INSECT BIOTECH CONFERENCE

Virtual Research Symposium

June 11, 2020

Schedule and Contributed Abstracts

Insect Biotech Conference – VRS June 11, 2020

Conference Schedule

8:50 am	Join Meeting on Zoom
8:55 am	Opening Remarks
9:00 am	**THE ROLE OF PYROKININS ON THE HINDGUT OF *AEDES AEGYPTI. [Page 6]*** Lajevardi, A., Paluzzi, J-P. [1]Department of Biology, York University, Toronto, ON, Canada.
9:20 am	**IN COLD BLOOD: DECIPHERING THE MECHANISMS UNDERLYING MOSQUITO-FROG INTERACTIONS. *[Page 7]*** [1]Reinhold, J., [2]McLeod, D., [1]Lahondere, C. [1]Department of Biochemistry, Virginia Polytechnic Institute and State University, VA, USA. [2]James Madison University, VA, USA
9:40 am	**THE *TRICHOPLAX ADHAERENS* CAV2 VOLTAGE GATED CALCIUM CHANNEL SHARES CONSERVED FUNCTIONAL FEATURES WITH HUMAN SYNAPTIC CAV CHANNELS. *[Page 8]*** Gauberg, J., Abdallah, S., Senatore, A. Department of Biology, University of Toronto Mississauga, Mississauga ON, Canada.
10:00 am	**CHARACTERIZATION OF THE FIRST SIFamide RECEPTOR IN A HEMIMETABOLOUS INSECT; THE CHAGAS DISEASE VECTOR, *RHODNIUS PROLIXUS. [Page 9]*** Ayub, M., Lange, A.B., Orchard, I. Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.
10:20 am	**SUGAR FEEDING BY INVASIVE MOSQUITO SPECIES ON ORNAMENTAL PLANTS. *[Page 10]*** Upshur, I.F., Savory, A., Lahondere, C. Department of Biochemistry, Virginia Polytechnic, Blacksburg, VA, USA.

10:40 am **LIFE WITHOUT WATER: A TALE OF PHYSIOLOGICAL PLASTICITY IN TWO ECOLOGICALLY DISTINCT AND EVOLUTIONARY DISTANT INSECTS. *[Page 11]***

[1,2]Thorat, L., [2]Nath, B.B.
[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Department of Zoology, S.P. Pune University, Pune, India.

11:00 am **DOES A BLOOD MEAL ALTER AQUAPORIN ABUNDANCE IN THE ADULT MOSQUITO, *AEDES AEGYPTI. [Page 12]***

Picinic, B., Paluzzi, J-P.V., Donini, A.
Department of Biology, York University, Toronto, ON, Canada.

11:20 am **VOLTAGE-GATED CALCIUM CHANNELS REGULATE ION TRANSPORT IN THE MALPIGHIAN TUBULES OF CATERPILLARS. *[Page 13]***

Kolosov, D., O'Donnell, M.J.
Department of Biology, McMaster University, Hamilton, ON, Canada.

11:40 am **ELUCIDATING THE ANTI-DIURETIC HORMONE SIGNALING SYSTEM OF CAPA NEUROPEPTIDES IN THE DISEASE VECTOR MOSQUITO, *AEDES AEGYPTI. [Page 14]***

Sajadi, F., Paluzzi, J-P.
Department of Biology, York University, Toronto, ON, Canada.

12:00 pm **REGULATION OF TREHALOSE TRANSPORTER BY INSULIN-LIKE PEPTIDE AND ADIPOKINETIC HORMONE IN *RHODNIUS PROLIXUS. [Page 15]***

Leyria, J., El-Mawed, H., Orchard, I., Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

12:20pm – 12:55pm Lunch break social and networking session

1:00 pm ***IXODES SCAPULARIS* MIALOME: A STEP TOWARDS IDENTIFICATION OF NOVEL TARGETS FOR TICK CONTROL. *[Page 16]***

[1]Reyes, J., [1]Sharma, A., [2]Petereit, J., [2]Tillett, R., [1]Chavez, C., [3]Nuss, A., [1]Gulia-Nuss, M.
[1]Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV, USA.

[2]Nevada Center for Bioinformatics, University of Nevada, Reno, NV, USA.
[3]Department of Agriculture, Veterinary and Rangeland Sciences, University of Nevada, Reno, NV, USA.

1:20 pm **ONTOLOGICAL MACHINE LEARNING MODELS FOR IDENTIFICATIONOF PITFALL TRAP-COLLECTED GROUND BEETLES AT THE CONTINENTAL SCALE.** ***[Page 17]***

[1]Blair, J., [2]Weiser, M.D., [2]Kaspari, M., [2]Miller, M., [2]Siler, C., [1]Marshall, K.E.
[1]Department of Zoology, University of British Columbia, Vancouver, BC, Canada.
[2]Department of Biology, University of Oklahoma, Norman, OK, USA

1:40 pm **SPATIAL AND TEMPORAL EXPRESSION OF CYTOCHROME P450 SUBFAMILY G MEMBERS IN *AEDES AEGYPTI. [Page 18]***

[1]Arshad, F., [1,2]Nuss, A., [1]Tittiger, C., [1]Blomquist, G.J., [1]Gulia-Nuss
[1]Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV, USA.
[2]Department of Agriculture, Veterinary and Rangeland Sciences, University of Nevada, Reno, NV, USA.

2:00 pm **NEUROENDOCRINE MODULATION OF DESICCATION AND COLD STRESS IN *DROSOPHILA.*** ***[Page 19]***

[1,4]Zandawala, M., [2]Nguyen, T., [3]Segura, M., [1]Johard, H., [1]Amcoff, M., [3]Wegener, C., [2]Paluzzi, J-P., [1]Nässel, D.
[1]Department of Zoology, Stockholm University, Stockholm, Sweden.
[2]Department of Biology, York University, Toronto, ON, Canada.
[3]Neurobiology and Genetics, Wurzburg Insect Research, Theodor-Boveri-Institute, Biocenter, University of Wurzburg, Germany.
[4]Department of Neuroscience, Brown University, Providence, USA.

2:20 pm **WHY ARE SOME MOSQUITOES INVASIVE?** ***[Page 20]***

Peach, D., Matthews, B.J.
Department of Zoology, University of British Columbia, Vancouver, BC, Canada.

2:40 pm **PREDICTING PHYSIOLOGICAL FUNCTIONS OF NEUROPEPTIDE F SIGNALING IN THE TOBACCO HORNWORM, *MANDUCA SEXTA. [Page 21]***

Kaur, G., Pooraiiouby, R., Tillett, R., Peterit, J., Nuss, A.B.
Department of Agriculture, Veterinary and Rangeland Sciences, University of Nevada, Reno, NV, USA.

3:00 pm **HOW TO FIND A MISSING PATHWAY: THE IMMUNE-DEFICIENCY (IMD) PATHWAY OF *RHODNIUS PROLIXUS*. *[Page 22]***

Salcedo-Porras, N., Lowenberger, C.
Centre for Cell Biology, Development and Disease, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada.

3:20 pm **ERYTHRITOL BASED SWEETENER SHORTENS THE LIFESPAN OF *AEDES AEGYPTI* POTENTIALLY BY N-LINKED PROTEIN GLYCOSYLATION. *[Page 23]***

[1]Sharma, A., [1]Reyes, J., [2]Nuss, A., [1]Gulia-Nuss, M.
[1]Department of Biochemistry and Molecular Biology, University of Nevada, Reno.
[2]Department of Agriculture, Veterinary & Rangeland Sciences, University of Nevada, Reno

3:40 pm **PLASTICITY OF COLD-HARDINESS IN THE EASTERN SPRUCE BUDWORM. *[Page 24]***

[1]Butterson, S., [2]Roe, A.D., [1]Marshall, E.
[1]Department of Zoology, University of British Columbia, Vancouver, BC, Canada.
[2]Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, ON, Canada.

4:00 pm **AMMONIUM TRANSPORTER EXPRESSION IN SPERM OF THE DISEASE-VECTOR *AEDES AEGYPTI* MOSQUITO INFLUENCES MALE FERTILITY. *[Page 25]***

Durant, A.C, Donini, A.
Department of Biology, York University, Toronto, ON, Canada.

4:20 pm End of IBC virtual research symposium

THE ROLE OF PYROKININS ON THE HINDGUT OF *AEDES AEGYPTI.*

Lajevardi, A., Paluzzi, J-P.

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

Aedes aegypti mosquitoes owe much of their success to processes that play important roles when faced with dietary and environmental challenges. The highly regulated excretory system helps alleviate risks posed to haemolymph homeostasis. The hindgut is part of this system, playing a vital role in reabsorbing water and ions before excreting urine through its contractile activity. Pyrokinin neuropeptides were initially identified to influence hindgut physiology of several other insects. The present study aimed to elucidate the role of two specific pyrokinin subtypes (PK1 and PK2) in A. aegypti. First, we characterized their authentic receptors (PK1-R and PK2-R) based on selectivity for these ligands. We then identified sites of receptor expression, which confirmed the hindgut as a prominent target for these peptides. Based on these distribution profiles, we assessed the role of PK1 on the posterior hindgut (rectum), which seemed to play no role in regulating contractile activity or sodium transport along this organ. Instead, we identified PK2 to have a myoinhibitory effect on the anterior hindgut (ileum), the only role that has been assigned for this peptide in any blood-feeding arthropod to date. Collectively, these findings suggest this neuropeptide family may function in regulating various processes in the mosquito, such as excretion, diuresis and reproduction.

IN COLD BLOOD: DECIPHERING THE MECHANISMS UNDERLYING MOSQUITO-FROG INTERACTIONS.

[1]Reinhold, J., [2]McLeod, D., [1]Lahondere, C.

[1]Department of Biochemistry, Virginia Polytechnic Institute and State University, VA, USA.
[2]James Madison University, VA, USA

Mosquitoes can feed on almost any animal, including cold-blooded vertebrates, annelids, and even other mosquitoes. While most research involves mosquitoes that feed on humans, one species, Culex territans, specializes in feeding on ectotherms, primarily amphibians and reptiles. With the decline of amphibians worldwide, it is important to expand our knowledge on this mosquito species, which is a known amphibian disease vector. Although Cx. territans is fairly widespread in North America and Europe, surprisingly little is known about its biology and ecology. For this project, we are particularly interested in understanding how these mosquitoes locate their cold blooded hosts and how they manage to imbibe cold, viscous blood. Mosquitoes typically use a variety of cues to locate a warm-blooded host, including carbon dioxide, odor, heat, and vision. However, carbon dioxide and thermal cues would not be particularly prevalent among amphibians. To better understand the cues that Cx. territans uses to find its hosts, we performed olfactometer experiments with green frogs and bullfrogs, two of the common hosts this mosquito feeds on in the wild. We also used solid-phase microextraction scent collection with gas chromatography coupled with mass spectrometry to analyze the scents of these two frog species. To better understand the ability of this mosquito to feed on cold blood, we performed feeding assays coupled with thermographic imaging and found some unexpected results. We hope this data will help to build a more complete picture of the evolution of blood feeding on warm-blooded as well as cold-blooded hosts and to learn about the role Cx. territans plays in the decline of amphibian populations worldwide.

THE *TRICHOPLAX ADHAERENS* CAV2 VOLTAGE GATED CALCIUM CHANNEL SHARES CONSERVED FUNCTIONAL FEATURES WITH HUMAN SYNAPTIC CAV CHANNELS.

Gauberg, J., Abdallah, S., Senatore, A.

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

n excitable cells, voltage-gated calcium (CaV) channels are necessary for rapidly converting electrical signals into cytoplasmic calcium signals. Most animals have three types of voltage gated calcium (CaV) channels, CaV1, CaV2, CaV3, which overlap in function but also exhibit unique specializations that are highly conserved among phyla. For example, CaV1 channels are expressed in muscle where they drive muscle contraction, while CaV2 channels are expressed in nerve terminals where they drive exocytosis of synaptic vesicles. Although these functions are conserved across many animal phyla, the evolution of this functional divergence is unclear. To gain a better understanding of the divergence of these channels, we are studying the most early-diverging animal to possess all three types of CaV channels, Trichoplax adhaerens. This animal lacks neurons, and only has six functionally distinct cell types. Despite this, Trichoplax demonstrates complex motile behaviour (e.g. feeding, chemotaxis, phototaxis) and expresses a large repertoire of genes typically associated with the nervous system. Because CaV2 channels drive synaptic exocytosis in animals with a nervous system, the Trichoplax CaV2 channel was examined to understand whether CaV2 channel properties and functions diverged prior to the evolution of the nervous system. This study examines the localization of the of Cav2 channel in Trichoplax and compares its biophysical properties to the human pre-synaptic CaV2 channel.

CHARACTERIZATION OF THE FIRST SIFamide RECEPTOR IN A HEMIMETABOLOUS INSECT; THE CHAGAS DISEASE VECTOR, *RHODNIUS PROLIXUS.*

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

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

Within arthropods, the SIFamide family of neuropeptides is responsible for the modulation of a host of physiological and behavioural processes. In *Rhodnius prolixus*, we have previously shown the presence of SIFamidergic processes in neurohemal release sites and provided evidence for a role of Rhopr-SIFa in modulating feeding behaviors. Here, the *R. prolixus* SIFamide receptor (RhoprSIFR) has been identified, cloned, and sequenced, from the central nervous system (CNS). Sequence analyses show high similarity and identity between RhoprSIFR and other cloned invertebrate SIFamide receptors, particularly the SIFamide receptor in the blood-sucking insect *Cimex lectularis*. The spatial expression profile of RhoprSIFR shows that the RhoprSIFR transcript is found in various tissues, including those involved in feeding and reproduction. In unfed insects, high transcript expression is observed in the CNS and anterior midgut, suggesting a role of Rhopr-SIFa in various processes related to feeding and digestion. Temporal expression of the RhoprSIFR transcript also varies between unfed, 24 h post-feed, and 7d post-feed conditions. Expression of RhoprSIFR transcript decreases in the AMG 24 h after feeding, allowing us to question involvement of SIFamide in post-feeding reabsorption of ions and solutes from the consumed blood meal. We also observed significantly increased expression of RhoprSIFR in the AMG and PMG 7d post-feeding, suggesting a role for Rhopr-SIFa in regulating long-term post-feeding osmotic balance and in coordinating feeding and post-prandial physiology.

SUGAR FEEDING BY INVASIVE MOSQUITO SPECIES ON ORNAMENTAL PLANTS.

Upshur, I.F., Savory, A., Lahondere, C.

Department of Biochemistry, Virginia Polytechnic, Blacksburg, VA, USA.

Feeding on plant-derived sugars is an essential component of mosquito biology that affects key aspects of their lives such as survival, metabolism, and reproduction. Mosquitoes locate plants to feed on using olfactory and visual cues. Aedes aegypti and Aedes albopictus are two mosquito species invasive to the US, and are vectors of diseases such as dengue fever, chikungunya, and Zika. These species live in urban, heavily-populated areas, where they have a high accessibility to human hosts as well as to plants in backyards and town landscapes. Therefore, it is important to understand what plants might attract / repel mosquitoes to inform citizens and authorities accordingly. Here, we observe Ae. aegypti and Ae. albopictus sugar-feeding behavior with eleven different commonly-planted ornamental plant species. We then assessed feeding activity using the anthrone method. The composition of volatiles in the headspace of each plant was then examined using gas-chromatography mass-spectroscopy, and how these volatiles may be mediating feeding interactions is discussed.

LIFE WITHOUT WATER: A TALE OF PHYSIOLOGICAL PLASTICITY IN TWO ECOLOGICALLY DISTINCT AND EVOLUTIONARY DISTANT INSECTS.

[1,2]Thorat, L., [2]Nath, B.B.

[1]Department of Biology, York University, Toronto, ON, Canada.
[2]Department of Zoology, S.P. Pune University, Pune, India.

Being ectotherms and inhabitants of diverse ecosystems, insects are susceptible to frequent dehydration-rehydration cycles. Under such conditions, desiccation tolerance serves as a crucial strategy for sustenance despite body water loss. Unless we are aware of the plethora of mechanisms governing desiccation tolerance in insect systems, the diversity of their survival tactics cannot be fully appreciated. In this study, I discuss few of the fundamental physiological adjustments in the aquatic midge, *Chironomus ramosus* and the terrestrial fruit fly, *Drosophila melanogaster*. These representative insects with contrasting habitats and an evolutionary gap of ~150 million years provided interesting cues of the differential strategies they have evolved to evade environmental dehydration regimes. Desiccation tolerance threshold of *Drosophila* larvae was found to be strikingly higher compared to *Chironomus.* Difference in their cuticular thickness provided one of the most likely explanations for the vulnerability of *Chironomus* to rapid water loss. The most intriguing part of this study was the identification of a novel role for glucosamine as a stress-alleviating metabolite in *Chironomus*, which was apparently absent in *Drosophila*. Furthermore, a common switch used by both the larval groups to withstand physiological water deficits was the induction of trehalose, which played an unconventional role as a redox regulator. Although trehalose happens to be one of the most extensively occurring stress protectants in animals, glucosamine has never been reported before for its role under stressful environments, including desiccation. In summary, these findings provide insights into the diversity of invertebrate desiccation tolerance from ecophysiological and evolutionary perspectives.

DOES A BLOOD MEAL ALTER AQUAPORIN ABUNDANCE IN THE ADULT MOSQUITO, *AEDES AEGYPTI.*

Picinic, B., Paluzzi, J-P.V., Donini, A.

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

The mosquito Aedes aegypti, found in sub-tropical regions of the world, are vectors of pathogens leading to deadly arboviral diseases such as Yellow fever and Zika virus, making them an important model for research. Female A. aegypti require a blood meal to provide the necessary proteins and amino acids for egg development; however, the blood meal poses an osmoregulatory challenge. Initially, excess water and sodium from the blood plasma requires excretion, while later mosquitoes must deal with excessive potassium released from digested blood cells. Osmoregulation involves the water and ion-flux across the epithelia of various organs such as the midgut, Malpighian tubules, and hindgut. The movement of water across the epithelia occurs through transmembrane proteins that are members of the Major intrinsic protein superfamily, which in insects can include aquaporins that function as selective water channels and entomoglyceroporins. It has been shown that due to the osmoregulatory stress that the mosquito faces upon ingesting a blood meal, the transcript abundance of AQPs (aquaporins and/or entomoglyceroporins) is affected; however, AQP protein localization and expression has not been studied. Here we report on the protein localization of several Aedes aegypti AQPs in organs and tissues of adult females and provide evidence that protein abundance of many of these AQPs increases in the Malpighian tubules after blood feeding. We show that protein abundance of at least one of these AQPs decreases in the fat body of adult females after a blood meal. This study begins to provide an essential map of AQP expression in adult female mosquitoes and implicates AQPs in regulating physiological processes upon ingestion of a blood meal.

VOLTAGE-GATED CALCIUM CHANNELS REGULATE ION TRANSPORT IN THE MALPIGHIAN TUBULES OF CATERPILLARS.

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

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

Epithelia of vertebrates and invertebrates are built to either secrete ions or to reabsorb ions. Malpighian tubules (MTs) of insects are ion-transporting epithelia that extend from the gut and form a part the functional insect kidney. In general, MTs of most insects consist of a distal portion that secretes ions/fluid and a proximal portion that reabsorbs ions/fluid. In contrast, MTs of larval Lepidoptera (caterpillars of butterflies and moths), are comprised of five regions that differ in their association with the gut, their structure and their ion/fluid transport function. Recent studies have also shown that several regions can rapidly and reversibly switch between ion secretion and reabsorption. The current study utilized microscopy, $[Ca2+]i$ measurements, and molecular, next-generation sequencing, and electrophysiological techniques to demonstrate that voltage-gated calcium (CaV) channels are involved in the regulation of ion transport in the non-contractile non-innervated MTs of caterpillars. The study provides evidence for direct involvement of voltage-gated ion channels in the regulation of epithelial ion transport of insect MTs and offers insight into how CaV channels may contribute to the rapid reversible switch in the direction of epithelial ion transport.

ELUCIDATING THE ANTI-DIURETIC HORMONE SIGNALING SYSTEM OF CAPA NEUROPEPTIDES IN THE DISEASE VECTOR MOSQUITO, *AEDES AEGYPTI.*

Sajadi, F., Paluzzi, J-P.

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

Haematophagus insects, such as female Aedes aegypti mosquitoes, face the challenge of excess water and ion intake after a blood meal. To cope with this, adult A. aegypti have a specialized excretory system, consisting of the Malpighian tubules (MTs), which are under rigorous control by several neuroendocrine factors. While CAPA exhibits variable response in insect species, the role and signaling pathway of these peptides remains unclear in adult Aedes mosquitoes. Given the CAPA receptor is highly enriched in insect MTs, the objectives of this study were to examine the effects of CAPA on adult female MTs stimulated with various diuretic factors. CAPA was found to inhibit secretion of MTs stimulated by select diuretic factors, 5HT and DH31, while having no effect on CRF-related peptides. Although CAPA elicits anti-diuretic activity, it does not influence the relative proportions of cations transported by adult MTs, thus maintaining the kaliuretic activity of 5HT and the natriuretic activity of DH31. Pharmacological inhibition of PKG/NOS signalling abolishes the anti-diuretic activity of CAPA, confirming its role in the CAPA signaling pathway. Furthermore, MTs treated with bafilomycin, a proton pump inhibitor, was found to inhibit fluid secretion stimulated by 5HT and DH31, while having no inhibitory action on CRF-stimulated MTs. Additionally, alkalization of the secreted fluid in response to CAPA suggests inhibition of the apical proton pump, which may lead to constrained entry of cations across the apical membrane of the MTs. Further understanding of the role of each specific hormone family, including both diuretic and anti-diuretic factors, will help resolve this complex regulatory network.

REGULATION OF TREHALOSE TRANSPORTER BY INSULIN-LIKE PEPTIDE AND ADIPOKINETIC HORMONE IN *RHODNIUS PROLIXUS.*

Leyria, J., El-Mawed, H., Orchard, I., Lange, A.B.

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

Trehalose homeostasis, the main blood carbohydrate in insects, is controlled by 1) trehalose synthase, 2) trehalases and 3) trehalose transporter (TRET). In insects, trehalose is stored in developing oocytes during vitellogenesis as a resource to support successful embryogenesis. In addition, starvation is a stressful event where energy consumption, provide by carbohydrate metabolism, is required to maintain the overall metabolism of the insect. Therefore, trehalose is useful for both anabolic (vitellogenesis) and catabolic (starvation) events. In insects, insulin-like peptides (ILPs) and adipokinetic hormone (AKH) are key hormones that regulate carbohydrate metabolism. Using Rhodnius prolixus, we studied the involvement of ILPs and AKH in carbohydrate metabolism, with focus on TRET regulation of the ovaries (OVs). We find that trehalose stores in OVs increase after feeding and decrease as starvation progresses. However, by qPCR we show that trehalose synthase is down-regulated in OVs of vitellogenic females and of insects undergoing starvation; in contrast, TRET mRNA increase in both situations. Using ex-vivo and in-vitro stimulation we demonstrate that TRET expression is up-regulated via ILP and AKH signaling, probably favouring anabolic and catabolic processes, respectively, according to metabolic needs. Also, using dsRNA to silence ILP/AKH signaling we find that TRET is down-regulated. Overall, the results support the hypothesis of a direct uptake and release of trehalose by TRET from the hemolymph and OVs, respectively, regulated by ILP and AKH signaling. In conclusion, these results show that trehalose metabolism and its hormonal regulation play critical roles in adapting to various nutritional conditions and stressful situations in this insect.

IXODES SCAPULARIS MIALOME: A STEP TOWARDS IDENTIFICATION OF NOVEL TARGETS FOR TICK CONTROL.

[1]Reyes, J., [1]Sharma, A., [2]Petereit, J., [2]Tillett, R., [1]Chavez, C., [3]Nuss, A., [1]Gulia-Nuss, M.

[1]Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV, USA.
[2]Nevada Center for Bioinformatics, University of Nevada, Reno, NV, USA.
[3]Department of Agriculture, Veterinary and Rangeland Sciences, University of Nevada, Reno, NV, USA.

Ixodes scapularis, commonly known as the black-legged tick, is one of the major vectors responsible for transmitting the bacterium, *Borrelia burgdorferi*, the causative agent of Lyme disease. Lyme disease is the most important vector-borne disease in the United States accounting for over 400,000 new cases every year. *Borrelia burgdorferi* spirochetes are ingested by the tick from an infected vertebrate host with a blood meal, the sole food source for all stages of ticks. Protein-rich blood meal provides nutrients for the tick development and reproduction and for the developing pathogen. The attachment to the vertebrate host and initiation of blood meal ingestion results in rapid changes in the transcriptome of the midgut to enable the proliferation of gut for the enormous blood meal (100x in body weight) taken over 5-10 days of feeding and for digestion. Tick blood feeding has been shown to occur in two phase: a slow feeding phase and a rapid feeding phase that lasts approximately 24 h. Most studies in tick blood digestion have focused on the early slow feeding phase and the knowledge of blood digestion post detachment is lacking. To understand the global changes in gene expression and protein levels throughout the feeding and digestion (post host detachment), we dissected midguts from the partially engorged (5-6 days on the host and detached prematurely), 1, 2, 7, and 14 d post detachment from the host. The combination of these data has led us to identify several enzymes that appear to be important for blood digestion that may be key in development of an anti-tick vaccine.

ONTOLOGICAL MACHINE LEARNING MODELS FOR IDENTIFICATIONOF PITFALL TRAP-COLLECTED GROUND BEETLES AT THE CONTINENTAL SCALE.

[1]Blair, J., [2]Weiser, M.D., [2]Kaspari, M., [2]Miller, M., [2]Siler, C., [1]Marshall, K.E.

[1]Department of Zoology, University of British Columbia, Vancouver, BC, Canada.
[2]Department of Biology, University of Oklahoma, Norman, OK, USA

Insect populations are rapidly changing, and monitoring these changes is essential for understanding the causes and consequences of these shifts. However, large scale insect identification projects are time-consuming and expensive when solely done by human identifiers. Machine learning offers a possible solution to help collect insect data quickly and efficiently. In this seminar, I present a methodology for training classification models using pitfall trap-collected ground beetles. I outline all the required procedures for image collection, image-data extraction, data preparation, and model training. Overall, I compare the performance of five machine learning algorithms and two classification methods (ontological and single-level) when identifying ground beetles from the species to subfamily level. The best performing algorithm, linear discriminant analysis (LDA), reached a top-1 accuracy comparable to human identifiers, which was further increased when classifications were limited by known local species pools. Model performance was negatively correlated with taxonomic specificity, with the LDA model reaching a top-1 accuracy of ~99% at the subfamily level. I also observed greater performance when classifications were made using the ontological classification method compared to the single-level classification method at taxonomic levels above species. The general methodology presented here serves as a proof-of-concept for classifying pitfall-trap collected organisms using machine learning algorithms, and the image data extraction methodology may be used for non-machine learning uses. I propose that integration of machine learning in large scale identification pipelines will increase efficiency and lead to a greater flow of insect macroecological data.

SPATIAL AND TEMPORAL EXPRESSION OF CYTOCHROME P450 SUBFAMILY G MEMBERS IN *AEDES AEGYPTI.*

[1]Arshad, F., [1,2]Nuss, A., [1]Tittiger, C., [1]Blomquist, G.J., [1]Gulia-Nuss

[1]Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV, USA.
[2]Department of Agriculture, Veterinary and Rangeland Sciences, University of Nevada, Reno, NV, USA.

Aedes aegypti is the major vector responsible for transmitting arboviruses such as the yellow fever virus, dengue fever, and Zika virus. Increasing insecticide resistance in mosquito populations requires novel targets for developing new control strategies. CYP4G is an insect specific subfamily of cytochrome P450 enzymes. These enzymes are oxidative decarboxylases and have been shown to regulate hydrocarbon (HCs) production for cuticle formation and may also be involved in phytochemical detoxification. A thicker cuticle with increased HCs is one way to avoid insecticide penetration in insects, therefore, manipulating HC synthesis can be a new target for mosquito control. Ae. aegypti genome encodes two CYP4Gs: CYP4G15 and CYP4G36. Transcript expression of CYP4G 15 and CYP4G 36 was determined in different mosquito life stages and female tissues using quantitative reverse transcription PCR (qRT-PCR). Results from these studies show that CYP4G15 and CYP4G36 are highly expressed in late pupal and adult stages supporting their importance in terrestrial life stage. CYP4G15 was highly expressed in olfactory tissues while CYP4G36 had ubiquitous expression. Transcript knockdown of CYP4G15, not CYP4G36, resulted in host avoidance suggesting different functions of these genes. This study provides a foundation for understanding of the functions of CYP4G subfamily members in Ae. aegypti. In contribution to basic scientific knowledge, strategies for functionally characterization of CYP4G genes in Ae. aegypti may facilitate the novel target discovery for a new insecticide.

NEUROENDOCRINE MODULATION OF DESSICATION AND COLD STRESS IN *DROSOPHILA.*

[1,4]Zandawala, M., [2]Nguyen, T., [3]Segura, M., [1]Johard, H., [1]Amcoff, M., [3]Wegener, C., [2]Paluzzi, J-P., [1]Nässel, D.

[1]Department of Zoology, Stockholm University, Stockholm, Sweden.
[2]Department of Biology, York University, Toronto, ON, Canada.
[3]Neurobiology and Genetics, Wurzburg Insect Research, Theodor-Boveri-Institute, Biocenter, University of Wurzburg, Germany.
[4]Department of Neuroscience, Brown University, Providence, USA.

Environmental factors challenge the physiological homeostasis in animals, thereby evoking stress responses. Various mechanisms have evolved to counter stress at the organism level, including regulation by neuropeptides. Although much progress has been made on the mechanisms and neuropeptides influencing nutritional stress, relatively little is known about the factors and pathways regulating osmotic and ionic stresses. Here, we uncover the neuropeptide Corazonin (Crz) as a neuroendocrine factor that modulates the release of an osmoregulatory peptide, CAPA, to regulate tolerance to osmotic and ionic stress. Both knockdown of *Crz* and acute injections of Crz peptide impact desiccation tolerance and recovery from chill-coma. Comprehensive mapping of the Crz receptor (*CrzR*) expression identified three pairs of *Capa*-expressing neurons (Va neurons) in the ventral nerve cord that mediate these effects of Crz. We further show that Crz is released during dry starvation (desiccation) and acts to restore homeostasis by inhibiting CAPA release via inhibition of cAMP production in Va neurons. Finally, knockdown of *CrzR* in Va neurons also affects CAPA release, and consequently influences desiccation tolerance and chill-coma recovery, considered proxies for diuretic state. Thus, Crz modulates Va neurons to maintain osmotic and ionic homeostasis, which in turn influences stress tolerance. Taken together with our previous work showing that systemic Crz signaling acts to restore nutrients levels by promoting food search and feeding, we propose that Crz signaling also ensures osmotic homeostasis by inhibiting the anti-diuretic CAPA peptides. Thus, Crz ameliorates stress-associated physiology through systemic modulation of both peptidergic neurosecretory cells and the fat body in *Drosophila*.

WHY ARE SOME MOSQUITOES INVASIVE?

Peach, D., Matthews, B.J.

Department of Zoology, University of British Columbia, Vancouver, BC, Canada.

Mosquitoes are the world's deadliest animals, due to the pathogens they spread. In addition, some species are highly successful invasive organisms, readily spreading between continents through anthropogenic transport. The ability to utilize diverse oviposition (egg-laying) sites and corresponding larval habitat are important characteristics that contribute to the likelihood of a mosquito becoming an invasive species. To study how oviposition behaviour contributes to invasiveness, I am taking a comparative approach using the coastal rock pool mosquito, Aedes togoi and the yellow fever mosquito Ae. aegypti. Ae. togoi breeds specifically in salty rock pools and is geographically restricted to coastal east Asia and the Pacific Northwest. Conversely, Ae. aegypti was once restricted to water-filled tree holes of sub-Saharan Africa but has now spread across the globe, using a wide range of habitats including water-filled containers associated with human habitation. There is substantial inter- and intraspecific variation in oviposition behaviour within each of these species, including evidence of some overlap in oviposition preference (i.e. Ae. aegypti using salt-water and Ae. togoi using containers). I am taking a multidisciplinary approach to compare and contrast the oviposition behaviour and larval habitats of these two mosquitoes and to identify the genetic pathways that influence oviposition behaviours. Ultimately, this work may help us understand how some species become widespread and invasive while others do not, allowing us to predict what mosquitoes might be future invaders and prepare for the pathogens that they might bring.

PREDICTING PHYSIOLOGICAL FUNCTIONS OF NEUROPEPTIDE F SIGNALING IN THE TOBACCO HORNWORM, *MANDUCA SEXTA.*

Kaur, G., Pooraiiouby, R., Tillett, R., Peterit, J., Nuss, A.B.

Department of Agriculture, Veterinary and Rangeland Sciences, University of Nevada, Reno, NV, USA.

Neuropeptides play a crucial role in regulating arthropod physiological processes. The most intensively studied are neuropeptides in the nervous system, but peptides released from insect midgut endocrine cells in paracrine or hormonal fashion also regulate diverse metabolic and behavior responses. Neuropeptide F (NPF) is the invertebrate homolog of vertebrate neuropeptide Y (NPY), and regulates a wide range of behaviors in insects, such as feeding and stress response. Much of the current knowledge on functions of NPF comes from *Drosophila melanogaster* studies of the nervous system yet there is much to be explored in other insect orders. For instance, in the corn earworm, *Helicoverpa zea*, NPF secreted from the midgut contributed to high hemolymph titers during the active feeding stage of caterpillars. We therefore chose to characterize NPF1 in the tobacco hornworm, *Manduca sexta*, a classic model organism for insect physiology. Immunohistochemistry was performed to detect tissue distribution of NPF1 in midgut and central nervous system of caterpillars. Comparative transcriptomics (RNA-seq) was conducted to detect differential transcript regulation in NPF1-injected versus saline-injected 4th instar caterpillars. We hypothesize that differential gene expression in response to NPF1 in the hemolymph will provide information about the downstream physiological processes induced by this signaling pathway. Results from this study will provide a foundation for understanding the functions of NPF in lepidopterans and aid in planning strategies for targeting the NPF signaling for insect pest management.

HOW TO FIND A MISSING PATHWAY: THE IMMUNE-DEFICIENCY (IMD) PATHWAY OF *RHODNIUS PROLIXUS.*

Salcedo-Porras, N., Lowenberger, C.

Centre for Cell Biology, Development and Disease, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada.

The immune system in insects is entirely innate and relies on a few well-characterized signaling pathways to control pathogens. These immune pathways were originally identified in holometabolous insects and have been reported in most insects. The hemimetabolous insect, *Rhodnius prolixus*, is a major vector of *Trypanosoma cruzi,* a protozoan that causes Chagas disease and kills 10,000 people annually. The analysis of the *R. prolixus* genome revealed that one of the main immune pathways, the Immune-deficiency pathway (IMD), was incomplete and presumed to be inactive. Silencing some of the few reported genes of this pathway, however, suggests that it is indeed active. Furthermore, the IMD-pathway in other hemimetabolous insects such as the pea aphid (*Acyrthosiphon pisum*) and the bedbug (*Cimex lectularius*) only lacks a few genes in contrast with the almost-inexistent IMD-pathway of *R. prolixus*. We proposed, based on phylogenetic analyses of individual genes of the IMD-pathway, that most of these genes are indeed present in *R. prolixus*. To test this hypothesis, we used bioinformatic analyses based on reciprocal BLAST and HMM-profile searches to search for the "missing" orthologs. We detected candidate genes for almost all the unreported IMD-pathway genes in *R. prolixus*; we recovered a wider repertoire of immune receptors and an almost complete intracellular signaling pathway. In addition, we used RNAi strategies to validate the functional role of some of these genes and determine if the IMD-pathway regulates the expression of antimicrobial peptides (AMPs). Our results indicate that the IMD-pathway regulates different AMPs in *R. prolixus* and that this pathway is triggered principally by Gram-negative bacteria, opening new avenues of research on *R. prolixus*-*T. cruzi* interactions.

ERYTHRITOL BASED SWEETENER SHORTENS THE LIFESPAN OF *AEDES AEGYPTI* POTENTIALLY BY N-LINKED PROTEIN GLYCOSYLATION.

[1]Sharma, A., [1]Reyes, J., [2]Nuss, A., [1]Gulia-Nuss, M.

[1]Department of Biochemistry and Molecular Biology, University of Nevada, Reno.
[2]Department of Agriculture, Veterinary & Rangeland Sciences, University of Nevada, Reno

Adult male and female mosquitoes consume sugar as floral and extrafloral nectar. Earlier work demonstrated that mosquito populations and their vector potential are dependent upon the availability of sugar sources. Thus, a novel method of vector control may involve targeting sugar-feeding mosquitoes. Multiple human-safe sugar substitutes are already approved by the U.S. Food and Drug Administration and are readily available. However, plant-based sugar substitutes such as stevia (erythritol) have been shown to affect lifespan in other flies. Therefore, the current study was carried out to test the potential of commercially available sugar substitutes to adversely affect the survival, fecundity, and metabolism of adult *Aedes aegypti* mosquitoes. Of the four sugar substitutes tested, erythritol (Stevia), sucralose (Splenda), aspartame (Equal), and saccharin (Sweet'N Low), only erythritol negatively affected mosquito longevity and fecundity. The effect on fecundity was probably due in part to a corresponding decrease in glycogen and lipid levels over time in mosquitoes fed on erythritol. Comparative mosquito head transcriptomes indicated upregulation of a gene in the mannose biosynthesis pathway in females fed on erythritol, suggesting that N-linked glycosylation might be responsible for the negative impact of erythritol feeding in mosquitoes. Mosquitoes preferred sucrose when a choice was given but were not averse to erythritol. Our results suggest the possibility of using erythritol alone or in combination with sucrose as a component of attractive toxic sugar baits for a human-safe approach for mosquito control.

PLASTICITY OF COLD-HARDINESS IN THE EASTERN SPRUCE BUDWORM.

[1]Butterson, S., [2]Roe, A.D., [1]Marshall, E.

[1]Department of Zoology, University of British Columbia, Vancouver, BC, Canada.
[2]Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, ON, Canada.

Of all abiotic factors that drive range boundaries, temperature is the best studied because of its pervasive influence on biological processes. For populations at high-latitudes, extreme cold and the populations' cold-hardiness set the range boundary. Local adaptation in phenotypic plasticity, which results in different responses in different populations, can assist in differences in temperatures across a species range and especially at range boundaries. We used the eastern spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae) as a model system for exploring local adaptation and phenotypic plasticity of insect cold-hardiness. The species is one of the most destructive forest pests in North America, therefore accurately predicting its range and population growth is essential for management. We show evidence for both local adaptation of seasonal and short-term plasticity cold-hardiness. Therefore, providing further evidence for the inclusion of phenotypic plasticity and local adaptation when modelling species distributions under climate change.

AMMONIUM TRANSPORTER EXPRESSION IN SPERM OF THE DISEASE-VECTOR *AEDES AEGYPTI* MOSQUITO INFLUENCES MALE FERTILITY.

Durant, A.C, Donini, A.

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

The ammonium transporter (AMT)/methylammonium permease (MEP)/Rhesus glycoprotein (Rh) family of ammonia (NH_3/NH_4^+) transporters have been identified in organisms from all domains of life. In animals, fundamental roles for AMT and Rh proteins in the specific transport of ammonia across biological membranes to mitigate ammonia toxicity and aiding in osmoregulation, acid-base balance, and excretion, has been well documented. Here, we observed enriched *Amt* (*AeAmt1*) mRNA levels within reproductive organs of the disease vector mosquito, *Aedes aegypti* prompting us to explore the role of AMTs in reproduction. We show that AeAmt1 is localized to sperm flagella during all stages of spermiogenesis and spermatogenesis in male testes. AeAmt1 expression in sperm flagella persists in spermatozoa that navigate the female reproductive tract following insemination and are stored within the spermathecae, as well as throughout sperm migration along the spermathecal ducts during ovulation to fertilize the descending egg. We demonstrate that RNAi-mediated AeAmt1 protein knockdown leads to significant reductions (~40%) of spermatozoa stored in seminal vesicles of males, resulting in decreased egg viability when these males inseminate non-mated females. Similar perturbations in egg viability occurred when female *A. aegypti* were inseminated by wild-type males prior to RNAi-mediated AeAmt1-knockdown. We suggest that AeAmt1 function in spermatozoa is to protect against ammonia toxicity, based on our observations of high NH_4^+ levels in the densely packed spermathecae of mated females. The presence of AMT proteins, in addition to Rh proteins, across insect taxa may indicate a conserved function for AMTs in sperm viability and reproduction, in general.