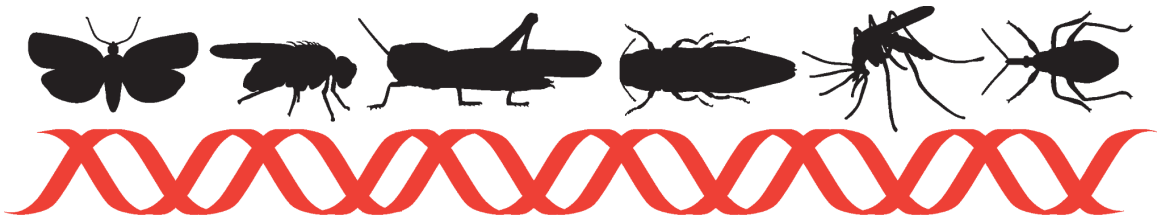

INSECT BIOTECH CONFERENCE

Annual Scientific Meeting

June 14-16, 2017

Schedule and Contributed Abstracts

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

Sponsored by:

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FroggaBio scientific solutions

Insect Biotech Conference – 2017

Conference Schedule

Wednesday Evening – June 14

6:00 pm	**Registration:** Outside Niagara Gardenview Room
7:00 pm	**Plenary Talk:** Gardenview Room **BEHAVIOURAL AND EVOLUTIONARY ECOLOGY OF INSECT PHEROMONES.** ***[Page 1]*** Jeremy Allison, Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, ON, Canada.
7:45 – 9:00 pm	**Reception**: Niagara Gardenview Room (Pizzeria and Beverages)

Thursday, June 15

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

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

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

9:10 am **CLONING, LOCALIZATION, AND PHYSIOLOGICAL EFFECTS OF SULFAKININ IN THE KISSING BUG, *RHODNIUS PROLIXUS*. *[Page 2]***
Al-Alkawi, H., Lange, A.B. and Orchard, I.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

9:30 am ***AEAMT2*, AN ANIMAL MEP/AMT AMMONIA TRANSPORTER – LIKE PROTEIN, CONTRIBUTES TO AMMONIA EXCRETION IN THE ANAL PAPILLAE OF THE LARVAL MOSQUITO, *AEDES AEGYPTI*. *[Page 3]***
Durant, A.C. and Donini, A.
Department of Biology, York University, Toronto, ON, Canada

9:50 am **THE TISSUE DISTRIBUTION OF CAPA-LIKE PEPTIDES IN THE BLACK-LEGGED TICK, *IXODES SCAPULARIS*. *[Page 4]***
Uyuklu, A. and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada

10:10 am **INVESTIGATING THE ENDOGENOUS REGULATION OF THE INSULIN-LIKE RESPONSE IN THE BLOOD-FEEDING HEMIPTERAN, *RHODNIUS PROLIXUS*. *[Page 5]***
[1]da Silva, S.R., [1]Defferrari, M., [1,2]Mollayeva, S. and [1,2]Lange, A.B.
[1]Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada
[2]Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada

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

Session Chair: **Peter Piermarini** (Niagara Gardenview Room)

10:50 am **REVEALING THE LOCALIZATION AND PHYSIOLOGICAL FUNCTION OF ION TRANSPORT PEPTIDE IN THE MOSQUITO, *AEDES AEGYPTI*. *[Page 6]***
[1]Matei, A., [2]Zandawala, M., [2]Dircksen, H., [2]Nässel, D. and [1]Paluzzi, J.P.
[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Department of Zoology, Stockholm University, Stockholm, Sweden.

11:10 am **CALCIUM TRANSPORT BY ISOLATED MALPIGHIAN TUBULES OF *ACHETA DOMESTICA*. *[Page 7]***
Browne, A.A. and O'Donnell, M.J.
Department of Biology, McMaster University, Hamilton, ON, Canada

11:30am **FUNCTIONAL INTERACTION BETWEEN LEUCOKININ AND CORTICOTROPIN-RELEASING FACTOR-LIKE DIURETIC HORMONE SIGNALING IN *DROSOPHILA.* *[Page 8]***
[1]Zandawala, M., [2]Marley, R., [2]Davies, S.A. and [1] Nässel, D.R.
[1]Stockholm University, Department of Zoology, Stockholm, Sweden
[2]Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

11:50 am **EVIDENCE THAT PRINCIPAL AND SECONDARY CELLS IN THE MALPIGHIAN TUBULES OF LEPIDOPTERANS ARE COUPLED VIA GAP JUNCTIONS. *[Page 9]***
O'Donnell, M.J. and Kolosov, D.
Department of Biology, McMaster University, Hamilton, ON, Canada

12:10 – 1:20 pm **Lunch Break**: Niagara Gardenview Room (Executive Deli)

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

1:20 pm **HELICOKININ ALTERS ION TRANSPORT IN THE DISTAL ILEAC PLEXUS OF THE MALPIGHIAN TUBULES OF THE LARVAL CABBAGE LOOPER. *[Page 10]***
Kolosov, D. and O'Donnell, M.J.
Department of Biology, McMaster University, Hamilton, ON, Canada

1:40 pm **WHEN NATURE CALLS: AQP1 EXPRESSION AND FUNCTION IN THE MALPIGHIAN TUBULES OF THE MOSQUITO, *AEDES AEGYPTI*. *[Page 11]***
Misyura, L., Yerushalmi, G. and Donini, A.
Department of Biology, York University, Toronto, ON, Canada.

2:00pm **REARING SALINITY ALTERS LOCALISATION OF IONOMOTIVE ATPASES AND ION TRANSPORT ACROSS THE GASTRIC CAECUM OF *AEDES AEGYTI* LARVAE. *[Page 12]***
[1]D'Silva, N.M., [2]Patrick, M.L. and [1]O'Donnell, M.J.
[1]Department of Biology, McMaster University, Hamilton, ON, Canada.
[2]Department of Biology, University of San Diego, CA, USA.

2:20 pm **GAP JUNCTIONS IN THE CROP OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*. *[Page 13]***
Calkins, T.L., DeLaat, A. and Piermarini, P.M.
Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH USA

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

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

3:00 pm **GLOBAL DISTRIBUTION OF IONOMOTIVE PUMPS AND CONTROL OF MALPIGHIAN TUBULE SECRETION IN THE ADULT TICK, *IXODES SCAPULARIS*. *[Page 14]***
Paez, M. and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada

3:20 pm **EXPRESSION ANALYSIS OF SMALL HEAT SHOCK PROTEIN GENES IN SPRUCE BUDWORM DURING DIAPAUSE.** ***[Page 15]***

[1]Quan, G., [1]Fick, W., [1,2]Duan, J., [1]Ladd, T. and [2]Krell, P.
[1]Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada
[2]Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada

3:40pm **IF IT'S K^+ YOU EXCRETE, THE COLD YOU SHALL BEAT! PHYSIOLOGICAL PLASTICITY OF THE GUT AND MALPIGHIAN TUBULES UNDERLIES COLD ACCLIMATION IN *DROSOPHILA*.** ***[Page 16]***

Yerushalmi, G., Misyura, L., MacMillan, H. and Donini, A.
Department of Biology, York University, Toronto, ON, Canada

4:00pm **HOW TO MINIMIZE ACCIDENTAL LEAKAGE: THERMAL ACCLIMATION MITIGATES COLD-INDUCED PARACELLULAR LEAK FROM THE *DROSOPHILA* GUT.** ***[Page 17]***

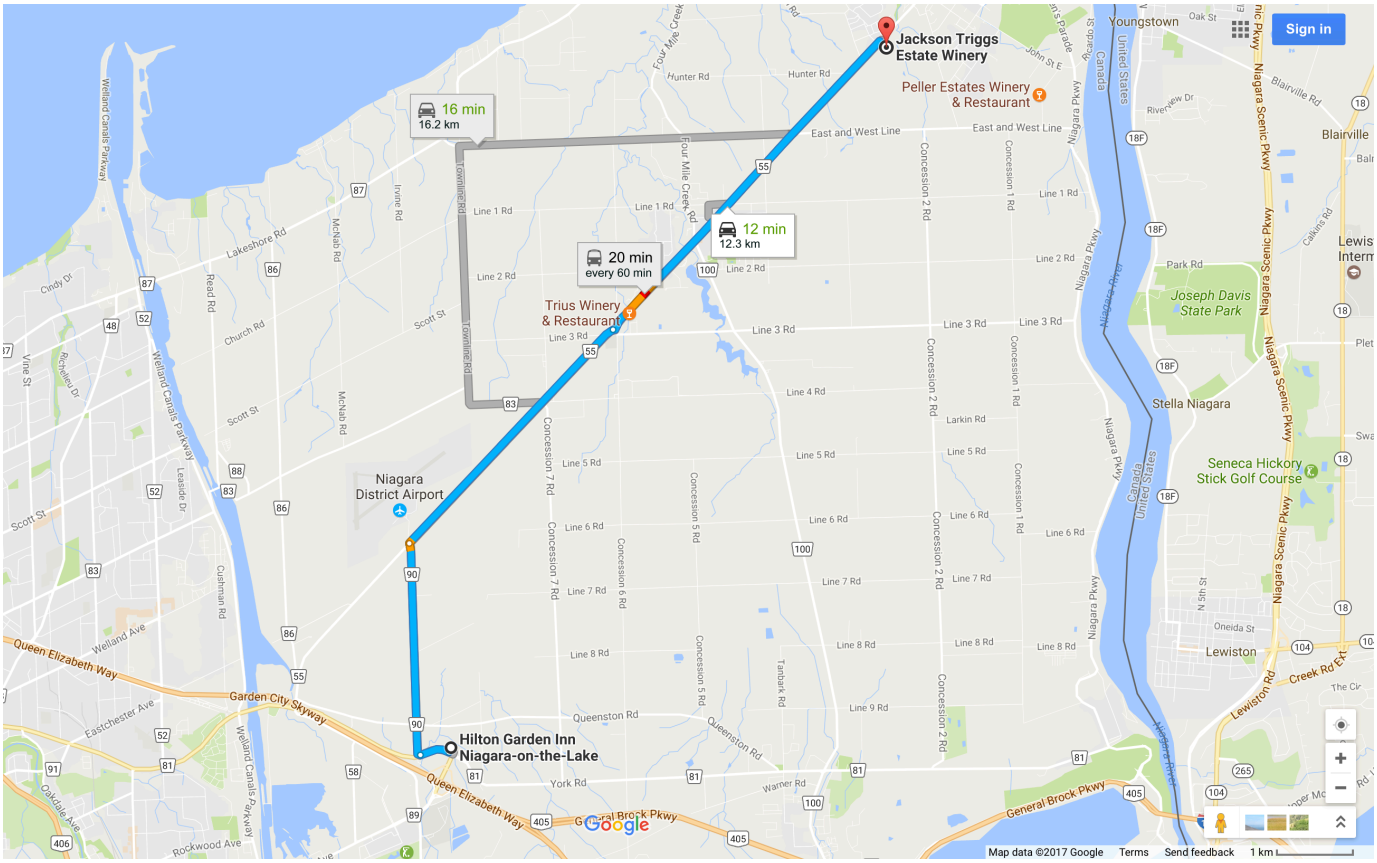
MacMillan, H.A., Yerushalmi, G., Jonusaite, S., Kelly, S.P. and Donini, A.
Department of Biology, York University, Toronto, Ontario, Canada, M3J 1P3

4:20pm **End of Session**

6:00 pm **Winery Tour**
Jackson-Triggs Niagara Estate, 2145 Niagara Stone Road, Niagara-on-the-Lake, Ontario, L0S 1JO

7:00 pm **Banquet Dinner in Barrel Cellar**
Jackson-Triggs Niagara Estate, 2145 Niagara Stone Road, Niagara-on-the-Lake, Ontario, L0S 1JO

Map and Directions for Banquet:



Estimated travel time ~12 min (12.3 km) via Niagara Stone Rd/Regional Rd 55

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 2nd exit and stay on Niagara Stone Rd/Regional Rd 55
  5.2 km

Jackson Triggs Estate Winery
2145 Niagara Stone Rd, Niagara-on-the-Lake, ON L0S 1J0

Friday, June 16

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

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

9:10 am **RNA INTERFERENCE OF MDR GENES IN *LEPTINOTARSA DECEMLINEATA* AND *TRICHOPLUSIA NI* BY INGESTION OF DOUBLE-STRANDED RNA.** ***[Page 18]***
Favell, G. and Donly, C.
Department of Biology, The University of Western Ontario, London, ON, Canada
London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada

9:30 am **UTILIZING ENTOMOPATHOGENIC FUNGI TO INVESTIGATE THE PHYSIOLOGY OF THE BROWN MARMORATED STINK BUG IMMUNE RESPONSE.** ***[PAGE 19]***
Radauskas, V.J., Mierlo, V.V., Tahir, I. and da Silva, R.
Department of Biology, McMaster University, Hamilton, ON, Canada

9:50 am **DIET EFFECTS ON TRANSCRIPTOMICS IN *TRICHOPLUSIA NI* AND ITS SUSCEPTIBILITY TO ACMNPV BACULOVIRUS.** ***[Page 20]***
Chen, E. and Donly, B.C.
London Research and Development Centre, Agriculture and Agri-food Canada, London, Ontario, Canada
Department of Biology, University of Western Ontario, London, Ontario, Canada

10:10 am **THE CHEMOSENSORY PROTEIN GENE FAMILY OF THE EMERALD ASH BORER: EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF CSP5.** ***[Page 21]***
[1]Doucet, D., [2]Zhou, Z., [1]Pavlik, L., [1]Duan, J., [1]Bowman, S., [1]Wen, F., [1]Quan, G. and [3]Krell, P.
[1]Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, Ontario, Canada
[2]Department of Plant Protection, College of Forestry, Henan University of Science & Technology, Luoyang, Henan, P.R. China.
[3]Department of Molecular and Cellular Biology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada.

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

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

SHORT EXPOSURES SESSION (5 MINUTE TALK and QUESTIONS)

10:50 am

IDENTIFICATION, EXPRESSION ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF TWO GNRH-RELATED PEPTIDE RECEPTORS IN THE MOSQUITO, *AEDES AEGYPTI*. *[Page 22]*
Oryan, A., Wahedi, A. and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada.

THE EFFECT OF *RHINELLA ICTERICA* TOAD VENOM ON HEART AND OVIDUCT CONTRACTIONS IN *LOCUSTA MIGRATORIA*. *[Page 23]*
[1]Oliveira, R.S., [2]dos Santos, D.S., [1]Leal, A.P., [1,2]Dal Belo, C.A. and [3]Lange, A.B.
[1]Laboratory of Neurobiology and Toxicology, University of Pampa, Rio Grande do Sul, Brazil
[2]Toxicology Biochemistry, University of Santa Maria, Rio Grande do Sul, Brazil
[3]Department of Biology, University of Toronto Mississauga, Toronto, Ontario, Canada

INSECTICIDAL TRAP PLANTS INCORPORATING RNA INTERFERENCE TECHNOLOGY. *[Page 24]*
Donly, C., Kaplanoglu, E., Ludba, K.K., Kolotilin, I., Menassa, R. and Scott, I.M.
London Research & Development Centre, AAFC, London, ON Canada and
Department of Biology, University of Western Ontario, London, ON Canada

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

12:00 – 1:10 pm **Lunch Break**: Niagara Gardenview Room (Picnic Lunch Buffet)

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

1:10 pm

IN THE BELLY OF THE BEAST: VECTOR IMMUNE RESPONSES IN THE MIDGUT DETERMINE WHICH INSECT VECTORS TRANSMIT WHICH PARASITES. THE SAGA CONTINUES…… *[Page 25]*
Lowenberger, C.
Department of Biological Sciences, Simon Fraser University, Burnaby BC V5A 1S6

1:30 pm

THE INVOLVEMENT OF RHOPR-CRF/DH IN FEEDING AND REPRODUCTION IN THE BLOOD-GORGING INSECT *RHODNIUS PROLIXUS*. *[Page 26]*
Mollayeva, S. Orchard, I. and Lange A.B.
Department of Biology, University of Toronto Mississauga, Toronto, ON, Canada

1:50 pm

OCTOPAMINE AND TYRAMINE MODULATE THE FEMALE REPRODUCTIVE SYSTEM IN THE MEDICALLY-IMPORTANT BUG, *RHODNIUS PROLIXUS*. *[Page 27]*
Hana, S., and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

2:10 pm

ELUCIDATING THE FUNCTIONAL ROLE OF AN ANCIENT NEUROENDOCRINE SYSTEM INVOLVING GPA2/GPB5 AND ITS RECEPTOR (LGR1) IN THE MOSQUITO, *AEDES AEGYPTI*. *[Page 28]*
Rocco, D.A. and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada.

2:30 – 2:50pm **Coffee Break**: Niagara Gardenview Room

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

2:50 pm **BIOCHEMICAL BASIS OF PUPAL SUSCEPTIBILITY TO IONIZING RADIATION IN *DROSOPHILA MELANOGASTER*. *[Page 29]***
Paithankar, J.G., Raghu, S.V and Patil, R.K.
Department of Applied Zoology, Mangalore University, Mangalore, 574199, India

3:10 pm **EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF TACHYKININ-RELATED PEPTIDES IN THE BLOOD-FEEDING BUG, *RHODNIUS PROLIXUS*. *[Page 30]***
Haddad, A.N., Defferrari, M., Hana, S. and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

3:30 pm **FUNCTIONAL CHARACTERIZATION OF THE ADIPOKINETIC HORMONE/CORAZONIN-RELATED PEPTIDE RECEPTOR IN *AEDES AEGYPTI*. *[Page 31]***
Wahedi, A. and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada.

3:50 pm **Closing Remarks:** Jean-Paul Paluzzi and Andrew Donini

BEHAVIOURAL AND EVOLUTIONARY ECOLOGY OF INSECT PHEROMONES.

Allison, J.

Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, ON, Canada.

The location or recruitment of a mate is a pivotal event in sexual reproduction. Although asexual reproduction has evolved independently several times in insects, for many insect groups sexual reproduction is by far the most common. It is typically preceded by long-distance attraction and short-range courtship, both of which are often mediated by pheromones. Because of their role as pests, contact and volatile pheromones have been identified for numerous species of bark and woodboring beetles. Fewer studies have documented variation in insect pheromone signals within and among individuals, populations and species. Unfortunately, this variation is usually incompletely documented and its causes and consequences are poorly understood. This talk will summarize some recent work examining the role of pheromones in the mating behaviour of bark and woodboring beetles with particular attention on their role in the maintenance of reproductive isolation of sympatric species.

CLONING, LOCALIZATION, AND PHYSIOLOGICAL EFFECTS OF SULFAKININ IN THE KISSING BUG, *RHODNIUS PROLIXUS.*

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

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

Sulfakinins (SKs) are a family of multifunctional neuropeptides that have been primarily shown to have myotropic activity on muscles of the digestive system and to function as feeding satiety factors. Our initial experiments have 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 unfed fifth-instar *R. prolixus*. Fluorescent in situ hybridization showed transcript expression only in neurons in the brain. Immunohistochemical staining of SK-like peptides was observed in the same neurons in the brain and in processes extending throughout the CNS, as well as over the posterior midgut and anterior hindgut. Rhopr-SK-1 induced contractions of the hindgut in a dose-dependent manner, but had no significant effect on heartbeat frequency. Injection of Rhopr-SK-1 significantly decreased the overall weight of the blood meal consumed, suggesting SK's role as a satiety factor in *R. prolixus*. A seven-transmembrane Rhopr-SK G-protein coupled receptor (GPCR) was examined via its cloning and characterization. RT-qPCR of the receptor revealed that the target tissues for Rhopr-SK-1 and/or –SK-2 are primarily located in the CNS, with further expression in the heart, gut, salivary glands, Malpighian tubules, as well as male and female reproductive systems. An oviduct contraction assay demonstrated that sulfakinin inhibits contractions of the oviduct in adult *R. prolixus* in a dose-dependent manner. These findings suggest that SKs are linked with the control of feeding in *R. prolixus*.

This work was supported by NSERC.

AEAMT2, AN ANIMAL MEP/AMT AMMONIA TRANSPORTER – LIKE PROTEIN, CONTRIBUTES TO AMMONIA EXCRETION IN THE ANAL PAPILLAE OF THE LARVAL MOSQUITO, _AEDES AEGYPTI_.

Durant, A.C. and Donini, A.

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

The mosquito, *Aedes aegypti* inhabits ammonia rich septic tanks in tropical regions of the world that make extensive use of these systems, explaining the prevalence of disease during dry seasons. Since ammonia (NH_3/NH_4^+) is toxic to animals, an understanding of how *A. aegypti* larvae can survive in this high ammonia environment is important. Aquatic animals typically excrete ammonia directly into the aqueous environment and the anal papillae of larval *A. aegypti,* in part, serve this function. Previously, we have shown that two Rhesus-like ammonia transporter proteins, *AeRh50-1*, *AeRh50-2*, and a MEP/Amt ammonia transporter-like protein, *AeAmt1,* participate in ammonia excretion by the anal papillae. The present study demonstrates that a fourth ammonia transporter – like protein, *AeAmt2,* is expressed in the anal papillae epithelium where it co-localizes with V-type H^+-ATPase on the apical membrane. By feeding larvae dsRNA targeting *AeAmt2*, protein abundance of this transporter was significantly reduced after two days post treatment. We assessed the effects of *AeAmt2* knockdown on ammonia fluxes at the anal papillae, as well as hemolymph ammonia levels and pH. We show that a decrease in AeAmt2 protein within the anal papillae results in a significant reduction in ammonia efflux at the anal papillae epithelium, but does not affect ammonia hemolymph or pH levels.

This work was supported by NSERC.

THE TISSUE DISTRIBUTION OF CAPA-LIKE PEPTIDES IN THE BLACK-LEGGED TICK, *IXODES SCAPULARIS*.

Uyuklu, A. and Paluzzi, J.P.

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

Ixodes scapularis is a chief vector for a range of diseases. Lyme disease, which can result due to infection with *Borrelia burgdorferi*, leads to arthritis-like symptoms as it causes swelling and pain in the large joints of the body. In the face of increasing cases of infection, very little is known about the neuroendocrine system that regulates key physiological processes of the black-legged ticks. Furthermore, as obligate blood feeders, it is crucial for *I. scapularis* to maintain ionic and osmotic homeostasis. In insects, CAPA peptides have been shown to influence fluid secretion by Malpighian tubules, acting as either diuretic or anti-diuretic hormones that control the movement of excess ions, water, and metabolic wastes secreted in the primary urine. In the present study, the distribution of CAPA-like peptides was localized in various tissues of adult *I. scapularis* by using immunohistochemical procedures. CAPA-like immunoreactivity was localized in cells and processes in the synganglion, midgut endocrine-like cells, reproductive tissues and the posterior region of the rectal sac. RT-PCR was used to verify the distribution of the CAPA peptide-encoding transcript in various tissues where it was detected exclusively in the synganglion. The transcript encoding a peptide sharing structural similarity at its C-terminus, short neuropeptide F (sNPF), demonstrated overlapping tissue distribution to that identified by the CAPA-like immunoreactive staining. Furthermore, an enzyme-linked immunosorbent assay (ELISA) was developed to quantify *I. scapularis* CAPA peptides present in the various tissues. These findings will help elucidate the physiological functions of the CAPA-related peptides in this important human disease vector.

Research supported by an NSERC Discovery Grant to J.P.P.

INVESTIGATING THE ENDOGENOUS REGULATION OF THE INSULIN-LIKE RESPONSE IN THE BLOOD-FEEDING HEMIPTERAN, *RHODNIUS PROLIXUS*.

[1]da Silva, S.R., [1]Defferrari, M., [1,2]Mollayeva, S. and [1,2]Lange, A.B.

[1]Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada
[2]Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada

Insulin signalling regulates glucose uptake and metabolism in vertebrate tissues. Similarly, invertebrates express and release a diverse series of insulin-like peptides (ILPs) from insulin-producing cells (IPCs) upon feeding. In contrast to vertebrate systems, ILPs stimulate both carbohydrate and lipid uptake and storage in the fat body and skeletal muscle, and their signalling is also crucial for ecdysis. We have previously identified a putative ILP, namely RhoprILP, within the blood-feeding insect *Rhodnius prolixus*. RNAi-mediated knockdown of RhoprILP triggers an increase in hemolymph carbohydrate and lipid levels, with a concomitant decrease in carbohydrate levels within fat bodies and skeletal muscles. However, the downstream signalling pathway induced by RhoprILP signalling is yet to be confirmed. In the present study, we have identified that injection of 5th instar animals with the vertebrate insulin receptor (IR) activator, BpV(phen), induced the phosphorylation and activation of the intracellular protein kinase AKT, which is one of the key regulators of the insulin signalling response in vertebrates and other invertebrates such as *Drosophila melanogaster*. We also observed an interesting pattern of AKT activation following a blood meal that matches a previously observed pattern of ILP release from IPC cells in 5th instar *R. prolixus*. The phosphorylation of AKT was accompanied by the phosphorylation of its downstream targets including glycogen synthase kinase 3 (GSK3) and the transcription factor FOXO1. These data suggest that endogenous RhoprILP operates on an IR-like tyrosine kinase receptor, and triggers a coordinated intracellular response similar to that observed in other invertebrate and vertebrate species.

REVEALING THE LOCALIZATION AND PHYSIOLOGICAL FUNCTION OF ION TRANSPORT PEPTIDE IN THE MOSQUITO, *AEDES AEGYPTI.*

[1]Matei, A., [2]Zandawala, M., [2]Dircksen, H., [2]Nassel, D. and [1]Paluzzi, J.P.

[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Department of Zoology, Stockholm University, Stockholm, Sweden.

In the locust, Ion Transport Peptide (ITP) was the first characterized antidiuretic factor shown to act on the hindgut, which was later uncovered in other insects including the mosquito, *Aedes aegypti*. My research aims to delineate the function of ITP within the hindgut of the mosquito, *A. aegypti* – the vector responsible for spreading a range of diseases, such as dengue and yellow fever. In order to generate a functional ITP recombinant peptide, we utilized an endocrine-derived cell culture system involving mouse anterior pituitary (AtT-20) cells to express both *D. melanogaster* ITP (DromeITP) and *A. aegypti* ITP (AedaeITP). Protein extracts were isolated from AtT-20 cells transiently expressing either AedaeITP or DromeITP, and samples were processed through western blot analysis using a primary antibody against the C-terminal region of DromeITP, which shares ~40% similarity to the AedaeITP C-terminal region. A band size of approximately 9 kDa was detected in protein samples from both ITP-transfected cells, however a higher molecular weight band at approximately 13 kDa was detected in protein samples from DromeITP-transfected cells. This higher molecular weight band was suggested to be a glycosylated variant of ITP, as shown by its loss upon PNGase treatment. Using wholemount immunohistochemistry, the central nervous system of four-day-old adult *A. aegypti* was surveyed for ITP-like immunoreactivity. Preliminary findings indicate ITP-immunoreactive cells located medioposteriorly and ventrally on each abdominal ganglia of the ventral nerve cord. Using Scanning Ion-selective Electrode Technique to measure ion transport across the hindgut epithelia, the influence of recombinant AedaeITP and suspected second messengers, cAMP and cGMP, were investigated. Results thus far indicate that treatment with cAMP generally promoted hemolymph directed Na^+ flux (i.e absorption), while it generally inhibited K^+ absorption. Conversely, cGMP generally promoted lumen-directed Na^+ flux (i.e secretion).

Research supported by an NSERC Discovery Grant to J.P.P.

CALCIUM TRANSPORT BY ISOLATED MALPIGHIAN TUBULES OF *ACHETA DOMESTICA*.

Browne, A.A. and O'Donnell, M.J.
Department of Biology, McMaster University, Hamilton, ON, Canada

Malpighian (renal) tubules are insect excretory organs that are thought to play a principal role in extracellular calcium regulation by removing excess Ca^{2+} from the blood. Previous studies have suggested there are two routes for Ca^{2+} removal: 1) by transporting Ca^{2+} into the lumen of the tubule where it then becomes a solute of the primary urine or 2) by sequestering calcium as mineral salts within the tubule. In the house cricket, *Acheta domesticus*, sequestration appears dominant as calcium-containing granules are abundant in intracellular vesicles of tubule cells. These granules are analogous to mammalian kidney stones leading many researchers to propose that insects may represent good model systems to study human nephrolithiasis. Of interest are the sites and mechanisms of calcium transport by insect Malpighian tubules. Using the scanning ion-selective microelectrode technique (SIET) to measure basolateral Ca^{2+} flux along the length of isolated tubules, Ca^{2+} uptake was found selectively in mid-tubule regions. Preliminary results indicate that cyclic nucleotides stimulate Ca^{2+} influx through calcium channels.

FUNCTIONAL INTERACTION BETWEEN LEUCOKININ AND CORTICOTROPIN-RELEASING FACTOR-LIKE DIURETIC HORMONE SIGNALING IN *DROSOPHILA*

[1]Zandawala, M., [2]Marley, R., [2]Davies, S.A. and [1] Nässel, D.R.

[1]Stockholm University, Department of Zoology, Stockholm, Sweden
[2]Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

The renal (Malpighian) tubules of the fly *Drosophila melanogaster* represent an excellent epithelial model for genetics and physiology, and in which to study the interaction between different signaling systems regulating diuresis and fluid homeostasis. In *Drosophila*, there are four major neuropeptide families that regulate ion and water homeostasis by acting on the Malpighian tubules (MTs). These include (1) corticotropin-releasing factor-like diuretic hormone (diuretic hormone 44; DH44), (2) leucokinin (LK), (3) calcitonin-like diuretic hormone (diuretic hormone 31; DH31) and (4) CAPA (also known as CAP2b). In order to investigate functional relations between LK and DH44, especially in osmotic and metabolic stress tolerance, we have investigated the expression of these peptides using various GAL4 lines and immunocytochemistry to screen for possible colocalization and determined their cell-specific functions following RNAi-based knockdown. Our expression data show that DH44 and LK are co-localized in both larval and adult lateral segmental neurosecretory cells (ABLKs) of the ventral nerve cord, but not in any other neurons. Using cell specific drivers i.e., Lk- and DH44-GAL4 drivers, to knock down these peptides we assayed for effects on stress tolerance, feeding and water content. Finally, we tested for functional interaction between LK and DH44 at the level of MTs using the Ramsay fluid secretion assay on isolated, intact MTs. The application of both LK and DH44 results in an additive response in fluid secretion by MTs. Our results suggest that these two co-localized peptides could be simultaneously released from ABLKs as neurohormones and impact stress tolerance in a similar manner, but have different effects on feeding and organismal water content.

EVIDENCE THAT PRINCIPAL AND SECONDARY CELLS IN THE MALPIGHIAN TUBULES OF LEPIDOPTERANS ARE COUPLED VIA GAP JUNCTIONS

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The larvae of many species of Lepidoptera (butterflies and moths) are major agricultural pests. High rates of feeding and growth and maintenance of extremely alkaline conditions in the anterior midgut pose dramatic ionoregulatory challenges for these insects. Excretion in insects is accomplished by the combined actions of the Malpighian tubules and hindgut, which together form the functional kidney. The fluid secretory portion of the tubule is composed of principal and secondary cells. Na^+/K^+ ATPase, which energizes ion transport in vertebrate epithelia, has been assumed to play a minor role in this process. We show that Na^+/K^+ ATPase is localized to the basolateral membrane of principal cells in the Malpighian tubules of *Trichoplusia ni*, contrasting its localization to the secondary cells in dipteran tubules. Unexpectedly, blocking Na^+/K^+ ATPase activity in tubules of *T. ni* with ouabain resulted in diminished reabsorption of Na^+ and K^+ by the secondary cells (which lack Na^+/K^+ ATPase immunoreactivity), suggesting coupling of the two cell types via gap junctions. The effects of drugs which block gap junctions indicated coupling of ion transport in the two cell types. Transcript abundance of several gap junction proteins (innexins) in the Malpighian tubules changed in response to variations in dietary Na^+ and K^+. The coupling of ion transport between the two cell types is hypothesized to aid in recycling of ions necessary for constant fluid secretion and excretion of metabolic wastes in lepidopterans.

HELICOKININ ALTERS ION TRANSPORT IN THE DISTAL ILEAC PLEXUS OF THE MALPIGHIAN TUBULES OF THE LARVAL CABBAGE LOOPER

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Fluid secretion in the Malpighian tubules (MTs) of insects is under coordinated control of multiple diuretic and anti-diuretic hormones. Bloodfeeders ingest meals intermittently and the postprandial diuresis functions to eliminate excess water and sodium. By contrast, the role of diuretics in insects which feed constantly is less apparent. For a caterpillar, ingesting large amounts of food results in a constant need for active excretion of metabolites and potential difficulty in maintaining ion balance as haemolymph volume increases with larval growth. Helicokinin (HK) is a lepidopteran kinin isolated from the abdominal ventral nerve cord of adult Helicoverpa zea in the 1990's with reported regulatory effects on the fluid secretion rates of the Malpighian tubules in several lepidopteran species to date. In the current study, we demonstrate the presence of Helicokinin receptor (HK-R) in the distal ileac plexus of the larval cabbage looper *Trichoplusia ni*. HK-R mRNA abundance altered in response to in vivo variations in dietary Na+ and K+ or application of HK to isolated Malpighian tubules. Scanning ion-selective electrode and Ramsay assay techniques were used to demonstrate that HK regulates $[Na^+]/[K^+]$ ratio in the fluid secreted by the MTs of *T. ni* by altering K+ secretion via principal cells and Na^+ reabsorption via secondary cells. Quantitative real-time PCR was used to implicate NKCC, NKA, NHE-6 and NHE-8 as targets for HK action in the MTs of *T. ni*.

WHEN NATURE CALLS: AQP1 EXPRESSION AND FUNCTION IN THE MALPIGHIAN TUBULES OF THE MOSQUITO, *AEDES AEGYPTI*.

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The mosquito, *Aedes aegypti*, is the primary vector for the arboviral diseases Zika, dengue fever, chikungunya, and yellow fever that affect millions of people worldwide. The larvae of *A. aegypti* reside in hypo-osmotic freshwater habitats, where they face dilution of bodily fluids due to the influx of water. The Malpighian tubules (MTs) are one of the major contributors to osmoregulation, whereby they function in the removal of excess water from the hemolymph, thus maintaining ionic and osmotic homeostasis. The epithelium of the MTs actively transports ions from the hemolymph into the lumen which creates an osmotic gradient that drives paracellular and transcellular water movement in the same direction. Our previous study has shown that this transcellular water transport occurs through at least one of the six aquaporins (AQP) that *A. aegypti* possesses, AQP5. In the present study, the function of other AQPs with high relative mRNA abundance in the MTs were studied with the aid of double stranded RNA knockdown. The MTs' fluid secretion rate and ionic composition of secreted fluid were assessed using Ramsay assay and ion selective microelectrode (ISME), respectively. Hemolymph ion concentrations and larval survival were measured to gauge effects of AQP knockdown on osmoregulation and larval physiology. Water transport across the MTs of the larval mosquito is a critical physiological process because it is the first essential step in eliminating the excess water, a consequence of living in hypo-osmotic habitats. Our study sheds light on the relative contribution of aquaporins in this vital process.

This work was supported by NSERC.

REARING SALINITY ALTERS LOCALISATION OF IONOMOTIVE ATPASES AND ION TRANSPORT ACROSS THE GASTRIC CAECUM OF *AEDES AEGYTI* LARVAE.

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Larvae of *Aedes aegypti*, the yellow fever vector, inhabit a variety of aquatic habitats ranging from fresh water to brackish water. Understanding larval physiology and larval responses to salinity changes can provide the foundation for development of novel larvicides. Although previous studies have described changes in ion transport by the Malpighian tubules, hindgut and anal papillae in response to variations in ambient salinity, the effects of rearing salinity on ion transport by the gastric caecum have not been studied. 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. Moreover, we have shown that the regionalization of VA and NKA, and their ATPase activities, are dependent upon rearing salinity. Measurements of electrochemical gradients and transepithelial potentials for H^+, Na^+ and K^+ show that the caecum is functionally distinct from the adjacent anterior midgut and that Na^+ and K^+ concentrations differ dramatically in the lumen of the caecum of fresh versus brackish water larvae.

GAP JUNCTIONS IN THE CROP OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

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The crop is a diverticulum of the esophagus and a food storage organ conserved among insects of the Order Diptera (flies). The crop must pump its stored contents back into the alimentary canal for digestion and absorption by the animal. The pumping is mediated by peristaltic contractions of the crop musculature. In adult female mosquitoes, the crop selectively stores sugar solutions (e.g., nectar); protein-rich blood meals by-pass the crop and are directly transferred to the midgut for digestion. The mechanisms that mediate and regulate crop contractions have never been investigated in mosquitoes. However, these mechanisms are relatively well described in other flies, such as the blow fly (*Phormia regina*). Here we characterize the contractile nature of the mosquito crop, and investigate the potential involvement of gap junctions in crop contractions. We measured contraction rates of mosquito crops in vitro, and utilized qPCR and immunohistochemistry to characterize the expression of gap junctional proteins (i.e., innexins). We found that the mosquito crop is under similar physiological controls as other flies, with serotonin increasing crop contractions and a dromyosupressin mimic, benzethonium chloride, decreasing contractions. Moreover, for the first time in any insect, we demonstrated that the gap junction inhibitor carbenoxolone reduced crop contractions. Using qPCR, we identified the mRNA expression of several genes in the crop that encode gap junctional proteins (innexins), with innexin 2 (inx2) being the most highly expressed. Furthermore, we localized immunoreactivity of inx2 to muscle cells and inx3 to epithelial cells of the crop, consistent with a role of gap junctions in crop physiology.

GLOBAL DISTRIBUTION OF IONOMOTIVE PUMPS AND CONTROL OF MALPIGHIAN TUBULE SECRETION IN THE ADULT TICK, *IXODES SCAPULARIS*

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Osmoregulation and ion regulation in ticks are pivotal to their survival as their hematophagous feeding strategy results in the presence of excess ions and fluid during blood meals. The salivary glands in Ixodid ticks are directly involved in pathogen transmission and they play essential roles in absorption of water vapor from unsaturated air. They are key to tick hydromineral balance playing a vital role in excretion of excess ions and fluids back to the host during blood meal engorgement. The Malpighian tubules of ticks are also suggested to play a role in regulation since the salivary glands only account for 70% of water excretion back into the host. In order to further characterize tissues involved in ion and osmoregulation, we used immunohistochemical methods to examine the distribution of two ionomotive pumps, the Na^+/K^+ ATPase and V-type H^+ ATPase, as these are both suggested to coordinate transport within the salivary glands and Malpighian tubules. Specifically, Na^+/K^+ ATPase immunoreactivity was found predominantly in type I salivary gland acini, while V-type H^+ ATPase immunoreactivity was found in all acini. Na^+/K^+ ATPase and V-type H^+ ATPase immunoreactivity was found throughout the Malpighian tubules, which suggests a potential contribution in energizing ion transport. The effects of two prospective second messengers, cyclic AMP and cyclic GMP, were also examined on isolated Malpighian tubules to determine their effects on fluid secretion rates measured using the Ramsay assay. Interestingly, both second messengers resulted in an increase in Malpighian tubule secretion rates compared to basal secretion in unstimulated controls.

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EXPRESSION ANALYSIS OF SMALL HEAT SHOCK PROTEIN GENES IN SPRUCE BUDWORM DURING DIAPAUSE.

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Spruce budworm (SBW) survive the cold harsh conditions of winter by diapausing as second instar larvae. Winter survival greatly affects SBW population size and geographical distribution. Insect diapause is a complex state involving endocrine, neuroendocrine, metabolic, molecular, cellular, enzymatic and behavioral changes. It is well established that production of polyols and other low molecular weight compounds can enhance insect cold tolerance. Work on the molecular regulation of diapause, however, remains in its infancy. Recently studies have demonstrated that heat shock proteins (HSPs) may substantially contribute to cold tolerance during diapause. HSPs act as chaperones, assisting in correct folding of both nascent and stress-accumulated misfolded proteins, and thus preventing protein aggregation. Compared to other HSP families, sHSPs display structural and functional diversity among different insect species. Some of these proteins may be species-specific and have specific roles within the insect. Recently, we identified 15 sHSP genes, nine of which are temperature-sensitive and eight are likely to be unique to SBW. In the present study, we monitored, under laboratory conditions, gene expression in 14 sHSPs during the entrance into, maintenance of and termination of diapause. We also measured the accumulation of the sHSP in response to heat shock at the different diapause stages. Preliminary results indicated that the various sHSPs have different expression patterns and they response to heat shock differently at different points during diapause. These results indicated that sHSPs have important roles during SBW diapause.

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IF IT'S K^+ YOU EXCRETE, THE COLD YOU SHALL BEAT! PHYSIOLOGICAL PLASTICITY OF THE GUT AND MALPIGHIAN TUBULES UNDERLIES COLD ACCLIMATION IN *DROSOPHILA.*

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At low temperatures *Drosophila*, like most insects, lose the ability to regulate ion and water balance across the gut epithelia, which leads to a lethal accumulation of K^+ in the hemolymph (hyperkalemia). Cold-acclimation, prior to low temperature exposure, can mitigate or entirely prevent these ion imbalances, but the physiological mechanisms that facilitate this process are still not understood. Here, we investigate the potential modulation of Na^+/K^+-ATPase (NKA) and V-Type H^+-ATPase (VA) activities in the gut and the Malpighian tubules of *Drosophila* in cold acclimation. Upon adult emergence, *D. melanogaster* females were subjected to seven days at 25°C (warm acclimation) or 10°C (cold acclimation). Cold-acclimation reduced the critical thermal minimum (CT_{min}), sped up recovery from chill coma, improved survival following prolonged cold stress, and mitigated cold-induced hyperkalemia. NKA and VA activities were lower in the midgut and the Malpighian tubules of cold-acclimated flies. This coincided with increased Malpighian tubule fluid secretion and the maintenance of K^+ secretion in the cold, as well as reduced K^+ reabsorption in the gut of cold acclimated flies. Our results suggest that the modification of Malpighian tubule and gut activity mitigates cold-induced hyperkalemia in cold-acclimated flies and that this process is not driven by altered VA or NKA activities.

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HOW TO MINIMIZE ACCIDENTAL LEAKAGE: THERMAL ACCLIMATION MITIGATES COLD-INDUCED PARACELLULAR LEAK FROM THE *DROSOPHILA* GUT.

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Chill susceptible insects are incapacitated and killed by chilling, but adaptation and acclimation to low temperatures can facilitate substantial improvements in chilling tolerance. Cold exposure causes a gradual leak of ions and water down their concentration gradients. This loss of balance occurs most notably across the gut epithelia, where large ionic and osmotic gradients are normally maintained by high rates of transport. It is unclear whether this effect is related to a suppression of transcellular transport alone, or whether chilling also disrupts paracellular barrier function. In this talk we will demonstrate that some of the major proteins of the paracellular septate junctions of *Drosophila* are differentially expressed following cold acclimation. Cell-cell contact regions in the midgut epithelium also become more convoluted and contain more septate junctions in cold-acclimated flies. This plasticity in junction structure is associated with a reduction in the tendency for paracellular solute leak from the midgut before and during chronic cold stress. Thus, septate junction plasticity likely aids in the maintenance of solute and water balance and may represent an important mechanism of thermal acclimation.

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RNA INTERFERENCE OF MDR GENES IN *LEPTINOTARSA DECEMLINEATA* AND *TRICHOPLUSIA NI* BY INGESTION OF DOUBLE-STRANDED RNA.

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Colorado potato beetle (*Leptinotarsa decemlineata*) and cabbage looper (*Trichoplusia ni*) are two species of insects that are significant agricultural pests around the world. Numerous populations of these insects have developed resistances to various pest control techniques including chemical insecticides. Of these populations, many exhibit a multi-drug resistance phenotype whereby they are resistant to multiple, often unrelated insecticides. Overexpression of multidrug resistance (MDR) genes which code for transmembrane ATP-binding cassette efflux transporters has been associated with this multi-drug resistance phenotype in many other organisms, but not yet in *L. decemlineata* or *T. ni*, as their MDR genes have not been closely studied. In this project, three MDR genes in *L. decemlineata* and one MDR gene in *T. ni* are investigated. First, relative expression of these genes was measured in nerve, Malpighian tubule, and midgut tissue to determine their localization across tissues related to insecticide detoxification. Then, RNA interference mediated knockdown of each gene was attempted through ingestion of double-stranded RNA (dsRNA) to evaluate the effectiveness of this delivery method for dsRNA in each species. Finally, for successfully down-regulated genes, their relation to insecticide susceptibility was assessed by exposing the insects to an insecticide and measuring differences in mortality caused by the knockdown. This research provides valuable knowledge of MDR gene expression in *L. decemlineata* and *T. ni*, the relationship between that expression and insecticide susceptibility; and the potential of RNA interference as a control method for these pests.

UTILIZING ENTOMOPATHOGENIC FUNGI TO INVESTIGATE THE PHYSIOLOGY OF THE BROWN MARMORATED STINK BUG IMMUNE RESPONSE.

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The brown marmorated stink bug (BMSB) is one of the newest invasive insect species in Ontario that can cause millions of dollars in agricultural damage each year due to its extensive feeding habits. Most of the limited research on this pest has been focused on geographic distribution and chemical controls, with little work investigating how BMSB regulate their physiological processes. Since entomopathogenic fungi have been identified as a potential biocontrol tool for reducing BMSB populations, this study utilized infection of BMSB 5th instars with strains of entomopathogenic fungi in order to investigate and characterize the insect's immune response. This was done through repeated fungal inoculations of BMSB instars to determine mortality curves and immune response physiology. The main conclusions of this study were that relative production of fungal melanin may be a key factor influencing the virulence of entomopathogenic fungal strains, and that BMSB primarily localize their immune response to the dorsal vessel region. A secondary finding was that the relative level of melanization may change for a fungal strain after passage through an insect, implying a complex level of interaction with the BMSB immune system, that can influence how a fungal strain can interact with an insect host. Taken together, the results of this study open many new lines of inquiry that questions insect-fungal interactions, and the overall impact of these interactions on the integrated function of the immune and cardiac system of insects.

DIET EFFECTS ON TRANSCRIPTOMICS IN *TRICHOPLUSIA NI* AND ITS SUSCEPTIBILITY TO ACMNPV BACULOVIRUS.

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The insect peritrophic membrane (PM) is a permeable chitin matrix, lining the midgut with roles in food compartmentalization and biochemical defence against ingested plant compounds. It also has protective roles against ingested insect pathogens. However, the PM structure can be influenced by diet, which in turn can affect the susceptibility of insects to pathogens. In this study, we aim to investigate the extent of such an effect by examining PM structure and measuring larval susceptibility to ingested pathogens in *Trichoplusia ni* larvae raised on either cabbage or potato diet. We are studying the effects of different diets on PM structure, such as differences in formation, thickness, and organization, under scanning and transmission electron microscopy. In addition, through RNA sequencing experiments, we are investigating the gut transcriptome under each diet to provide further insights on candidate genes whose differential expression could result in the variability of PM structure observed. Furthermore, we are studying the effect of different diets on *T. ni* growth and its susceptibility to the baculovirus *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). Our primary results show prolonged larval development and lower susceptibility to AcMNPV in potato-fed insects compared to cabbage-fed insects. Together, our results should further our knowledge in dietary influences on insect susceptibility to pathogens, helping to develop improved strategies in pest control.

THE CHEMOSENSORY PROTEIN GENE FAMILY OF THE EMERALD ASH BORER: EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF CSP5.

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Chemosensory proteins (CSPs) are small, soluble proteins present in many species of arthropods. Some CSPs are strongly expressed in sensory organs and are therefore hypothesized to play major roles in olfaction, for instance in mate-finding and host plant detection. Using a draft EAB genome and multiple transcriptome resources, we characterized the genomic organization and expression of 17 CSPs genes from the Emerald Ash Borer (EAB, *Agrilus planipennis*). CSP expression was monitored in adult legs, antennae, head, thorax and abdomen. Q-PCR results demonstrate that mRNAs encoding two CSPs (AplaCSP4 and AplaCSP5) accumulate at higher levels in antennae than in other tissues, therefore pointing to their possible roles in volatile odorant detection and/or processing. The AplaCSP5 protein was further expressed in vitro using E. coli and purified and will be used in competitive binding assays with various volatile odorant chemicals. Binding assay results should help provide a better understanding of the functional role of AplaCSP5 in olfaction.

IDENTIFICATION, EXPRESSION ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF TWO GNRH-RELATED PEPTIDE RECEPTORS IN THE MOSQUITO, AEDES AEGYPTI

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To cope with stressful events such as flight, organisms have evolved various regulatory mechanisms, including control by factors that are part of the endocrine system. One such mechanism is through the employment of two gonadotropin-releasing hormone related peptides in insects known as adipokinetic hormone (AKH) and corazonin (CRZ). AKH has a variety of functions, but is best known to act as a substrate liberator of proteins, lipids and carbohydrates. In certain species, CRZ has been shown to have a role in either pigmentation, ecdysis or acts as a cardiostimulatory factor. However, a universal function for CRZ has not yet been elucidated. Although both AKH, CRZ and their respective receptors (AKHR and CRZR) have been characterized in several organisms, very little is known about their roles within the disease vector, *Aedes aegypti*. Here, we re-validated and obtained two *A. aegypti* AKHR variants (AKHR-I and AKHR-II) which arise due to the alternative splicing of a 10 exon spanning mRNA. Our results mainly coincide with what was previously identified, albeit with notable differences within the AKHR-II variant. In addition, we have identified a novel third truncated variant dubbed AKHR-Ib, which involves a cryptic splice site within the fifth exon that results in the deletion of 17 nucleotides causing a frameshift that yields a smaller receptor isoform. We have also identified the *A. aegypti* CRZR receptor, which spans 5 exons and encodes a receptor comprised of 505 amino acids. Transcript expression profiling and functional deorphanization of the receptors was investigated, indicating that AKHR and CRZR exhibited a highly specific response for their native ligands in a dose-dependent manner. In contrast, the truncated receptor AKHR-Ib did not elicit a response even with the native AKH peptide, most likely due to this variant lacking the necessary structural domains required to transduce the signaling cascade normally mediated by its extracellular ligand. Spatial expression profiles reveal an enrichment of CRZR in the head of both males and females, whereas greater AKHR transcript abundance was observed in the thorax/abdomen of male and female mosquitoes.

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THE EFFECT OF *RHINELLA ICTERICA* TOAD VENOM ON HEART AND OVIDUCT CONTRACTIONS IN *LOCUSTA MIGRATORIA*

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Toads have poisonous glands around the body, which produce a toxic secretion of biotechnological interest. The toad skin's secretions are rich in different chemical compounds, which have a range of pharmacological activities. Toads of the species *Rhinella icterica* (Spix, 1824) are native of South America, and the pharmacological and toxicological effects of their venom have not been extensively studied. This study used poison from *R. icterica* prepared by methanol extraction followed by lyophilization (MERIV). The effects of MERIV on spontaneous contractions of the visceral muscles of the oviduct and heart of adult female *Locusta migratoria* were examined. MERIV at high concentrations (10^{-4} and 10^{-6} g/animal) induced an increase in the amplitude of contraction and basal tonus in *L. migratoria* oviducts and decreased the heart rate. MERIV is a collection of toxic compounds that include cardiac glycosides that are known to inhibit Na^{+}/ K^{+}-ATPase which might lead to increases in intracellular calcium levels. The observed effects in *L. migratoria* contractions are likely the result of such activity.

INSECTICIDAL TRAP PLANTS INCORPORATING RNA INTERFERENCE TECHNOLOGY.

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RNA interference (RNAi) holds immense promise as a potential crop protection tool. In recent years, numerous demonstrations of the effectiveness of this system in providing insect protection for crop species from cotton to corn and potato have emerged. These have taken two forms: through external application where double-stranded RNA (dsRNA) is applied either with a spray-on or soak approach, or conversely, the dsRNA is produced within the plant after transformation of the genome. The first option is beset by the high cost of production of the dsRNA necessary, and the second is challenged by lingering suspicions on the part of consumers regarding genetically modified crops. Our intention is to deploy the highly efficient strategy of genetic modification to achieve RNAi, but to do so in trap plants designed to attract pests away from crops destined for market. This will result in death of the pest insects while avoiding any stigma of modification in the products for human consumption. Several strategies for implementing dsRNA production are possible and the resulting fit with different pest classes will be discussed. Optimal matching of strategy and pest will ultimately be necessary for this novel IPM tool to be efficiently applied in crop protection.

IN THE BELLY OF THE BEAST: VECTOR IMMUNE RESPONSES IN THE MIDGUT DETERMINE WHICH INSECT VECTORS TRANSMIT WHICH PARASITES. THE SAGA CONTINUES……

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Vectors that feed on vertebrate blood ingest multiple parasites and pathogens, yet few are transmitted. Molecular interactions between the innate immune responses of the vector and the parasite determine the transmission outcome. Vectors recognize and eliminate most parasites, while successful parasites circumvent host immune responses. The mosquito *Aedes aegypti* is the principle vector of Dengue and Zika viruses. In Cali Colombia, however, 30% of feral *Ae. aegypti* kill all 4 serotypes of Dengue. We sequenced (RNA-seq) the midgut transcriptome of mosquitoes that are susceptible (S) or refractory (R) to dengue viruses, and designated differentially expressed genes as pro-viral (overexpressed in S) or anti-viral (over expressed in R). We used RNAi to knockdown the expression of selected genes and determined the S or R phenotype. While we understand some of the mechanisms involved we do not understand the "logic" of becoming refractory to a virus that has no significant impact on the vector, and which the vector is unlikely to encounter in its lifetime, especially when there are fitness costs associated with the R phenotype. If we could "convince" all *Ae. aegypti* to become refractory to Dengue and other viruses, we might significantly reduce human disease.

THE INVOLVEMENT OF RHOPR-CRF/DH IN FEEDING AND REPRODUCTION IN THE BLOOD-GORGING INSECT *RHODNIUS PROLIXUS.*

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Rhodnius prolixus is a blood-gorging insect and a vector for human Chagas disease. The insect transmits the disease following feeding, when it excretes urine and feces contaminated with the *Trypanosoma cruzi* parasite. A corticotropin-releasing factor-like peptide acts as a diuretic hormone in *R. prolixus* (Rhopr-CRF/DH); however, its distribution throughout the insect's central nervous system (CNS) and the expression of its receptor in feeding-related tissue as well as the female reproductive system suggests a multifaceted role for the hormone beyond that of diuresis. Here we investigate the involvement of Rhopr-CRF/DH in feeding and reproduction in *R. prolixus*. Immunohistochemistry of the CNS showed diminished CRF staining in neurosecretory cells of the brain and mesothoracic ganglionic mass immediately following feeding, and partial restocking of those same cells two hours later, implicating Rhopr-CRF/DH stores in those regions in control of feeding. Elevating haemolymph Rhopr-CRF/DH titres by injection of Rhopr-CRF/DH prior to feeding resulted in the intake of a significantly smaller blood meal in fifth instars and adults. When adult females were injected with Rhopr-CRF/DH, they produced and laid significantly fewer eggs. Finally, in vitro oviduct contraction assays illustrate that Rhopr-CRF/DH inhibits the amplitude of contractions of the lateral oviducts, highlighting a potential mechanism via which the hormone diminishes reproductive capacity. To conclude, the study of the Rhopr-CRF/DH pathway, its components and mechanisms of action, has implications for vector control by highlighting targets to alter feeding, diuresis, and reproduction of this disease vector.

OCTOPAMINE AND TYRAMINE MODULATE THE FEMALE REPRODUCTIVE SYSTEM IN THE MEDICALLY-IMPORTANT BUG, *RHODNIUS PROLIXUS*.

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Octopamine and tyramine are neuroactive chemicals involved in a wide range of physiological processes acting as neurotransmitters, neuromodulators and neurohormones. Octopamine and tyramine have been shown to play a crucial role in modulating reproductive processes in insects. Both octopamine and tyramine modulate visceral muscle contractions in various insects. In *Rhodnius prolixus*, octopamine decreased the amplitude of spontaneous muscle contractions and reduced the RhoprFIRFa-induced contraction of the oviducts in a dose-dependent manner, whereas tyramine only reduced the RhoprFIRFa-induced contractions. At the bursa, both octopamine and tyramine reduced the frequency of spontaneous contractions and abolished contractions at higher concentrations. These events are mediated by G-protein coupled receptors with cyclic AMP or calcium acting as second messengers. The cDNA sequences of two distinct receptors, Octβ-R and Tyr-R, has been cloned from *R. prolixus* and the transcript is shown to be expressed in all female reproductive tissues. Injection of octopamine into mated and fed adult females results in a higher number of eggs produced and ovulated when compared to control insects. Overall, it appears that octopamine and tyramine modulate the female reproductive tissues leading to successful ovulation, fertilization and the oviposition of eggs.

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ELUCIDATING THE FUNCTIONAL ROLE OF AN ANCIENT NEUROENDOCRINE SYSTEM INVOLVING GPA2/GPB5 AND ITS RECEPTOR (LGR1) IN THE MOSQUITO, *AEDES AEGYPTI*.

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GPA2/GPB5 is a glycoprotein hormone which, together with its receptor LGR1 (leucine-rich repeat containing G protein-coupled receptor 1), represents a novel neuroendocrine glycoprotein hormone-signaling system found in both vertebrate and invertebrate genomes. However, to date, the function of GPA2/GPB5 remains unknown. In insects, transcript expression of the GPA2/GPB5 subunits and LGR1, indicate a role in hydromineral balance as well as development. To elucidate additional roles of GPA2/GPB5 in the adult mosquito, we aimed to identify physiological targets by examining the expression profile of its receptor, LGR1. A heterologous system expressing *A. aegypti* LGR1 was used to characterize an *A. aegypti* LGR1 custom antibody, from which immunoblot analyses confirmed a 112 kDa band associated with membrane-protein fractions. A second heterologous system using HEK 293T cells stably expressing a fusion construct of *A. aegypti* LGR1-EGFP (LGR1: 105 kDa + EGFP: 27 kDa) yielded a 139 kDa band that also associated with membrane-protein fractions, and upon deglycosylation, migrated to the predicted fusion-protein molecular weight of 132 kDa. To verify specificity of the custom antibody, immunocytochemical analysis of HEK 293T cells stably expressing LGR1-EGFP confirmed EGFP fluorescence and LGR1-like staining colocalized to the plasma membrane. In adult mosquitoes, immunohistochemical analyses revealed LGR1-like staining localizes to basolateral surfaces of epithelia associated with various regions of the gut, suggesting a possible role in feeding processes and/or hydromineral balance for GPA2/GPB5. Interestingly, modest levels of LGR1 transcript and strong immunostaining was also identified in reproductive tissues. Specifically, regionalization of LGR1 to distal regions of the testes suggests a potential role related to spermatogenesis in adult males. Furthermore, in the ovaries of non-blood fed females, LGR1-like staining implies a potential role associated with previtellogenesis, a reproductive stage whereby females prepare for engorgement of a blood meal.

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BIOCHEMICAL BASIS OF PUPAL SUSCEPTIBILITY TO IONIZING RADIATION IN *DROSOPHILA MELANOGASTER*

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The fruit fly *Drosophila melanogaster* is a radio-resistant insect. The adult flies have LD_{50} that is about 250 times more than the lethal dose for human beings. Our recent study demonstrated that the early pupal stage of *Drosophila* displays an interesting phenomenon of a significant fall in radiation tolerance. The non-feeding third instar larvae were observed to have a LD_{50} (1 hr post-irradiation) of 1590 Gy. This drops to 50 Gy in early pupae. The adults recover from this radiation susceptibility phase to have LD_{50} of 1228 Gy (Male) and 1250 Gy (Female). Being a holometabolus insect, *Drosophila* provides opportunities to test the existing hypotheses of resistance and also permits a genetic approach.
Indirect mechanisms of radiation damage are through generation of free radicals. To counter this, antioxidant mechanisms are necessary. In early pupae, the levels of GSH and Catalase were found to increase. SOD and GST levels were found to decrease. This reduces the efficiency to scavenge superoxides and peroxides. Protein carbonylation is believed to lead to DNA damage and senescence. It was observed that the levels of protein carbonylation increases during early pupal stage. The levels of protein carbonylation in life stages are also radiation-dose dependent. Protein carbonyls increase more in early pupal stage compared to other life stages following irradiation. It is therefore likely that DNA repair mechanisms are weakened during early pupal stage leading to a susceptible phase. Trehalose, the major blood sugar in insects is known to protect the antioxidant mechanisms. A fall in the level of trehalose in early pupae was observed which may lead to inefficient antioxidant mechanisms, resulting in protein carbonylation and eventual DNA damage and therefore susceptibility.

EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF TACHYKININ-RELATED PEPTIDES IN THE BLOOD-FEEDING BUG, *RHODNIUS PROLIXUS.*

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Tachykinins (tachykinin-related peptides, TRPs) are multifunctional neuropeptides that have widespread distribution in the nervous system and gastrointestinal tract in many insects, including the Chagas disease vector, *Rhodnius prolixus*. Most TRPs have been shown to stimulate contraction of visceral muscles of insects. Invertebrate TRPs carry a characteristic conserved C-terminal pentapeptide FXGXR-amide. Most TRPs share this evolutionary conserved C-terminal pentapeptide motif, and share some limited sequence similarities (approx. 45%) to the vertebrate and mammalian tachykinin family. We have cloned and functionally characterized *R. prolixus* tachykinins (Rhopr-TKs). The gene encodes 8 Rhopr-TKs that have the characteristic FX1GX2R-amide C-terminus domain, where X1 is F, V, Q or M and X2 is either M or V. Spatial expression of the Rhopr-TK transcript reveals highest abundance in CNS and lower expression in foregut, midgut and hindgut. Interestingly, salivary glands and fat body showed higher expression than the gut. Salivary gland contraction assays using Rhopr-TK 1, 2 and 5 showed a significant increase in frequency and amplitude of the peristaltic waves of contractions. Hindgut muscle contraction assays displayed dose-dependent increases in contraction in response to Rhopr-TK1. To study the effects of feeding on Rhopr-TK spatial expression in salivary glands and fat body, four groups of 5th instar *R. prolixus* were used; unfed, 4h, 24h and 5 days post-feeding. Results showed a gradual decrease in expression level at 4 h post-feeding followed by a significant increase at 5 days post-feeding. This suggests a possible role of Rhopr-TKs in the control of feeding.

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FUNCTIONAL CHARACTERIZATION OF THE ADIPOKINETIC HORMONE/CORAZONIN-RELATED PEPTIDE RECEPTOR IN *AEDES AEGYPTI*

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Adipokinetic hormone/corazonin-related peptide (ACP) is an insect neuropeptide that is structurally intermediate between the corazonin (CRZ) and adipokinetic (AKH) hormones. Various studies have characterized the corazonin and AKH signalling systems within many insect species, and putative cardioacceleratory and energy mobilization functions, respectively, have been proposed. In contrast, the ACP peptide and its receptor, ACPR, have only been identified in few insect species and its function remains unknown. Despite ACP/ACPR being structurally related to AKH and CRZ and their receptors, studies have shown it to be functionally unrelated to the two later signalling pathways. Here, we aim to identify and functionally characterize the ACP/ACPR signalling system in the dengue and yellow fever mosquito, *Aedes aegypti*. Thus far, three ACP receptor variants have been identified, one functional receptor (ACPR-I; 577 residues, 7 TM domains) and two nonfunctional truncated receptor isoforms (ACPR-II and ACPR-III; 328 residues, 5-TM domains and 243 residues, 3-TM domains, respectively). Functional assays testing ACP, AKH, CRZ, and other *A. aegypti* peptides have demonstrated the specificity of ACPR-I for ACP with an EC50 value in the low nanomolar range. Transcript expression profiles of the receptor in different developmental stages of the mosquito as well as individual tissues of the adult *A. aegypti* will allow for the identification of possible physiological roles for the ACP/ACPR signalling system. On the whole, the outcomes of this research will improve our understanding of the ACP/ACPR signalling pathway to elucidate its function.

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