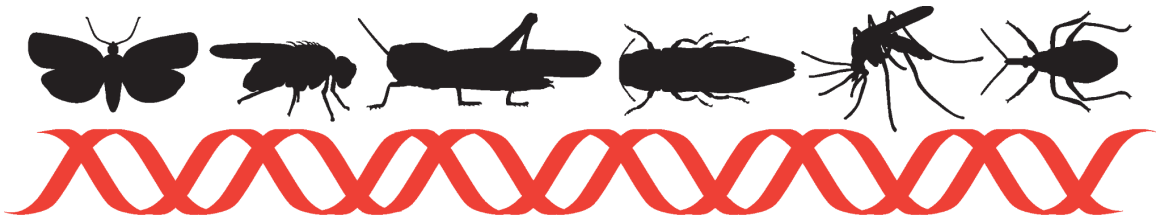

INSECT BIOTECH CONFERENCE

Annual Scientific Meeting

June 8-10, 2016

Schedule and Contributed Abstracts

Four Points Suites, Sheraton, St. Catharines, ON

Sponsored by:

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FroggaBio scientific solutions

Insect Biotech Conference – 2016

Conference Schedule

Wednesday Evening – June 8

6:00 pm **Registration:** Outside Brock Ballroom

7:00 pm **Plenary Talk:** Brock Ballroom
"**INSECT MOLECULAR PHYSIOLOGY IN A TIME OF ZIKA"***[Page 1]*
Peter M. Piermarini,
Department of Entomology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, USA.
http://entomology.osu.edu/our-people/peter-piermarini

7:45 – 9:00 pm **Reception**: Brock Ballroom (Pizzeria, Beer and Wine)

Thursday, June 9

7:45 – 9:00 am **Breakfast:** Decew Room (The All Canadian Breakfast Buffet)

9:00 am **Opening Remarks:** Brock Ballroom: **Jean-Paul Paluzzi**

Session Chair: **Andrew Donini**

9:10 am **INSULIN-LIKE PEPTIDES IN *RHODNIUS PROLIXUS*: THE VECTOR OF CHAGAS DISEASE**.*[Page 2]*
Lange, A.B., Defferrari, M.S. and Orchard, I.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

9:30 am **OCTOPAMINE AND TYRAMINE ARE ESSENTIAL IN REGULATING FEMALE REPRODUCTIVE PROCESSES IN *RHODNIUS PROLIXUS.***[Page 3]
Hana, S., and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

9:50 am **dsRNA KNOCKDOWN OF AQUAPORINS AaAQP3a AND AaAQP3b AFFECTS LARVAL MOSQUITO MALPIGHIAN TUBULE FUNCTION.**
[Page 4]
Misyura, L. and Donini A.
Department of Biology, York University, Toronto, ON, Canada.

10:10 am **A NOVEL INSIGHT TO THE FUNCTIONAL ROLE OF THE GLYCOPROTEIN HORMONE GPA2/GPB5 AND ITS RECEPTOR LGR1 IN THE MOSQUITO, *AEDES AEGYPTI*.** *[Page 5]*
Rocco, D.A. and Paluzzi J.P.
Department of Biology, York University, Toronto, ON, Canada.

10:30 – 10:50 am **Coffee Break**: Brock Ballroom

Session Chair: **Jean-Paul Paluzzi** (Brock Ballroom)

10:50 am **A SALTY DIET CONFERS COLD TOLERANCE IN *DROSOPHILA.***[Page 6]
Yerushalmi, G., Misyura, L., Donini, A. and MacMillan, H.
Department of Biology, York University, Toronto, ON, Canada

11:10 am **COLD ACCLIMATION OF CHILL COMA MECHANISMS IN THE LOCUST CNS.***[Page 7]*
Srithiphaphirom, P., Soo Lum, D. and Robertson, R.M.
Department of Biology, Queen's University, Kingston, ON, Canada

11:30am **EVIDENCE FOR A PERMISSIVE EFFECT OF HEMOLYMPH SUGAR ON ECDYSIS IN THE AMERICAN COCKROACH (*PERIPLANETA AMERICANA*).***[Page 8]*
Steele, J.E.
Department of Biology, The University of Western Ontario, London, ON, Canada

11:50 am **REGULATION OF THE CARDIAC SYSTEM OF THE BROWN MARMORATED STINK BUG BY THE GASEOUS SIGNALLING MOLECULE NITRIC OXIDE.***[Page 9]*
[1]Tahir, I., [2]Peters, R.J., [3]Lange, A.B. and [2]da Silva, R.
[1]School of Interdisciplinary Science, McMaster University, Hamilton, ON, Canada
[2]Department of Biology, McMaster University, Hamilton, ON, Canada
[3]Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

12:10 – 1:20 pm **Lunch Break**: Decew Room (The Deli)

Session Chair: **Heath MacMillan** (Brock Ballroom)

1:20 pm **_DROSOPHILA_ MODELS OF DEGENERATIVE DISEASES, AND THEIR POTENTIAL FOR DRUG DISCOVERY.***[Page 10]*
Hilliker, A.J., Mukai, S., Belozerov, K.
Department of Biology, York University, Toronto, ON, Canada

1:40 pm **NEUROMODULATION AT THE _DROSOPHILA_ LARVAL NEUROMUSCULAR JUNCTION.***[Page 11]*
Ormerod, K.G. and Littleton, T.J.
Massachusetts Institute of Technology, The Picower Institute for Learning and Memory, Department of Biology, Cambridge, MA, USA

2:00pm **INVESTIGATION OF SYNAPTOTAGMIN 4 ASSOCIATED VESICLE TRAFFICKING.***[Page 12]*
Zhang, Y.V. and Littleton, T.J.
Massachusetts Institute of Technology, The Picower Institute for Learning and Memory, Department of Biology, Cambridge, MA, USA

2:20 pm **K^+ AND H^+ TRANSPORT MECHANISMS OF THE _DROSOPHILA_ GUT EPITHELIA.***[Page 13]*
D'Silva, N.M. and O'Donnell, M.J.
Department of Biology, McMaster University, Hamilton, ON, Canada.

2:40 – 3:00 pm **Coffee Break** : Brock Ballroom

Session Chair: **Michael O'Donnell** (Brock Ballroom)

3:00 pm **CALCIUM TRANSPORT BY ISOLATED MALPIGHIAN TUBULES OF _ACHETA DOMESTICA_.***[Page 14]*
Browne, A.A. and O'Donnell, M.J.
Department of Biology, McMaster University, Hamilton, ON, Canada

3:20 pm **NEUROPEPTIDE F AND ITS RECEPTOR IN REGULATING THE REPRODUCTION OF THE BLOOD-GORGING HEMIPTERAN _RHODNIUS PROLIXUS_.***[Page 15]*
Sedra, L. and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

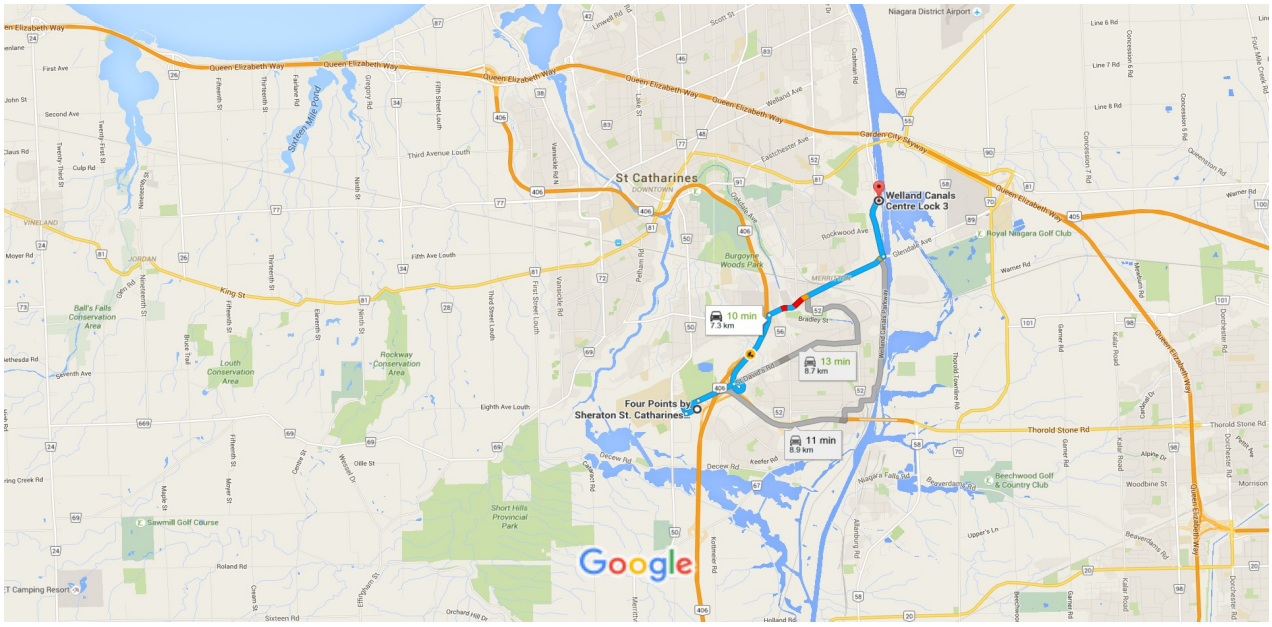
3:40pm **REPRODUCTIVE PHYSIOLOGY IN THE BLOOD FEEDING INSECT, _RHODNIUS PROLIXUS_: SPERM TRANSFER.***[Page 16]*
Chiang, G. and Chiang, J.
Biology Department, Redeemer University College, Ancaster, ON, Canada

4:00pm **AN RhGP HOMOLOG, AeRH50-2, CONTRIBUTES TO AMMONIA EXCRETION BY THE ANAL PAPILLAE IN LARVAL MOSQUITO, _AEDES AEGYPTI_.***[Page 17]*
Durant, A., Chasiotis, H., Misyura, L. and Donini, A.
Department of Biology, York University, Toronto, ON, Canada

4:20pm **End of Session**

6:00 pm **St. Catharines Museum and Welland Canals Centre, 1932 Welland Canals Parkway, St. Catharines, ON, L2R 7K6, (Museum Tour)**

7:00 pm **Conference Dinner, The Lockview Lounge, Welland Canals Centre, 1932 Welland Canals Parkway, St. Catharines, ON, L2R 7K6.**
Catering by Kristin's Fine Foods



Four Points by Sheraton St. Catharines Niagara Suites
3530 Schmon Parkway, Thorold, ON L2V 4Y6

Continue to Sir Isaac Brock Way/Regional Rd 71

1.	Head northwest toward Schmon Pkwy	1 min (400 m)
2.	Turn right onto Schmon Pkwy	260 m
		130 m

Take Glendale Ave/Regional Rd 89 to Welland Canals Parkway in St. Catharines — 9 min (6.9 km)

3.	Turn right onto Sir Isaac Brock Way/Regional Rd 71	
4.	Continue onto St David's Rd	300 m
5.	Use the middle lane to stay on St. David's Rd and follow signs for ON-406N	400 m
6.	Use the right lane to take the ramp onto ON-406N	350 m
		1.8 km
7.	Take the Regional Road 89/Glendale Avenue exit	350 m
8.	Turn right onto Glendale Ave/Regional Rd 89	
		2.5 km
9.	Turn left onto Welland Canals Parkway	
	Destination will be on the right	

Friday, June 10

7:45 – 9:10 am **Breakfast:** Decew Room (The All Canadian Breakfast Buffet)

Session Chair: **Peter Krell:** Brock Ballroom

9:10 am **THE ROLE OF RHOPR-CRF/DH IN FEEDING AND REPRODUCTION IN *RHODNIUS PROLIXUS*.** *[Page 18]*
Mollayeva, S. and Lange A.B.
Department of Biology, University of Toronto Mississauga, Toronto, ON, Canada

9:30 am **THE EFFECT OF AN ESTERASE/PROTEASE GENE OF *AMSACTA MOOREI* ENTOMOPOXVIRUS ON VIRUS PRODUCTION.***[PAGE 19]*
Ozsahin, E., Sezen, K., Demirbag, Z.
Department of Biology, Karadeniz Technical University, Trabzon, Turkey

9:50 am **NEONICOTINOID RESISTANCE IN THE COLORADO POTATO BEETLE.** *[Page 20]*
Kaplanoglu, E., Chapman, P., Scott, I., 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

10:10 am **A ROLE FOR SEPTATE JUNCTION PROTEIN GLIOTACTIN IN THE MAINTENANCE OF SALT AND WATER BALANCE IN LARVAL MOSQUITO (*AEDES AEGYPTI*).***[Page 21]*
Jonusaite, S., Kelly, S.P. and Donini, A.
Department of Biology, York University, Toronto, ON, Canada

10:30 – 10:50 am **Coffee Break**: Brock Ballroom

Session Chair: **Ian Orchard:** (Brock Ballroom)
SHORT EXPOSURES SESSION I (5 MINUTE TALK and QUESTIONS)

10:50 am **CAPA-LIKE IMMUNOREACTIVITY IN THE SYNGANGLION AND PERIPHERAL TISSUES OF THE BLACK-LEGGED TICK, *IXODES SCAPULARIS*.***[Page 22]*
Uyuklu, A., and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada

CHARACTERIZING THE ROLE OF TISSUES INVOLVED IN ION AND FLUID TRANSPORT IN *IXODES SCAPULARIS*.*[Page 23]*
Paez, M., and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada

CHARACTERIZATION OF THE IMMUNE SYSTEM OF THE BROWN MARMORATED STINK BUG.*[Page 24]*
[1]Peters, R.J., [2]Tahir, I. and [1]da Silva, R.
[1] Department of Biology, McMaster University, Hamilton, ON, Canada
[2] School of Interdisciplinary Science, McMaster University, Hamilton, ON, Canada.

FATAL ATTRACTION: THE VOLATILE INFLUENCES THAT WILL LEAD WHITEFLIES TO DEADLY ENCOUNTERS AND THE RNAi RESPONSIBLE.*[Page 25]*

Ludba, K.K., Scott, I.M., Thompson, G.J., Donly, B.C. and Perceival-Smith, A.
Department of Biology, University of Western Ontario, London, ON, Canada
London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada

11:30 - 12:00 noon **Check out of hotel rooms**

11:45 – 1:00 pm **Lunch Break**: Decew Room (Mediterranean Lunch Buffet)

Session Chair: **Mel Robertson** (Brock Ballroom)

1:00 pm **MEASURING THE *MAMESTRA CONFIGURATA* NUCLEOPOLYHEDROSIS VIRUS TRANSCRIPTOME: PERSONALIZED INSECT GENOMICS.** *[Page 26]*

[1]Donly, C., [1]Kaplanoglu, E., [2]Theilmann, D.A., [3]Hegedus, D.D., [3]Baldwin, D.D., [3]Sieminska, E. and [3]Erlandson, M.A.
[1]London Research and Development Centre, AAFC, London, ON, Canada
[2]Summerland Research and Development Centre, AAFC, Summerland, BC, Canada
[3]Saskatoon Research and Development Centre, AAFC, Saskatoon, SK, Canada.

1:20 pm **CHARACTERIZATION OF CHEMOSENSORY PROTEIN GENES FROM THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS*.***[Page 27]*

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

1:40 pm **SMALL HEAT SHOCK PROTEIN GENES IN SPRUCE BUDWORM AND THEIR RESPONSE TO HEAT SHOCK, STARVATION AND VIRUS INFECTION.***[Page 28]*

[1]Quan, G., [1,2]Duan, J., [1]Ladd, T., [1]Doucet, D., [3]Cusson, M. 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]Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec City, QC, Canada

2:00 pm **"CRYOPROTECTANTS" IN THE HEMOLYMPH OF CHILL-TOLERANT *DROSOPHILA* PROTECT AGAINST CHILLING INJURY THROUGH OSMOPROTECTION.***[Page 29]*

Olsson, T., Malmendal, A., MacMillan, H., Nyberg, N., Staerk, D. and Overgaard, J.
University of Aarhus, Denmark.

2:20 pm **THE INVOLVEMENT OF SULFAKININS IN THE CONTROL OF FEEDING IN *RHODNIUS PROLIXUS.*** *[Page 30]*
Al-Alkawi, H., Lange, A.B. and Orchard, I.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

2:40 – 3:00pm **Coffee Break**: (Brock Ballroom)

Session Chair: **Angela Lange:** (Brock Ballroom)
SHORT EXPOSURES SESSION II (5 MINUTE TALK and QUESTIONS)

3:00 pm **STUDY OF THE EFFECT OF ABNORMAL SYSTEMIC METABOLISM ON TUMOR GROWTH AND METASTASIS IN A *DROSOPHILA* MODEL.**
[Page 31]
Belozerov, K., Mukai, S. and Hilliker, A.J.
Department of Biology, York University, Toronto, ON, Canada

INVESTIGATING THE FUNCTION OF THE C4 ZINC FINGER DOMAIN OF BACULOVIRUS AcMNPV PROTEIN ME53. *[Page 32]*
Ralph, R.E., Liu, Y., de Jong, J. and Krell, P.J.
Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada

AEDES-CAPA1 MODULATION OF DIURESIS STIMULATED WITH NEUROENDOCRINE FACTORS IN *AEDES AEGYPTI.* *[Page 33]*
Curcuruto, C. and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada

ECDYSTEROID TARGETS IN AN ADULT INSECT: PRESENCE AND CYCLING OF THE ECDYSTEROID RECEPTOR IN TISSUES OF ADULT *RHODNIUS PROLIXUS.* *[Page 34]*
Cardinal-Aucoin, M., Rapp, N., Saroiu, T., Hindley-Smith, M. and Steel, C.G.H.
Department of Biology, York University, Toronto, ON, Canada

3:40 pm **Closing Remarks:** Jean-Paul Paluzzi, Andrew Donini

INSECT MOLECULAR PHYSIOLOGY IN A TIME OF ZIKA.

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

Mosquitoes are the most dangerous animals on the planet; they are responsible for infecting hundreds of millions of people each year with debilitating and sometimes deadly illnesses, such as malaria and dengue fever. The most recent mosquito-borne disease to emerge around the globe is Zika virus (ZIKV), which is spread by the yellow fever mosquito Aedes aegypti. Although ZIKV does not usually produce an illness with severe symptoms, it has been linked to causing 1) birth defects (e.g., microcephaly) in infants born to mothers who were infected during pregnancy and 2) a rare autoimmune disorder (i.e., Guillan-Barre syndrome). While therapeutics and vaccines are unavailable to treat and prevent ZIKV, the only means to control the spread of the virus is to reduce interactions between mosquitoes and humans: i.e., mosquito control. A cornerstone to mosquito management is the use of insecticides, most of which target the nervous system (e.g., pyrethroids) and are not selective to mosquitoes. However, mosquitoes are evolving resistance to these insecticides, which is eroding the number of available tools in an already limited toolbox for vector control. Thus, the identification of novel molecular and physiological targets in mosquitoes for disrupting their life cycle and guiding development of next-generation mosquitocides is needed. The goal of this talk is to highlight a role of addressing fundamental questions in insect molecular physiology for improving our capabilities to control mosquito vectors. In particular, recent effects by our group to elucidate the molecular mechanisms of urine production in Malpighian tubules of Aedes mosquitoes and to develop novel mosquitocides targeting these mechanisms will be reviewed.

INSULIN-LIKE PEPTIDES IN *RHODNIUS PROLIXUS*: THE VECTOR OF CHAGAS DISEASE.

Lange, A.B., Defferrari, M.S. and Orchard, I.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

Insulin-like peptides (ILPs) are functional analogs of insulin and have been identified in many insect species. The insulin/insulin-like growth factor (IGF) pathway is a conserved regulator of metabolism, and in insects, as well as in other animals, it can modulate physiological functions associated with growth, development and metabolism of lipids and carbohydrates. In the present study, we have identified one ILP and one IGF and investigated their involvement in R. prolixus metabolism and growth. We have identified the peptides within the R. prolixus genome and have cloned their cDNA sequences. Expression profile analyses showed that the ILP transcript is predominantly present in the brain while the IGF is distributed among a variety of tissues, mostly in the fat body, the dorsal vessel and the central nervous system. Using RNAi, we have knocked-down the expression of both transcripts separately and examined the effects on metabolism and growth. We observed that the absence of the ILP transcript increased the levels of lipids and carbohydrates in the hemolymph, while the lipid content in the fat body was increased. At the same time, the carbohydrate level was decreased in the fat body and the leg muscles, indicating that this peptide is involved in energy homeostasis. The absence of the IGF transcript resulted in defective molting of fifth instars into adults. Compared to the control, insects lacking IGF display abnormal morphological features such as smaller wings and reduced body size. Further experiments are being conducted on the physiological significance and downstream signaling of both peptides.

This work was supported by NSERC.

OCTOPAMINE AND TYRAMINE ARE ESSENTIAL IN REGULATING FEMALE REPRODUCTIVE PROCESSES IN *RHODNIUS PROLIXUS.*

Hana, S., and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

Octopamine (OA) is a biogenic amine involved in many physiological processes acting as a neurohormone, neuromodulator and a neurotransmitter in insects. Recently, its precursor, tyramine (TA), has also been found to be a neuroactive chemical. Work in Drosophila melanogaster has established that OA, through direct innervation, regulates reproductive processes and egg-laying. These actions are mediated by the G protein-coupled receptors (GPCRs), Oamb and Octβ2R. Mutated flies that lack OA or its receptors have been found to be sterile. In Rhodnius prolixus, OA reduced the spontaneous lateral oviduct burst amplitude by 60% and decreased the peptide-induced basal tonus by about 50%. Tyramine produced a less dramatic reduction of lateral oviduct contraction than that observed for OA. At a concentration of 5×10-7 M, OA and TA completely abolished bursa contractions. Immunohistochemical data reveals octopaminergic neurons innervating the oviducts and bursa, possibly from dorsal unpaired median neurons located in the mesothoracic ganglionic mass. Injection of OA into mated fed adult females increased egg-laying. In silico data has provided insights into the existence of both OA and TA GPCRs in R. prolixus. These receptors are likely to be expressed on different regions in the reproductive system mediating the observed physiological effects. Preliminary data suggest the involvement of cAMP, an Octβ receptor specific second messenger, in OA effects on the oviducts and bursa. We plan to utilize molecular tools to clone an Octβ receptor which will allow us to silence the OA signaling pathway and investigate the role of OA in the physiological phenomena observed.

This work was supported by NSERC.

dsRNA KNOCKDOWN OF AQUAPORINS AaAQP3a AND AaAQP3b AFFECTS LARVAL MOSQUITO MALPIGHIAN TUBULE FUNCTION.

Misyura, L. and Donini A.
Department of Biology, York University, Toronto, ON, Canada.

The mosquito, Aedes aegypti, is the primary arboviral vector for zika virus, dengue fever, chikungunya, and yellow fever. The larvae reside in hypo-osmotic freshwater habitats, where they face dilution of body fluids due to the osmotic gradient. The Malpighian tubules help maintain ionic homeostasis by removing excess water from the hemolymph. Active ion transport is used to facilitate transcellular and paracellular water movement by solvent drag into the tubule lumen. Aquaporins are transmembrane channels thought to permit transcellular transport of water from the hemolymph into the Malpighian tubules. Immunolocalization of Aedes aegypti Aquaporin 3a (AaAQP3a) and 3b (AaAQP3b) revealed their expression by principal cells of the Malpighian tubules. AaAQP3b localized to both the apical and basolateral membranes, while AaAQP3a was located at the brush border of the apical membrane. Larvae were fed double stranded RNA (dsRNA), targeting AaAQP3a and AaAQP3b to knockdown their expression by RNA interference. As a result of the AaAQP3b knockdown, fluid secretion rate of the Malpighian tubules decreased. The reduction in fluid secretion resulted in lower in vitro K+ and Na+ transport rate across the Malpighian tubule epithelium. AaAQP3a knockdown also resulted in decreased rate of transepithelial Na+ transport. Overall survival of the dsRNA treated larvae decreased in both AaAQP3a and AaAQP3b groups. These findings give rise to potential targets for development of novel mosquito control agents aimed at the larval life stage prior to emergence into the disease vector adult stage.

This work was supported by NSERC.

A NOVEL INSIGHT TO THE FUNCTIONAL ROLE OF THE GLYCOPROTEIN HORMONE GPA2/GPB5 AND ITS RECEPTOR LGR1 IN THE MOSQUITO, *AEDES AEGYPTI*.

Rocco, D.A. and Paluzzi J.P.
Department of Biology, York University, Toronto, ON, Canada.

GPA2/GPB5 is a glycoprotein hormone found in most bilateral metazoans including the mosquito, Aedes aegypti. Transcript expression of the GPA2/GPB5 subunits and its receptor in insects, the leucine-rich repeat-containing G protein-coupled receptor 1 (LGR1), provide evidence for involvement iono- and osmoregulation and a role in development. To further elucidate the function of GPA2/GPB5, we have characterized the LGR1 protein-level expression for the first time in the invertebrates, and analyzed its tissue- and sex-specific distribution patterns in adult A. aegypti. Western blot analyses on protein isolated from HEK 293T cells expressing LGR1 yielded an appropriate-sized band at ~105kDa associated with membrane-protein fractions. Immunohistochemical analysis in adult mosquitoes revealed LGR1-like immunoreactivity is widespread in the alimentary canal. Staining localized specifically within basolateral regions of the epithelium and appears as punctate staining dispersed intracellularly. Interestingly, strong LGR1-like immunoreactivity was also identified in reproductive tissues including the testes and ovaries, which suggests a role related to spermatogenesis and oogenesis in males and females, respectively. RNA interference techniques to downregulate LGR1 transcript expression has been successful in adult mosquitoes achieving over 90% knockdown. Ongoing studies implementing this knockdown approach will further investigate this putative reproductive role and will build upon the established osmoregulatory and ionoregulatory role by examining GPA2/GPB5 activity related to diet-specific challenges.

Research in this study was funded by an NSERC Discovery Grant to J.P.P.

A SALTY DIET CONFERS COLD TOLERANCE IN *DROSOPHILA*.

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

At low temperatures, Drosophila, like most other insects, lose the ability to regulate water and ion homeostasis across the gut epithelia. A resulting hemolymph water loss leads to a hyperkalemic state that leads to onset of chilling injuries. To better understand the importance of diet in determining cold tolerance, we exposed adult D. melanogaster to 24-hours on diets highly enriched in sucrose, KCl, or NaCl. Supplementation with KCl improved chill coma recovery time and both NaCl and KCl enrichment improved survival rates following cold stress. No difference in critical thermal minimum was found among diets, suggesting that high salt intake is beneficial for survival and recovery but not for coma onset. Additionally, no change in chill tolerance was observed in flies on a high sucrose diet, suggesting this effect is not strictly osmotic in nature. Measurements of hemolymph [K+] revealed that both the KCl, and NaCl enriched diets led to an increase in baseline hemolymph [K+] and that cold exposure similarly disrupted [K+] balance in all but the sucrose-enriched diet. We thus propose that acute NaCl and KCl supplementation confers benefits to cold tolerance that are not clearly related to extracellular [K+].

This work was supported by an NSERC Discovery Grant to A.D.; A Banting PDF to H.M. and the Faculty of Science, York University.

COLD ACCLIMATION OF CHILL COMA MECHANISMS IN THE LOCUST CNS.

Srithiphaphirom, P., Soo Lum, D. and Robertson, R.M.
Department of Biology, Queen's University, Kingston, ON, Canada

Temperature has profound effects on the neural function and behaviour of insects. When exposed to low temperature, migratory locusts (Locusta migratoria) enter chill coma, a state of complete neuromuscular paralysis, and can resume normal body functions after returning to normal temperature. The mechanisms underlying chill coma and recovery are not well understood and in particular the role of the CNS is unclear. With an implanted thermocouple in the thorax, we measured the low temperature that induced loss of coordination and responsiveness (CTmin) in intact male adult locusts. In parallel experiments, we also recorded field potential in the metathoracic ganglion in dissected preparations to determine the low temperature that induced neural shutdown. Acclimation at 10 ˚C for 10 days or rapid cold hardening (RCH) at 4 ˚C for 4 hours reduced CTmin and chill coma recovery time. RCH also reduced the temperature at neural shutdown (in dissected preparation) by an amount approximately equal to the reduction of CTmin (in intact animal preparation). These results suggest that the CNS has an important role in determining entry into and exit from chill coma in locusts.

EVIDENCE FOR A PERMISSIVE EFFECT OF HEMOLYMPH SUGAR ON ECDYSIS IN THE AMERICAN COCKROACH (*PERIPLANETA AMERICANA).*

Steele, J.E.
Department of Biology, The University of Western Ontario, London, ON, Canada

Molting of larval insects comprises a series of events initiated by a rising titer of the ecdysteroid hormone 20-hydroxyecdysone (20E). Characteristically, the titer of 20E in the hemolymph peaks during the latter part of the stadium but ecdysis, the final event in the molting sequence, can only occur after ecdysteroid in the hemolymph has been returned to a low level. The decrease in 20E to a low level occurs rapidly and is an obligatory event that triggers the release of ecdysis triggering hormone (ETH). The mechanism that returns 20-hydroxyecdysone to a low level late in the stadium is therefore vitally important if the molt is to be successful. Strangely, the literature has little to say about this important aspect of insect development, especially the mechanism which facilitates a rapid changeover from accumulation of the ecdysteroid to its expedited removal. The efficacy of this mechanism is underlined by studies showing that although the ecdysteroid concentration in *Periplaneta americana* just prior to ecdysis is approximately 15 percent of the peak value a day earlier, the prothoracic gland is still producing ecdysteroid at half the maximal rate. The temporal relationship between the peak levels of ecdysteroid and of sugar in the hemolymph prior to ecdysis suggests that hemolymph sugar may be functionally associated with molting. The results support the contention that a rising level of sugar in the hemolymph prior to ecdysis is part of a mechanism that enables ecdysis to proceed in a timely fashion.

This work was supported by NSERC.

REGULATION OF THE CARDIAC SYSTEM OF THE BROWN MARMORATED STINK BUG BY THE GASEOUS SIGNALLING MOLECULE NITRIC OXIDE.

[1]Tahir, I., [2]Peters, R.J., [3]Lange, A.B. and [2]da Silva, R.
[1]School of Interdisciplinary Science, McMaster University, Hamilton, ON, Canada
[2]Department of Biology, McMaster University, Hamilton, ON, Canada
[3]Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

The Brown Marmorated Stink Bug (*Halyomorpha halys*) is an invasive agricultural pest species originating in East Asia that is rapidly expanding its range into Ontario and Quebec. The insect is a novel target for research and so, very little is currently known about the insect's physiology. Our study investigated the role of the gaseous signaling molecule nitric oxide (NO) on the regulation of the H. halys cardiac system. Using the fungal toxin phalloidin, the morphology of the dorsal vessel and surrounding alary muscles was characterized. Next, immunohistochemistry with an antibody against NO Synthase (NOS) identified NOS expressing hemocytes in the alary muscles supporting the dorsal vessel and in the lumen of the dorsal vessel. We propose that circulating and stationary hemocytes produce and deliver NO to the dorsal vessel and alary muscle tissues. To investigate this hypothesis, NO levels were exogenously increased using the NO donor MAHMA-NONOate which showed a dose dependent decrease in heart rate. Furthermore, application of 8-bromo-cGMP, an analog of the downstream signaling molecule guanosine 3′,5′-cyclic monophosphate (cGMP) led to a similar decrease in heart rate, suggesting that cGMP is conserved as the second messenger in the stink bug NO signaling pathway.

DROSOPHILA MODELS OF DEGENERATIVE DISEASES, AND THEIR POTENTIAL FOR DRUG DISCOVERY.

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Drosophila has become an immensely powerful model to uncover the molecular mechanisms of a variety of human diseases, and more recently, has shown considerable promise in pre-clinical pharmaceutical discovery. Given the high degree of molecular pathway conservation between mammals and flies, one of the advantages of using molecularly defined disease models in drug screening is the feasibility of extremely large-scale in vivo screens aimed at identifying novel axes of therapeutic intervention. In the last decade, our lab has developed, validated, and molecularly and pharmacologically explored a series of Drosophila models of various neuro- and myodegenerative diseases, including amyotrophic lateral Sclerosis (ALS), Huntington's disease, Friedreich's ataxia, Menkes disease, and sporadic inclusion body myositis. A common thread connecting these diseases, and one of the main interests of the lab is the involvement of Reactive Oxygen Species (ROS) in the development and progression of degenerative pathologies. To this end, we explored the perturbation of ROS homeostasis caused by genetic and transgenic manipulation of the activity of cytoplasmic and mitochondrial superoxide dismutases, ATP7A copper transporter, the mitochondrial iron chaperone fraxaxin, carnitine palmitoyltransferase I, and p38 MAP kinase. All of these ROS modulators are known to play key roles in human degenerative diseases, but the detailed knowledge of the molecular pathways involved and suitable therapeutic intervention points is often lacking. Here we present a summary overview of these models developed in our lab, many of which rely on transgenic technologies, and discuss their utility and potential for drug discovery.

NEUROMODULATION AT THE *DROSOPHILA* LARVAL NEUROMUSCULAR JUNCTION.

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Chemical signalling plays a critical role in the nervous system where communication between neurons occurs through the release of chemical signals following synaptic or dense core vesicle fusion. One of the major mechanisms by which synaptic communication can be altered is through the release of small molecules, e.g. neuromodulators. These molecules can affect cellular events both pre-and postsynaptically. We are using molecular and genetic tools available in the fruit fly (*Drosophila melanogaster*) to examine how exogenous application of neuromodulators alters synaptic efficacy, with the goal to identify the underlying molecular mechanisms. We use the Drosophila neuromuscular junction of third instar larvae as it is a heavily investigated and well characterized model synapse. Using optical quantal imaging and electrophysiology, we are exploring how neuromodulators alter synaptic release at individual active zones. These techniques enable us to not only quantitatively assess how synaptic efficacy is altered, but also to examine release probability of individual active zones and determine how they are influenced by exogenous application of neuromodulators.

This work is supported by NSERC and NIH.

NEUROMODULATION AT THE *DROSOPHILA* LARVAL NEUROMUSCULAR JUNCTION.

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Synaptotagmins are a large family of membrane trafficking proteins. Though sharing similar molecular structure, Synaptotagmins have distinct functions and unique subcellular localization. The most well characterized member of the Synaptotagmin family, Synaptotagmin1 (Syt1), is associated with synaptic vesicles and functions as a Ca2+ sensor during exocytosis. In contrast, Synaptotagmin 4 (Syt4), a close homolog controls retrograde signaling at the Drosophila neuromuscular junction (NMJ). Syt4 is not present on synaptic vesicles, and instead resides on membrane compartments with the postsynaptic muscle. However, it is still unclear if the postsynaptic expression of Syt4 is predominantly due to transcription and translation occurring in the muscle, or whether it is delivered to the postsynaptic compartment via exosome-mediated delivery from the presynaptic neuron. With the help of the recently developed Crispr-Cas9 induced double strand break, we have generated knockin lines with the endogenous Syt4 locus tagged with fluorescent proteins. We are using this tool to determine how Syt 4 is trafficked and functions within the postsynaptic compartment to regulate retrograde signaling at synapses.

K^+ AND H^+ TRANSPORT MECHANISMS OF THE *DROSOPHILA* GUT EPITHELIA.

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K+ and H+ transport along the midgut of third instar Drosophila larvae was characterized using the Scanning Ion-Selective Electrode Technique (SIET), immunohistochemistry and ATPase activity assays. The regions studied were the caeca, anterior midgut (AMG), copper cells of the middle midgut (MMG (CC)), large flat cells of the middle midgut (MMG (LFC)), neutral zone of the posterior midgut (PMG (N)), and the alkaline zone of the posterior midgut (PMG (A)). The roles of transport ATPases in energizing ion transport across the larval midgut were investigated using blockers like bafilomycin, a V-type H+ ATPase blocker, and ouabain, a Na+/ K+-ATPase blocker. Addition of bafilomycin to the basal membrane led to a decrease in proton absorption along all regions except the MMG (LFC). Bafilomycin also led to decreased K+ absorption across the caeca, the AMG, and the MMG (CC), suggesting proton-dependent transport of K+. Proton absorption was decreased by acetazolamide, indicating carbonic anhydrase activity in all regions except the AMG and MMG (LFC). Addition of ouabain led to the increase of K+ absorption along the caeca, the AMG, and MMG (LFC), suggesting a role for the Na+/K+-ATPase in these regions. Immunohistochemical evidence and ATPase activity assays also show the presence of V-type H+-ATPases and Na+/ K+-ATPase along the caeca and midgut.

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

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Malpighian (renal) tubules are insect excretory organs that are thought to play a principal role in extracellular calcium regulation by removing excess Ca2+ from the blood. Previous studies have suggested there are two routes for Ca2+ removal: 1) by transporting Ca2+ 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 domestica*, 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 a modified Ramsay assay to measure Ca2+ concentrations in both secreted fluid and bathing saline over time, transepithelial and basolateral Ca2+ fluxes of isolated Malpighian tubules were determined, respectively. Preliminary results indicate that Ca2+ transport is region-specific and the majority (~ 90%) of Ca2+ taken up across the basolateral surface is sequestered within the tubule.

NEUROPEPTIDE F AND ITS RECEPTOR IN REGULATING THE REPRODUCTION OF THE BLOOD-GORGING HEMIPTERAN *RHODNIUS PROLIXUS*.

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Long neuropeptide F (NPF) is a member of the FMRFamide-like peptide (FLP) superfamily and has been implicated in digestion and reproduction of many insects. I have previously shown the cloning and characterization of NPF (RhoprNPF) and its receptor (RhoprNPFR) in *Rhodnius prolixus*. Previous studies have shown that only the last 8 amino acids of RhoprNPF (truncated RhoprNPF - AVAGRPRFa) are required to activate RhoprNPFR. Quantitative PCR (qPCR) shows that RhoprNPF is predominantly expressed in the male testes as well as the female oviduct/spermathecae; whereas the RhoprNPF receptor is found in all male and female reproductive tissues tested. Fluorescent in situ hybridization images show that RhoprNPF and RhoprNPFR are expressed in 12 bilaterally-paired neurons in the adult brain and that RhoprNPF is found in cells along the lateral oviduct whereas its receptor is present in putative pre-follicular cells along each of the 7 telotrophic ovarioles. This suggests that RhoprNPF may play a role in vitellogenesis and egg movement. Injection of truncated RhoprNPF into mated, fed, female adult *R. prolixus* resulted in a decrease in oocytes retained in the ovaries (increase in ovulation) coupled with an increase in eggs produced (increase in oogenesis). Other FLPs screened in this biological egg-laying assay show that characterized myostimulators such as the N-terminally extended FM/L/IRFamides increase the rate of oogenesis, whereas myoinhibitors such as myosuppressin decrease the rate of oogenesis. This suggests that FLPs are involved in the regulation of egg production in the female *R. prolixus*.

This research was funded by NSERC.

REPRODUCTIVE PHYSIOLOGY IN THE BLOOD FEEDING INSECT, *RHODNIUS PROLIXUS*: SPERM TRANSFER.

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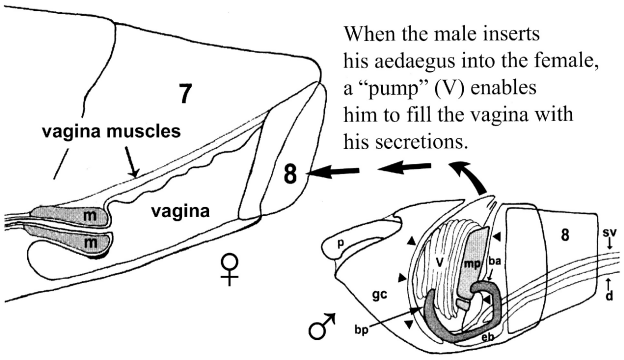
An early study of mating in *Rhodnius prolixus* described how the male forms the spermatophore within a spermatophore sac that everts from his aedaegus into the genital chamber of the female during copulation. This work was based on observations of mating couples who were sacrificed and fixed during copulation. For the present investigation, we examined living tissue and observed that the structure previously identified as the spermatophore sac may actually serve a sightly different role in sperm transfer. Rather than forming a sac for the spermatophore, this structure remains within the male when he inserts his aedaegus into the female. By allowing dye to diffuse from the abdominal cavity into the aedaegus, we discovered that the male secretions do not enter this sac, but course between its dorsal surface and an overlying rigid plate of cuticle (mp in the diagram below). In fixed tissue, this bellows-shaped structure (V) gives the impression of being an extendable sac, but in living tissue, this sac cannot be stretched beyond the aedaegus. However, it can be compressed and expanded dorso-ventrally indicating that it could serve as a pump to push the male secretions into the female and as a valve to prevent secretion back flow as the vagina fills.



AN RhGP HOMOLOG, AeRH50-2, CONTRIBUTES TO AMMONIA EXCRETION BY THE ANAL PAPILLAE IN LARVAL MOSQUITO, *AEDES AEGYPTI.*

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Ammonia (NH3/NH4+) is an important nutrient for microorganisms and plants, but is toxic to animal tissues. Depending on the habitat, various strategies of ammonia detoxification and nitrogenous waste excretion are employed by different groups of insects. The mosquito *Aedes aegypti* is the primary vector for the transmission of dengue and chikungunya viruses. While larvae are commonly found in freshwater habitats, it was shown that ammonia rich septic tanks in the Caribbean serve as an aquatic habitat for larval development and adult emergence. This study aimed to elucidate the epithelial transport mechanisms of ammonia excretion by the anal papillae of larval A. aegypti. Three putative ammonia transporter genes have been identified in *A. aegypti*. The transcripts of two Rhesus-like proteins, AeRh50-1 and AeRh50-2, were detected in the anal papillae of larval *A. aegypti*. We show that at 6 days post treatment with Rh2 dsRNA, a decrease in protein levels of AeRh50-2 in the anal papillae occurs. Immunolocalization of AeRh50-1 and AeRh50-2 to the apical and basal membranes of the anal papilla epithelium in both control and AeRh50-1 and AeRh50-2 dsRNA treated larvae is shown. Using the scanning ion selective electrode technique (SIET), it was found that ammonia efflux at the anal papillae is significantly reduced with Rh2 dsRNA treatment, but no change was observed with Rh1 dsRNA treatment. Interestingly, no significant change in hemolymph NH4+ levels or larval mortality was observed with dsRNA treatment.

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THE ROLE OF RHOPR-CRF/DH IN FEEDING AND REPRODUCTION IN *RHODNIUS PROLIXUS*.

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Feeding and reproduction are interrelated processes in the blood-feeding insect *Rhodnius prolixus*. Nutrition is a determinant of mating motivation, nutrient allocation toward egg production, and oviposition. The molecules behind these interactions are yet to be fully identified and explored. Corticotropin-releasing factor (CRF) is a neuropeptide involved in the stress response in mammals. In *R. prolixus*, a CRF-related peptide (Rhopr-CRF/DH) is a diuretic hormone, released from neurosecretory cells found in the mesothoracic ganglionic mass. Rhopr-CRF/DH is, however, also found in cell bodies throughout the central nervous system, including medial neurosecretory cells in the brain, suggesting it has additional roles. In the locust, CRF/DH is co-localized with ovary maturating parsin; moreover, the two neurohormones are encoded on the same gene. The existing data on CRF/DH and the interdependence of feeding and reproductive processes drive speculation of Rhopr-CRF/DH's potentially multifaceted role in R. prolixus. Feeding experiments were carried out to determine the effects of injected Rhopr-CRF/DH on satiety. To study the role of Rhopr-CRF/DH in reproduction, oviduct contraction assays and egg-laying assays were performed. The preliminary results demonstrate that Rhopr-CRF/DH alters feeding behaviour by inducing premature satiety, and alters reproduction by decreasing egg-laying capacity and interfering with the timing and duration of oviposition.

THE EFFECT OF AN ESTERASE/PROTEASE GENE OF *AMSACTA MOOREI* ENTOMOPOXVIRUS ON VIRUS PRODUCTION.

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Amsacta moorei entomopoxvirus (AMEV) is in the species Amsacta moorei entomopoxvirus, the type species of the genus Betaentomopoxvirus encompassing viruses infecting insects belong to Lepidoptera (moths) and Orthoptera (grasshoppers). AMEV replicates in nearly the entire body of the insect, but especially in the adipose tissue. Lipolytic and proteolytic enzymes have been investigated in several viruses and found to play a role in various functions including the production of DNA replication metabolites, rescue from endosomes, and membrane fusion. AMEV has an open reading frame, amv133, which encodes an active esterase enzyme with protease activity. Therefore, amv133 may play an essential role in virus production. In this study, we investigated the effects of amv133 on virus production and viral DNA replication. For this purpose, we constructed an amv133 knockout virus (amv133KO) by homologous recombination, purified the recombinant virus through serial plaque assays. We then determined the virus titers of both wild type and the amv133KO viruses at 0, 24, 48, 72 and 96 hours post infection via end point dilution assay. Viral DNA replication was also compared between the amv133KO and wild type viruses at 0, 12, 18, 24, 36, 48 h.p.i. using quantitative PCR. Our results showed that deletion of the amv133 gene from the virus genome reduced the infectious virus production by 96% compare to wild type virus. Quantitative PCR results showed that viral DNA replication of the knockout virus dropped by 82%. The results suggest that amv133 is an important, though non essential, gene for virus growth. It is not a structural protein so it may have a role in DNA replication.

NEONICOTINOID RESISTANCE IN THE COLORADO POTATO BEETLE.

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The Colorado potato beetle (*Leptinotarsa decemlineata*) is a significant pest of potato in most potato-growing areas of the world. Left unmanaged, the beetle can completely defoliate potato plants and reduce the yield by up to fifty percent. Currently, the management of *L. decemlineata* relies on use of insecticides, neonicotinoids in particular. However, emergence of insecticide resistance is a concern. In insects, the most common cause of insecticide resistance is elevated detoxification of insecticide molecules, caused by quantitative changes in protein levels of detoxifying enzymes such as cytochrome P450s, uridine 5'-diphospho-glucuronosyl transferases (UDP-GTs), esterases, glutathione S-transferases (GSTs), and ATP-binding cassette (ABC) transporters. Therefore, we postulated that overexpression of these proteins contributes to neonicotinoid resistance in *L. decemlineata*. Using RNA sequencing and qPCR analyses, we identified multiple detoxifying enzyme and ABC transporters genes which are transcriptionally upregulated in a neonicotinoid resistant strain of *L. decemlineata* compared to a sensitive strain. To reveal their potential role in neonicotinoid resistance, we used RNA interference (RNAi) to knock-down the transcript levels of several of these genes in the resistant insects. We demonstrated that RNAi knock-down of transcription for a cytochrome P450 and a UDP-GT enzyme gene results in a significant increase in susceptibility of resistant insects to imidacloprid (a neonicotinoid insecticide), indicating contribution of these genes to resistance. Together, our results suggest that RNAi knock-down of resistance-related genes, in combination with chemical insecticides, can offer a novel control strategy for economically important pests, including *L. decemlineata*

A ROLE FOR SEPTATE JUNCTION PROTEIN GLIOTACTIN IN THE MAINTENANCE OF SALT AND WATER BALANCE IN LARVAL MOSQUITO (*AEDES AEGYPTI*).

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Septate junctions (SJs), composed of transmembrane and cytosolic scaffolding proteins, occlude the paracellular pathway and function as paracellular diffusion barriers within invertebrate epithelia but their contribution to the maintenance of salt and water balance in aquatic invertebrates is not known. In this study, a role for transmembrane SJ protein gliotactin (Gli) in the ionoregulatory strategies of larval mosquito (Aedes aegypti) was examined. Gli exhibited tissue specific differences in transcript abundance in the midgut, Malpighian tubules (MT), hindgut and anal papillae (AP), which are tissues that participate in larval mosquito osmoregulation. Western blotting of Gli revealed its presence in monomer, putative dimer and phosphorylated forms. Gli was localized to SJ domain between the midgut epithelial cells and along the edges of the epithelial cells of the rectum and syncytial AP epithelium. In the MT, Gli immunolocalization was confined to SJs between the stellate and principal cells. Rearing larvae in 30% seawater caused tissue-specific changes in Gli protein abundance and in the midgut, increased Gli levels occurred in conjunction with increased paracellular permeability. To further characterize Gli function, larvae were fed gli-targeting dsRNA which resulted in reduced Gli protein abundance and paracellular permeability in the midgut. Taken together, data support the hypothesis that SJ protein Gli is important for the maintenance of salt and water balance in the aquatic larvae of A. aegypti and provide evidence to suggest that one role for Gli is to regulate the paracellular permeability of the midgut in response to changes in environmental ion and/or osmotic conditions.

This work was supported by NSERC.

CAPA-LIKE IMMUNOREACTIVITY IN THE SYNGANGLION AND PERIPHERAL TISSUES OF THE BLACK-LEGGED TICK, *IXODES SCAPULARIS*.

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Ixodes scapularis serves as a vector for a range of animal ailments including Lyme disease in humans, which results due to infection with *Borrelia burgdorferi*. In the face of increased prevalence of I. scapularis in Canada, very little is known about the neuroendocrine factors that govern key physiological processes in black-legged ticks. Furthermore, as a terrestrial arthropod and obligate blood feeder, it is crucial for *I. scapularis* to maintain ionic and osmotic homeostasis. In insects, CAPA peptides have been shown to have a dose-dependent antidiuretic or diuretic actions, controlling the removal of excess ions and water that are excreted as 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 organs and the posterior region of the rectal sac. These findings will be useful in permitting the discovery of physiological functions for the CAPA-related peptides in this important human disease vector.

This work was supported by NSERC.

CHARACTERIZING THE ROLE OF TISSUES INVOLVED IN ION AND FLUID TRANSPORT IN *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 derived from the imbibed blood meal. The salivary glands play an essential role in Ixodid ticks as they facilitate absorption of water vapor from unsaturated air and pathogen transmission. More importantly, the salivary glands are essential for tick hydromineral balance as they play the principal 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 the regulation of hydromineral balance 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 examined immunoreactivity of the Na+/K+ ATPase and V-type H+ ATPase, as these are both suggested to coordinate transport within the salivary glands and Malpighian tubules. Future studies will examine putative regulatory factors in the control of these tissues in order to further characterize the mechanisms of hydromineral balance in *I. scapularis*.

This work was supported by NSERC.

CHARACTERIZATION OF THE IMMUNE SYSTEM OF THE BROWN MARMORATED STINK BUG.

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The insect immune system is well developed and highly complex, and allows for widespread and rapid responses to foreign materials. Insects are able to regulate this response through the humoral and cell-mediated components of their innate system. The humoral response includes the production of antimicrobial peptides, complement-like proteins and radical oxygen species, as well as enzymatic cascades that lead to melanization and the coagulation of hemolymph. The cellular component of innate immunity includes phagocytosis, nodulation and encapsulation. The Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys*, is an invasive pest species from East Asia. With its history of inflicting extensive agricultural damage on a variety of host species, biomanagement of *H. halys* has become important, making the immune system a viable target. We have characterized morphologically distinct hemocyte subtypes within the hemolymph of *H. halys*. Currently, we are investigating the cellular response of these hemocytes to biotic pathogens, including GFP-expressing DH5-alpha *E.coli* and the encapsulated yeast, *Cryptococcus neoformans*. In vitro analysis of BMSB hemocytes challenged with *E. coli* have shown morphological change, aggregation and phagocytic activity. Through an understanding of BMSB specific immunological defenses it will be possible to identify putative gene targets for effective immunosuppression.

FATAL ATTRACTION: THE VOLATILE INFLUENCES THAT WILL LEAD WHITEFLIES TO DEADLY ENCOUNTERS AND THE RNAi RESPONSIBLE.

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In this day and age, food is being produced at an arithmetic rate, while human population is growing at an exponential rate, making crop yield and protection increasingly important. Currently, insect pests and pathogens are responsible for up to 30% of crop loss in Europe and the United States, and the incidence of pesticide resistance is increasing. One alternative to chemical pesticides are pest-specific 'trap crops', which attract target pest species; this can be done using olfactory responses in insects which can result in an attractant or arrestant effect, depending on the insect species. This has been observed in transgenic Micro-Tom tomatoes (Solanum lycopersicum cv.), which have enhanced carotenoid cleaving deoxygenase genes (CCD genes) and have an increased release of volatile organic compounds (VOCs); this change to the VOC profile has increased the oviposition preference by T. vaporariorum (Westwood) for the transgenic tomato compared to wild-type tomato. By combining these attractive plants with RNA interference (RNAi), a novel lethal trap crop model, which first lure, and then kill by silencing conserved targets, can be developed. This model can be used in future research in lethal trap crop development, which can benefit greenhouse production by decreasing chemical insecticide use, plant pathogen transmission and increase crop yield.

MEASURING THE *MAMESTRA CONFIGURATA* NUCLEOPOLYHEDROSIS VIRUS TRANSCRIPTOME: PERSONALIZED INSECT GENOMICS.

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We are investigating transcription by a baculovirus during infection of its host, the Bertha armyworm, Mamestra configurata, a significant pest of canola production in Western Canada. Knowledge of gene expression by the virus and host will be crucial to understanding how the two interact at the molecular level, and necessary to ultimately tap the potential of these viruses as biopesticides.
Previously, the cascade of gene expression that occurs during infection by baculoviruses has traditionally been measured in insect cells cultured and infected in vitro. This system provides robust information on transcription but is not fully representative of the in vivo environment in the larval midgut where the initiation of infection naturally occurs. Characterizing viral gene expression in the insect is difficult due to the low levels of virus transcripts at early time points relative to a very high background of host gene expression. To achieve this we have previously used both RNA sequencing and digital PCR approaches, which have revealed a specific suite of open reading frames (ORFs) that are detected in the initial phase of infection.
Recent studies exploring viral transcription using individual insects has, however, uncovered additional complexities to this story, and the potential implications of this personalized genomic approach will be discussed.

Supported by a Genomics Research & Development Initiative grant from AAFC.

CHARACTERIZATION OF CHEMOSENSORY PROTEIN GENES FROM THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS*.

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Chemosensory proteins (CSPs) are small, soluble proteins widely present among arthropods, but whose function is still not entirely clear. 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 from 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. Interestingly, one EAB CSP has a primary amino acid sequence that is approximately twice as long as all other CSPs. Although not particularly strongly expressed in sensory organs, homologous, "long" CSPs are also found in other beetle species. The results obtain so far will help decipher the role of this interesting gene family in EAB olfaction.

SMALL HEAT SHOCK PROTEIN GENES IN SPRUCE BUDWORM AND THEIR RESPONSE TO HEAT SHOCK, STARVATION AND VIRUS INFECTION.

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Heat shock proteins (HSPs), or stress proteins, are a highly conserved group of proteins expressed in response to diverse stresses, including heat, cold, starvation, anoxia, infection, ultraviolet light and exposure to a wide range of chemicals. HSPs exhibit chaperone-like activity by assisting the correct folding of nascent and stress-accumulated misfolded proteins, and preventing their aggregation. Many studies have suggested that HSPs are the major contributors to cold tolerance during the insect over wintering process. However, no HSPs have been investigated in spruce budworm, an important native forest pest in North America. HSPs have been classified into six families: HSP100, HSP90, HSP70, HSP60, HSP40 and small heat shock proteins (sHSP) based on the molecular weight. In this study, 16 sHSP genes encoding 18.6 to 28.4 kD proteins were identified from the spruce budworm transcriptome. The identified proteins have a common α-crystalline domain (ACD) located in the C-terminal region. Most of the sHSPs displayed low similarities to sHSPs from other organisms. The mRNA expression profiles in different developmental stages and tissues under normal conditions were examined; the accumulation of the sHSP in response to heat shock, starvation and virus infection was also investigated. The results indicated that the sHSPs mRNA levels were varied in different developmental stages and tissues under normal conditions. The spruce budworm specific sHSPs appeared to be very sensitive to heat shock stress implying the importance of these genes in response to temperature.

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"CRYOPROTECTANTS" IN THE HEMOLYMPH OF CHILL-TOLERANT *DROSOPHILA* PROTECT AGAINST CHILLING INJURY THROUGH OSMOPROTECTION.

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Like most insects, *Drosophila* succumb to biochemical effects of chilling that are unrelated to freezing, but species in this genus can widely vary in their chill tolerance. For susceptible species, cold exposure causes a loss of extracellular ion and water homeostasis, leading to hyperkalemia, chilling injury, and ultimately death. Chill-tolerant species instead maintain ion and water homeostasis during cold exposure and recover from an identical cold stress uninjured. Here, I will describe how these tolerant species maintain comparatively low hemolymph [Na^+], and how this "missing" Na^+ is "replaced" by other compatible osmolytes that may help to maintain osmotic balance at low temperatures. We used NMR to compare the metabolite profiles of the hemolymph of five drosophilid species at their rearing temperature (20°C) and immediately following cold exposure (4h at 0°C). Chill tolerant species constitutively maintain higher levels of specific organic osmolytes in their hemolymph and better maintain metabolic homeostasis during cold stress. Levels of these classical "cryoprotectants" in chill-tolerant species suggest a non-colligative or osmotic role for these compounds in determining chilling tolerance.

THE INVOLVEMENT OF SULFAKININS IN THE CONTROL OF FEEDING IN *RHODNIUS PROLIXUS*.

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Neuropeptides play influential roles in feeding and digestion-related activities in insects, including *Rhodnius prolixus*. Sulfakinins (SKs) are one family of such multifunctional neuropeptides that have been shown to influence digestive, diuretic, and myotropic activities in various insects. In this study, the presence of two sulfakinin peptide sequences, namely *Rhopr-SK-1* and *Rhopr-SK-2*, was confirmed within the sequenced open-reading frame (ORF) of the corresponding Rhopr cDNA. Immunohistochemical straining of SK-like peptides was observed in cells in the CNS as well as processes on the midgut and hindgut of 5th instars and adults. Reverse transcriptase quantitative PCR (RT-qPCR) revealed that the *Rhopr-SK* transcript is primarily localized throughout the brain, and possibly the subesophageal ganglion (SOG), in 5th instars. Fluorescent *in situ* hybridization (FISH) revealed that the cells expressing the *Rhopr-SK* transcript are located in the brain. Bioassays revealed an increase in contractions of the hindgut following the application of Rhopr-SK-1. A heart contraction assay has shown that heartbeat frequency was not affected by Rhopr-SK-1. The Rhopr-SK G-protein coupled receptors will be examined via their cloning, expression, and characterization. RT-qPCR will also be performed to examine the SK receptors' spatial and temporal expression patterns. Using double stranded RNA (dsRNA) sequences of the SK peptides and GPCRs, RNAi will be performed to examine the role of the SK signaling pathway in feeding-related activities, such as satiety. Moreover, a feeding bioassay involving the injection of Rhopr-SK-1 will be performed to examine the peptide's direct role as an inhibitor of food intake in *R. prolixus*.

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STUDY OF THE EFFECT OF ABNORMAL SYSTEMIC METABOLISM ON TUMOR GROWTH AND METASTASIS IN A *DROSOPHILA* MODEL.

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A large number of epidemiologic studies suggest a link between metabolic diseases, such as obesity and type 2 diabetes, and the aggressiveness of certain tumor types. For instance, enhanced lymph node metastasis was documented in obese breast cancer patients with progesterone receptor mutations. Despite these clear epidemiologic associations, little is known about the specific molecular mechanisms linking cancer progression and metabolic dysfunction. As dietary manipulations in Drosophila induce metabolic states similar to human diseases, such as hyperglycemia, insulin resistance, and accumulation of body fat, flies offer an excellent system for examining these mechanistic connections. We generated larvae carrying oncogenically transformed eye imaginal disc epithelia, and examined the progression of these tumors in animals fed a variety of macronutrient regimens. Striking changes in the overall tumor volume and the number of individual micro-metastases were observed in animals fed high-sugar diet, and to a lesser extent high-protein and fat diets. Importantly, the type of oncogenic transformation was found to be a critical determinant of tumor response to dietary manipulations, suggesting that systemic hyperglycemia and hyperinsulinemia exert context-dependent effects on tumor progression. For instance, tumors driven by the activated Notch pathway are exquisitely sensitive to hyperglycemic state, whereas tumors containing aberrations in the Hippo pathway appear significantly less affected by high-sugar diet. A molecular model consistent with the observed differences will be presented, and the applicability of our in vivo assay to studying metabolic sensitivity of specific oncogenic signatures from human cancers will be discussed.

INVESTIGATING THE FUNCTION OF THE C4 ZINC FINGER DOMAIN OF BACULOVIRUS AcMNPV PROTEIN ME53.

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All sequenced lepidopteran alpha- and betabaculoviruses encode me53, a conserved immediate early/late gene that is under the influence of a dual early/late promoter. ME53 is not essential for virus production however, its removal results in a ~10 000 fold reduction of budded virus production. The 449 amino acid ME53 polypeptide from the baculovirus Autographa californica nucleopolyhedrovirus (AcMNPV) contains a nuclear translocation sequence (NTS) at aa 109-137, and a conserved putative C4 zinc finger motif (ZnF) at the C-terminus at aa 379-399. C4 zinc fingers are commonly found associated with transcription factors. While ME53 localizes in viral GP64-containing membrane foci of infected cells it also localizes to the nucleus. The nuclear translocation of ME53 and its requirement for optimal virus production suggests that it may play a role in transcription. Viral growth curves and qRT-PCR were used to determine if the presence of the NTS or zinc finger affect virus production and viral gene transcript levels. Deletion of the NTS reduced levels of virus production to those for a completely deleted ME53 virus emphasizing the nuclear importance of ME53. These results were expected due to the inability of ME53 to localize to the nucleus in the absence of the NTS. Deletion of the zinc finger domain affected budded virus production from 12 to 24 hours post transfection and had a repressive effect on some viral gene transcript levels. These results were contradictory to the presumed transcriptional activating effect of a typical C4 zinc finger. This research suggests that ME53 plays a complex role in regulation of gene transcript levels and virus production.

AEDES-CAPA1 MODULATION OF DIURESIS STIMULATED WITH NEUROENDOCRINE FACTORS IN *AEDES AEGYPTI.*

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The global disease vector, *Aedes aegypti*, transmits pathogenic species into the host during the process of engorging on a blood meal. Intrinsic osmoregulatory mechanisms controlled by neuroendocrine factors are responsible for the regulation of diuresis across life stages. A number of diuretic and antidiuretic factors have been identified that regulate the secretion and absorption of ions in the Malpighian tubules of insects. CAPA peptides elicit distinct dose-dependent secretion effects in the Malpighian tubules, functioning as a diuretic at high concentrations and an antidiuretic at low concentrations. The objective of this research was to investigate the effects of a low, anti-diuretic concentration of AedesCAPA-1 on larval *A. aegypti* Malpighian tubules stimulated with various diuretic factors including 5-hydroxytryptamine (5-HT), calcitonin-like peptide (DH31), corticotropin-releasing factor (CRF)- related peptide, or culekinin. This study also investigated the effects of AedesCAPA-1 on adult female *A. aegypti in vivo*. The results suggest that a low concentration of AedesCAPA-1 inhibits larval Malpighian tubules stimulated with 5-HT and DH31, but not with CRF-related or culekinin. Based on previous studies, AedesCAPA-1 likely elicits anti-diuretic effects through a cGMP-dependent phosphodiesterase, reversing the diuretic effects of 5-HT and DH31, which utilize cAMP to promote secretion. Research into the osmoregulatory mechanisms of *A. aegypti* holds great potential for the development of novel pest control strategies.

This work was supported by NSERC.

ECDYSTEROID TARGETS IN AN ADULT INSECT: PRESENCE AND CYCLING OF THE ECDYSTEROID RECEPTOR IN TISSUES OF ADULT *RHODNIUS PROLIXUS*.

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Ecdysteroids, the insect molting hormones, are important regulators of insect larval development that act via the ecdysteroid receptor (EcR). In larvae, most tissues express EcR and are targets of ecdysteroids. Ecdysteroids are also present in adult insects, though generally in smaller amounts, and their role in adult physiology is less clearly understood. Few studies have examined the distribution of EcR in adult tissues and the targets of ecdysteroids in adult insects are mostly unknown. We recently showed that ecdysteroids are present in hemolymph of adult Rhodnius prolixus and that the hemolymph ecdysteroid titer changes with a daily rhythm that is under circadian control. To illuminate the function of ecdysteroids in adult Rhodnius we investigated the distribution of EcR in adult tissues using immunohistochemistry and confocal microscopy. In adult Rhodnius, ecdysteroids are undetectable in unfed insects; a blood meal triggers ecdysteroid production. EcR was never detected in unfed insects. In fed animals EcR was observed in tissues including fat body, testes, Malpighian tubules, brain, and ovaries, indicating that these are targets of ecdysteroids. Other tissues such as muscle, crop, gut, and salivary glands never expressed observable EcR. There appear to be fewer ecdysteroid-responsive tissues in adult than larval Rhodnius. EcR relative fluorescence intensity cycled with a daily rhythm in all tissues in which the receptor was observed, with peak fluorescence during the scotophase. Moreover, EcR favored a nuclear location during the night and a cytoplasmic location during the day. These results indicate that ecdysteroids and their receptor remain under circadian control in the adult stage and suggest several possible roles for ecdysteroids in the adult insect.

NOTES

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