, G., [1]Misyura, L., [1]Donini, A and [1]Paluzzi, J.P.

[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Department of Biology, Carleton University, Ottawa, ON, Canada.

For insects, chilling injuries that occur in the absence of freezing are thought to be caused by a systemic loss of ion and water balance that leads to extracellular hyperkalemia, cell depolarization, and the triggering of apoptotic signalling cascades. The ability of insect ionoregulatory organs (e.g. the Malpighian tubules) to maintain ion balance in the cold has been linked to improved chill tolerance, and many endocrine factors are known to influence ion transport rates of these organs. Micromolar doses of CAPA (an insect neuropeptide) have been demonstrated to improve Drosophila cold tolerance, but the mechanisms through which it does, and whether this effect is dose-dependent remain unclear. Here, we provide evidence that low (fM) and high (μM) doses of CAPA impair and improve chill tolerance, respectively, via two different effects on tubule ion and water transport. By quantifying CAPA peptide levels in the central nervous system, we estimated the maximum achievable hormonal titres of CAPA in Drosophila haemolymph, and found evidence to suggest that contrary to long-held opinions, CAPA may function as an anti-diuretic peptide in this species, with negative effects on chill tolerance.

NOTES

NOTES

List of Participants and Contact Information

Hussain Al-Alkawi
University of Toronto Mississauga
Department of Biology
3359 Mississauga Road North
Mississauga, ON, L5L 1C6
h.m@alumni.utoronto.ca

Sonia Aksamit
Brock University
Department of Biological Sciences
500 Glenridge Ave
St. Catharines, ON, L2S 3A1
sonia_aksamit@hotmail.com

Mahnoor Ayub
University of Toronto Mississauga
Department of Biology
3359 Mississauga Road North
Mississauga, ON, L5L 1C6
mahnoor.ayub@mail.utoronto.ca

Jacqueline Bede
McGill University
Department of Plant Biology
Raymond Building, 21111 Lakeshore Road
Ste. Anne de Bellevue, Quebec H9X 3V9
jacqueline.bede@mcgill.ca

Mark Brown
University of Georgia
Department of Entomology
413 Biological Sciences Building
Athens, GA, USA
mrbrown@uga.edu

Kaylen Brzezinski
Carleton University
Department of Biology
209 Nesbitt Biology Building
1125 Colonel By Drive
Ottawa, ON, K1S 5B6
kaylenbrzezinski@cmail.carleton.ca

William Burke
University of Western Ontario
Department of Biology
London, ON N6A 3K7
wburke@uwo.ca

Chun Chih Chen
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
kupo@yorku.ca

Adam Chippindale
Queen's University
Department of Biology
Kingston, ON K7L 3N6
chippind@queensu.ca

Laura Ana Cuciureanu
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
lcuciure@my.yorku.ca

Rosa da Silva
McMaster University
Department of Biology, Life Sciences Building
1280 Main Street West
Hamilton, ON, L8S 4K1
rosa.dasilva@mcmaster.ca

Ken Davey
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
davey@yorku.ca

Erik Demers
McGill University
Department of Plant Biology
Raymond Building, 21111 Lakeshore Road
Ste. Anne de Bellevue, Quebec H9X 3V9

List of Participants and Contact Information

Natalie D'Silva
McMaster University
Department of Biology, Life Sciences Building
1280 Main Street West
Hamilton, ON, L8S 4K1
dsilvanm@mcmaster.ca

Andrew Donini
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
adonini@yorku.ca

Cam Donly
London Research and Development Centre
Agriculture and Agri-Food Canada
1391 Sandford Street
London, ON, N5V 4T3
cam.donly@agr.gc.ca

Daniel Doucet
Great Lakes Forestry Centre
1219 Queen St. E.
Sault Ste. Marie, ON, P6A 2E5
ddoucet@NRCAN.gc.ca

Andrea Durant
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
adurant@yorku.ca

Grant Favell
London Research and Development Centre
Agriculture and Agri-Food Canada
1391 Sandford Street
London, ON, N5V 4T3
grant.favell@agr.gc.ca

William Fick
Great Lakes Forestry Centre
1219 Queen St. E.
Sault Ste. Marie, ON, P6A 2E5
william.fick@canada.ca

Julia Gauberg
University of Toronto Mississauga
Department of Biology
3359 Mississauga Road North
Mississauga, ON, L5L 1C6
j.gauberg@mail.utoronto.ca

Chris Gillen
Kenyon College
Higley Hall 310
Gambier, OH 43022
gillenc@kenyon.edu

Sam Hana
University of Toronto Mississauga
Department of Biology
3359 Mississauga Road North
Mississauga, ON, L5L 1C6
sam.hana@mail.utoronto.ca

Jaehwan Jung
Brock University
Department of Biological Sciences
500 Glenridge Ave
St. Catharines, ON, L2S 3A1
jj10qw@brocku.ca

Jinyuan Ji
McGill University
Department of Plant Biology
Raymond Building, 21111 Lakeshore Road
Ste. Anne de Bellevue, Quebec H9X 3V9

Alex C. Keene
Florida Atlantic University
Department of Biological Sciences
Jupiter, FL, 33458 USA
keenea@fau.edu

Arshad Mohammed Imrit
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
imarshad@my.yorku.ca

List of Participants and Contact Information

Emine Kaplanoglu
London Research and Development Centre
Agriculture and Agri-Food Canada
1391 Sandford Street
London, ON, N5V 4T3
Emine.Kaplanoglu@AGR.GC.CA

Scott P. Kelly
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
spk@yorku.ca

Dennis Kolosov
McMaster University
Department of Biology, Life Sciences Building
1280 Main Street West
Hamilton, ON, L8S 4K1
kolosovd@mcmaster.ca

A. Kornel
Brock University
Department of Biological Sciences
500 Glenridge Ave
St. Catharines, ON, L2S 3A1
ak12af@brocku.ca

Peter J. Krell
University of Guelph
Department of Molecular and Cellular Biology,
College of Biological Science,
Summerlee Science Complex
Guelph, ON, N1G 2W1
pkrell@uoguelph.ca

Angela B. Lange
University of Toronto Mississauga
Department of Biology
3359 Mississauga Road North
Mississauga, ON, L5L 1C6
angela.lange@utoronto.ca

Aryan Lajevardi
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
aryanlaj@my.yorku.ca

Sifang Liao
Stockholm University
Department of Zoology
S-10691 Stockholm Sweden
sifang.liao@zoologi.su.se

Alice Lin
Massachusetts Institute of Technology
Department of Biology
Cambridge, MA, USA
alicelin@mit.edu

Troy Littleton
Massachusetts Institute of Technology
Department of Biology
Cambridge, MA, USA
troy@mit.edu

K.K Ludba
University of Western Ontario
Department of Biology
London, ON N6A 3K7
kludba@uwo.ca

Heath MacMillan
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
macmilla@yorku.ca

H. Mansour
Brock University
Department of Biological Sciences
500 Glenridge Ave
St. Catharines, ON, L2S 3A1

List of Participants and Contact Information

Rima Menassa
London Research and Development Centre
Agriculture and Agri-Food Canada
1391 Sandford Street
London, ON, N5V 4T3
rima.menassa@agr.gc.ca

Joffre Mercier
Brock University
Department of Biological Sciences
500 Glenridge Ave
St. Catharines, ON, L2S 3A1
amercier@brocku.ca

Megan Meuti
The Ohio State University
Department of Entomology
Room 400 Aronoff Laboratory, 1680 Madison Ave, Wooster, OH 44691
meuti.1@osu.edu

Victoria Van Mierlo
McMaster University
School of Interdisciplinary Science
1280 Main Street West
Hamilton, ON, L8S 4K1
vanmieva@mcmaster.ca

Lidiya Misyura
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
lidiyam@my.yorku.ca

Dick R. Nässel
Department of Zoology
Stockholm University,
Svante Arrhenius väg 18B
S-106 91 STOCKHOLM, Sweden
dnassel@zoologi.su.se

Aleksander Necakov
Brock University
Department of Biological Sciences
500 Glenridge Ave
St. Catharines, ON, L2S 3A1
anecakov@brocku.ca

Fargol Nowghani
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
nowghani@my.yorku.ca

Kiel G Ormerod
Massachusetts Institute of Technology
Department of Biology
Cambridge, MA, USA
kormerod@mit.edu

Michael O'Donnell
McMaster University
Department of Biology,
Life Sciences Building
1280 Main Street West
Hamilton, ON, L8S 4K1
odonnell@mcmaster.ca

Raquel Soares Oliveira
Laboratory of Neurobiology and Toxicology
University of Pampa
Rio Grande do Sul, Brazil
raquelsoaresoliveira@yahoo.com.br

Ian Orchard
University of Toronto Mississauga
Department of Biology
3359 Mississauga Road North
Mississauga, ON, L5L 1C6
ian.orchard@utoronto.ca

Emine Özşahin
University of Guelph
Department of Molecular and Cellular Biology
451 University Centre
eozsahin@uoguelph.ca

List of Participants and Contact Information

Jean-Paul Paluzzi
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
paluzzi@yorku.ca

Peter M. Piermarini
The Ohio State University
Department of Entomology
224 Thorne, 1680 Madison Ave,
Wooster, OH 44691
piermarini.1@osu.edu

Guoxing Quan
Great Lakes Forestry Centre
1219 Queen St. E.
Sault Ste. Marie, ON, P6A 2E5
gquan@nrcan.gc.ca

Victoria Radauskas
McMaster University
School of Interdisciplinary Science
1280 Main Street West
Hamilton, ON, L8S 4K1
radausvj@mcmaster.ca

Robyn Ralph
University of Guelph
Department of Molecular and Cellular Biology
451 University Centre
rmccann@uoguelph.ca

R. M. Robertson
Queen's University
Department of Biology
Kingston, ON K7L 3N6
robertrm@queensu.ca

David Rocco
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
davrocco@yorku.ca

Farwa Sajadi
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
farwa@my.yorku.ca

Himeshi Samarasinghe
McMaster University
Department of Biology
1280 Main Street West
Hamilton, ON, L8S 4K1

Vishal Sangha
University of Toronto Mississauga
Department of Biology
3359 Mississauga Road North
Mississauga, ON, L5L 1C6
vishal.sangha@mail.utoronto.ca

Ian Scott
London Research and Development Centre
Agriculture and Agri-Food Canada
1391 Sandford Street
London, ON, N5V 4T3
ian.scott@agr.gc.ca

Adriano Senatore
University of Toronto Mississauga
Department of Biology
3359 Mississauga Road North
Mississauga, ON, L5L 1C6
adriano.senatore@utoronto.ca

Ryan Smith
McGill University
Department of Plant Biology
Raymond Building, 21111 Lakeshore Road
Ste. Anne de Bellevue, Quebec H9X 3V9

Phinyaphat Srithiphaphirom
Queen's University
Department of Biology
Kingston, ON K7L 3N6
p.srithiphaphirom@queensu.ca

List of Participants and Contact Information

Hongliang Su
McGill University
Department of Plant Biology
Raymond Building, 21111 Lakeshore Road
Ste. Anne de Bellevue, Quebec H9X 3V9

Trudy Watson-Leung
Ontario Ministry of the Environment and
Climate Change
Etobicoke, ON
trudy.watsonleung@ontario.ca

Chengfeng Xiao
Queen's University
Department of Biology
Kingston, ON K7L 3N6
xiao.c@queensu.ca

Jianping Xu
McMaster University
Department of Biology
1280 Main Street West
Hamilton, ON, L8S 4K1
jpxu@mcmaster.ca

Gil Yerushalmi
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
gili@my.yorku.ca

Meet R. Zandawala
Department of Zoology
Stockholm University
Svante Arrhenius väg 18B
S-106 91 STOCKHOLM, Sweden
meet.zandawala@zoologi.su.se

Amro Zayed
York University
Department of Biology
4700 Keele Street
Toronto, ON, M3J 1P3
zayed@yorku.ca