5th International Symposium on the Environmental Physiology of Ectotherms and Plants

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SABLE SYSTEMS INTERNATIONAL

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Physiological and Biochemical Zoology

Contents

Welcome to ISEPEP5!

ISEPEP5 is the latest in a succession of ISEPEP meetings and their predecessors, dating back to the 1980s. Held every two years, we benefit from an inclusive approach to taxa, methods and questions; a truly international group of participants and a welcoming and social atmosphere. The simple fact that this series of meetings has existed for three decades, without any formal management or societal structure, is testament to the enthusiasm that the participants bring to ISEPEP, and the meeting has been the seed for innumerable international scientific collaborations. It is a pleasure to host the 2013 edition at Western, and I look forward to a week of new ideas, unexpected syntheses and new collaborations. I am delighted to especially welcome graduate students and postdocs to the meeting – I hope that you will find the meeting as valuable and fulfilling as I did when I was first welcomed into this community.

I wish to thank the sponsors of the meeting – particularly the *Journal of Experimental Biology*, Sable Systems International and Western University for extremely generous material support, as well as the minor sponsors whose contributions you will see acknowledged throughout the meeting. I am very grateful to Melanie Harvey and Patty Scheerer of Western's Conference Services for their excellent and responsive help and experience behind the scenes. Finally, my lab have provided outstanding support – I have been wonderfully supported by their enthusiasm, attention to detail and creativity in solving the myriad small problems that come up when organizing such an event, so thanks to Hiroko Udaka, Laura Ferguson, Lauren Des Marteaux, Heath MacMillan, Annegret Nicolai, Golnaz Salehipour, David Li, and Ruth Jakobs.

I wish you all a fun, stimulating and creative meeting, and an enjoyable time in London, Ontario and at Western University.

Dr. Brent J. Sinclair

General information

Emergency contact numbers

- **Call 911 in general emergency** (Police, fire and ambulance)
- **519-661-3300** (Campus Community Police Service: Non-emergencies or inquiries, open 24h)
- University Hospital is located at 339 Windermere Road, about 15 minutes walk from Elgin Hall.
- Conference Service: Room 150, Lambton Hall, Tel: 519 661-3545

Accommodations

Elgin Hall
1151 Richmond Street, London, Ontario, Canada N6A 5B9
Tel: 519 661-3476 or 1 888 661-3545 (toll free)

The Windermere Manor
200 Collip Circle**,** London, Ontario, Canada N6G 4X8
Tel: 519-858-1414 or 1-800-997-4477 (toll free)

Ivey Spencer Leadership Centre
551 Windermere Road, London, Ontario, Canada N5X 2T1
Tel: 519-679-4547 or 1-800-834-7410

Hotel Metro
32 Covent Market Place, London, Ontario, Canada N6A 1E8
Tel: 519-518-9000 or 1-866-626-3876

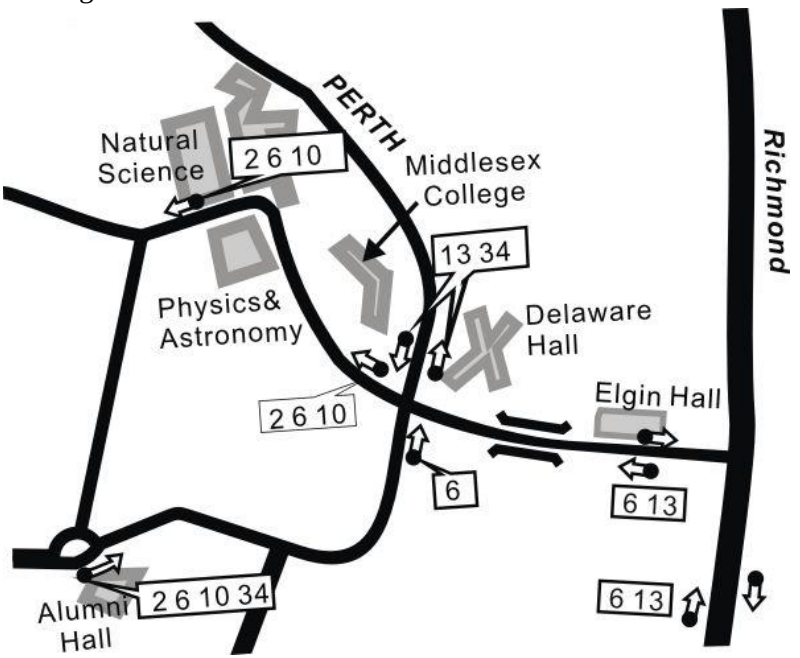
Hilton London Ontario
300 King Street, London, Ontario, Canada N6B 1S2
Tel: 519-439-1661

Delta London Armouries Hotel
325 Dundas Street, London, Ontario, Canada N6B 1T9
Tel: 519-679-6111 or 1-800-668-9999

Station Park All Suite Hotel
242 Pall Mall Street, London, Ontario, Canada N6A 5P6
Tel: 519-642-4444 or 1-800-561-4574 Ext. 0

Local transportation

Bus: LTC (London Transit Commission) controls the bus service in London and schedules and for a complete London map with the bus routes go to www.ltconline.ca



To go to downtown (10-15min)

In front of Natural Science / Alumni Hall, 2 (Dundas), 6 (Richmond)
Delaware Hall / University Hospital, 13 (Wellington)

To Masonville shopping mall (~10 min)

Delaware Hall / University Hospital, 10 (Wonderland), 13, 34 (Medway), Alumni Hall, 10, 34

Taxi: Fare from University campus to downtown is approximately $ 10-12 (+ tips, usually 10 to 15 percent of the price charged) and a taxi from London Airport to University takes about 20 min and costs ~$35. Credit card is available.

Aboutown Taxi Service	519-432-2222
Checker Limousine	519-659-0400
U-Need-A Taxi Service	519-438-2121
Yellow London Taxi	519-657-1111

Parking

Parking for participants is complimentary in the parking lot behind Elgin Hall (Medway Lot, University Dr.)

Banks and banking machines

Bank close to campus

TD Canada Trust 1137 Richmond St. (near Elgin Hall)

Machines on campus

UCC main floor (TD, Bank of Montreal, Royal Bank)
UCC lower level (CIBC, Bank of Montreal, Scotiabank)
Natural Science Centre, main floor (Royal Bank)

Automatic Teller Machines (ATMs)

Elgin Hall (Residence), Althouse college, Spencer Engineering Building, Law School, Somerville House, Talbot College, Medical Science, Natural Sciences, UCC, Thompson Recreation centre, Social Science Building (Food services locations)

Restaurants

On campus

The Grad club (lower level, Middlesex College)
The Wave (2nd floor, UCC)
Spoke (main floor of UCC)

Off Campus

There are many restaurants downtown (Bus routes 2 and 6) and along the following bus stops: "Dundas Street/Talbot Street", "Dundas Street/ Richmond Street" (Route 2) and between "Richmond Street/Oxford" and "Richmond/ Dundas" (Route 6).

Conference information

Presentation

Talks

All talks will be held in Middlesex College, Room 110

Poster presentations

Tuesday, 13th August, the Physics and Astronomy Atrium

The presenters are able to put posters up at the coffee breaks on Monday.

Internet

If you will stay at Elgin Hall during the conference, user name and passwords are provided at front desk and username and passwords are provided at the registration desk for participants not staying in residence.

Social events

Registration

Sunday, August 11th 5-7pm, the Grad Club (Middlesex College**)**

Monday, August 12th 8am - 10:30 am, (Middlesex College, TA106)

Icebreaker

Sunday, August 11th -5-7pm, the Grad Club (Middlesex College**)**

Reception

Monday, August 12th 5-7pm, the Grad Club (Middlesex College**)**

Excursion

Wednesday, August 14th 7:45 am, Meeting place: Elgin Hall (Lobby) (University Dr), expected returning time is 8 pm.

Banquet

Thursday, August 15th, Windermere Manor (200 Collip Circle). Shuttle van is available from Elgin Hall.

5 pm- Drinks

6:30 pm- Dinner

Detailed Programme

All talks will be held in Middlesex College Room 110

Sunday, August 11th	
5:00 – 7:00 pm	**Registration & Ice Break** [Grad Club/lower level, Middlesex College]

Monday, August 12th	
8:00 – 10:30 am	**Registration** [Middlesex College, TA106]
9:00 – 9:30 am	Early Refreshments [Middlesex College, TA104]
9:30 – 10:00 am	Opening remarks [Middlesex College, Room110]
Session 1: Plenary talk I	
10:00 – 11:00 am	**David Denlinger** Shutting down for the winter: an insect perspective
11:00 – 11:30 am	Coffee Break [Physics & Astronomy Atrium]
Session 2: Evolutionary Physiology ***Chair*** **Jesper Sørensen**	
11:30 – 11:45 am	**Andrew Rosendale** Hibernation physiology, freezing adaptation, and extreme freeze tolerance in a northern population of wood frog
11:45 – 12:00 pm	**Caroline Williams** Maintenance of metabolic networks during cold stress in cold-adapted *Drosophila melanogaster*.
12:00 – 12:15 pm	**Evan Pacey** Increasing genome size enhances desiccation tolerance in ectotherms and plants
12:15 – 12:30 pm	**Alison Egge** Genetics of short-term and long-term acclimation in *Drosophila melanogaster*
12:30 – 2:00 pm	Lunch [Physics & Astronomy Atrium]

Session 3: Sinclair lab extravaganza	
2:00 – 3:15 pm	Sinclair lab extravaganza
3:15 – 3:45 pm	Coffee Break [Physics & Astronomy Atrium]
Session 4: Brief Presentations ***Chair* Scott Hayward**	
3:45 – 4:30 pm	**Tomáš Ditrich** Heritability of SCP in *Microvelia reticulata*
	Fengliang Jin An integrated analysis of miRNA and mRNA related to immune response of *Plutella xylostella* to pesticide chlorantraniliprole
	Tanya Dann Physiological responses underpinning fast/slow life histories and species interactions.
	Xiaoxia Xu Characterization of antibacterial peptide genes from the diamond back moth and their expression in response to fungal and destruxin-A challenge
5:00 – 7:00 pm	**Reception** [Grad Club, Middlesex College]

Tuesday, August 13th	
9:00 – 9:30 am	Early Refreshments [Middlesex College, TA104]
Session 5: Insect Environmental Physiology ***Chair* Dan Hahn**	
9:30 – 9:45 am	**Elena Lopatina** Thermal reaction norms for development in ants: inter- vs. intraspecific variation
9:45 – 10:00 am	**Claire Hemmer-Brepson** Soma vs. Germ line: protection of the offspring at all costs in warmer environment
10:00 – 10:15 am	**Qian Long** Oxygen exchange in galls of *Eurosta solidaginis* (Diptera: Tephritidae)

10:15 – 10:30 am	**Nicholas Levis** Mild desiccation rapidly increases freeze tolerance of the goldenrod gall fly, *Eurosta solidaginis*: evidence for drought-induced RCH
10:30 – 10:45 am	**Elizabeth Dahlhoff** Adaptation to thermal stress in a Finnish butterfly threatened by climate change
10:45 – 11:15 am	Coffee Break [Physics & Astronomy Atrium]
Session 6: Water and Ion Balance ***Chair* Heath MacMillan**	
11:15 – 11:30 am	**Kévin Hidalgo** Ecophysiological plasticity assisting dry-season survival in the malarial mosquitoes *Anopheles gambiae* s.l.
11:30 – 11:45 am	**David Wharton** Ionic regulation in the Antarctic nematode, *Panagrolaimus davidi*
11:45 – 12:00 pm	**Martin Holmstrup** Drought tolerance of Collembola revisited: are "hygrophilic" species really restricted to high humidity soils?
12:00 – 12:15 pm	**Richard Cornette** Understanding the evolution of anhydrobiosis in the sleeping Chironomid, *Polypedilum vanderplanki*, by comparative genomics
12:15 – 12:30 pm	**Hang Nguyen** Insect Malpighian tubules: a comparative look at their structure, function and regulation
12:30 – 2:00 pm	Lunch [Physics & Astronomy Atrium]
Session 7: Molecular Physiology I ***Chair* Elizabeth Dahlhoff**	
2:00 – 2:15 pm	**Jantina Toxopeus** Functional differentiation of small heat shock proteins in diapause embryos of *Artemia franciscana*
2:15 – 2:30 pm	**Jose-Luis Martínez-Guitarte** *Chironomus riparius* telomeric transcriptome and its response to heat shock
2:30 – 2:45 pm	**Jesper Sørensen** The effects of developmental thermal environment on the transcriptome and heat knock down tolerance phenotype in *Drosophila melanogaster*

2:45 – 3:00 pm	**Jantina Toxopeus** The role of Late Embryogenesis Abundant (LEA) proteins in a stress-tolerant crustacean
3:00 – 3:30 pm	Coffee Break [Physics & Astronomy Atrium]
Session 8: Molecular Physiology II ***Chair* Greg Ragland**	
3:30 – 3:45 pm	**Scott Hayward** Metabolomic and lipidomic characterization of diapause in the blue bottle fly *Calliphora vicina*
3:45 – 4:00 pm	**Alexander McKinnon** Examining the genetic components implicated in maintaining inducible defences in *Daphnia lumholtzi.*
4:00 – 4:15 pm	**Johannes Johnsen** Localisation of antifreeze proteins in *Rhagium mordax* by immunofluorescence
4:15 – 4:30 pm	**Craig Marshall** Functional significance of post translational modifications to *Tenebrio* THP
4:30 pm	**Group photo**
5:00 – 8:00 pm	**Poster Session** [Physics & Astronomy Atrium]

Wednesday, August 14th	
7:45 am – 8:00 pm	**Conference Excursion** Niagara Falls Meet at Elgin Hall

Thursday, August 15th	
9:00 – 9:30 am	Early Refreshments [Middlesex College, TA104]
Session 9: Diapause ***Chair* Katie Marshall**	
9:30 – 9:45 am	**Shin Goto** Locomotor activity rhythm and circadian clock gene expression in the Antarctic midge *Belgica antarctica*
9:45 – 10:00 am	**Greg Ragland** In Synch: Genomic change associated with the evolution of seasonal synchrony via adaptation across the life cycle
10:00 – 10:15 am	**Kazuhiro Tanaka** Why the onion fly avoids daytime adult eclosion?
10:15 – 10:30 am	**George Yocum** The long winter: the synergy between fluctuating thermal regime and quiescence physiology in the pollinator, *Megachile rotundata*
10:30 – 10:45 am	**Daniel Hahn** Repeated anoxia exposures during the immature stages have hormetic effects that extend into adulthood
10:45 – 11:15 am	Coffee Break [Physics & Astronomy Atrium]
Session 10: Insect Cold Tolerance ***Chair* Hiroko Udaka**	
11:15 – 11:30 am	**Emily Owen** Can winter-active bumblebees survive the cold? Assessing *Bombus terrestris audax* cold tolerance and the effects of pollen feeding
11:30 – 11:45 am	**Natalia Li** Cold adaptation of insects inhabiting East Siberia, Russia: from physiology to practical perspectives
11:45 – 12:00 pm	**Oldřich Nedvěd** Delayed freezing events: distribution in time and relationship between SCP and LLT
12:00 – 12:15 pm	**Matthew Everatt** Life of a non-native midge in the maritime Antarctic
12:15 – 12:30 pm	**Karina Fisker** Roles of carbohydrate reserves for local adaptation to low temperatures in the freeze tolerant *Enchytraeus albidus*

12:30 – 2:00 pm	Lunch [Physics & Astronomy Atrium]
Session 11: Temperature and Performance ***Chair* Annegret Nicolai**	
2:00 – 2:15 pm	**Dmitry Kutcherov** Geographic variation and environmental plasticity of growth and development in the Colorado potato beetle
2:15 – 2:30 pm	**Megan Coombs** The thermal biology and thresholds of the spider mite predator *Phytoseiulus macropilis*
2:30 – 2:45 pm	**Sergey Balashov** Effects of some internal and external factors on growth of the linden bug *Pyrrhocoris apterus*
2:45 – 3:00 pm	**Jonas Andersen** How to measure *Drosophila* cold tolerance: Chill coma temperature predicts distribution and tolerance limits
3:00 – 3:30 pm	Coffee Break [Physics & Astronomy Atrium]
Session 12: Mechanisms Underlying Cold Tolerance ***Chair* Caroline Williams**	
3:30 – 3:45 pm	**Nicola White** The importance of sex, developmental stage, acclimation and using different indices of cold tolerance: a case study with *Tetranychus urticae*
3:45 – 4:00 pm	**Johannes Overgaard** Why do insects enter and recover from chill coma? Low temperature and high extracellular potassium compromises muscle function in *Locusta migratoria*
4:00 – 4:15 pm	**Vladimír Koštál** On the roles of so far neglected structural components of biological membranes–lyso-phospholipids, sterols and tocopherols–in insect overwintering
4:15 – 4:30 pm	**Stine Slotsbo** Cold tolerances of drosophilids correlate with membrane phospholipid fatty acid composition
5:00 pm –	**Drinks & Banquet** [Windermere Manor] 5 pm- Drinks, 6:30 pm-Dinner

Friday, August 16th	
9:30 – 10:15 am	Early Refreshments [Middlesex College, TA104]
10:15 – 10:45 am	Business meeting (ISEPEP 6&7, Prizes for the best student presentation)
Session 13: Plenary Talk II	
10:45 – 11:45 am	**Allison McDonald** Alternative oxidase: respiratory flexibility of mitochondria in eukaryotes in response to environmental stresses
11:45 – 12:00 pm	Farewell
12:00 – 1:00 pm	Lunch [Physics & Astronomy Atrium]

Talks

David L. Denlinger has broad interests in insect dormancy, stress tolerance and photoperiodism. After completing his Ph.D. from the University of Illinois, he worked in the Netherlands, Kenya, and at Harvard before joining the faculty of Ohio State University, where he currently holds the rank of Distinguished University Professor.

Shutting down for the winter: an insect perspective

David L. Denlinger
Departments of Entomology and Evolution, Ecology and Organismal Biology, Ohio State University

The shortening days and lower temperatures of late summer and early autumn are widely used as environmental cues to program an overwintering developmental arrest (diapause) in insects. This presentation will offer a quick overview of insect diapause and then focus on recent work implicating the insulin signaling pathway in coordinating diapause in mosquitoes and other invertebrates. Insulin signaling and the downstream transcription factor FOXO appear to be key elements in generating enhanced stress resistance, increased longevity, accumulation of fat reserves, and other features of the diapause phenotype. Though several themes are common to diverse forms of dormancy, there are relatively few shared "diapause transcripts", suggesting that diapause has arisen independently numerous times in the insect world.

Dr. Allison McDonald is an Assistant Professor in the Department of Biology at Wilfrid Laurier University in Waterloo, Ontario. She is a physiologist and molecular biologist with expertise in respiratory electron transport systems and investigates the physiological role(s) of the enzyme alternative oxidase (AOX) in the acclimation of organisms to environmental stresses. She employs a comparative and integrative approach in her work in order to explore this enzyme in bacteria, plants, yeast, and animals.

Alternative oxidase: respiratory flexibility of mitochondria in eukaryotes in response to environmental stresses

Allison McDonald
Department of Biology, Wilfrid Laurier University, Waterloo, Ontario

Biological electron transport chains (ETCs) power life on this planet. In most eukaryotic organisms, ETCs in mitochondria generate energy via oxidative phosphorylation. Typically, linear ETCs are depicted in textbooks, however this is misleading, as most organisms have additional protein complexes that increase the points of entry and/or exit of electrons. My research program focuses on the alternative oxidase (AOX), a terminal oxidase that introduces a branch-point at the level of ubiquinol in the respiratory ETC. Our journey has involved crossing biological kingdom boundaries by working on bacteria, fungi, plants, protists, and animals, and examining different aspects of AOX at the level of ecosystems all the way down to molecules. An underlying hypothesis is that flexibility in the respiratory ETC allows for the utilization of a wide variety of metabolic substrates to generate energy and permits organisms to respond effectively to limitations placed on respiration by environmental stress.

Hibernation physiology, freezing adaptation, and extreme freeze tolerance in a northern population of wood frog

Costanzo, Jon P.; do Amaral, M. Clara F.; Rosendale, Andrew J.; Lee, Jr., Richard E.
Laboratory for Ecophysiological Cryobiology, Department of Zoology, Miami University, Oxford, OH 45056 USA

We investigated hibernation physiology and freeze tolerance in a population of the wood frog, *Rana sylvatica*, indigenous to Interior Alaska, near the northernmost limit of the species' range. Winter acclimatization included a 233% increase in the hepatic glycogen depot that was subsidized by catabolism of fat body and skeletal muscle, and accrual of urea and an unidentified solute. In contrast, frogs from a cool-temperate population (southern Ohio) amassed much less glycogen, had a lower uremia, and lacked the unidentified solute. Alaskan frogs survived freezing at –16°C, some 10-13°C below temperatures tolerated by southern conspecifics, and endured a two-month bout of freezing at –4°C. Profound freeze tolerance was associated with high levels of urea and glucose, a comparatively high proportion of bound water, and limited cryoinjury. Post-glacial colonization of high latitudes by *R. sylvatica* required a substantial increase in freeze tolerance that was achieved by enhancing their cryoprotectant system.

Maintenance of metabolic networks during cold stress in cold-adapted *Drosophila melanogaster*.

Williams, Caroline M.[1]; Watanabe, Miki[2]; Guarracino, Mario R.[3]; Edison, Arthur S.[1]; Morgan, Theodore J.[4]; Boroujerdi, Arezue[2]; Hahn, Daniel A.[1]

[1]University of Florida; [2]Claflin University; [3]High Performance Computing and Networking Institute Naples; [4]Kansas State University

Low temperatures induce in insects a state of paralysis (chill coma), and the time taken to recover (CCR) varies widely both inter- and intra-specifically although the underlying mechanisms are largely unknown. We are using laboratory selected lines of tolerant and susceptible *Drosophila melanogaster* to investigate the hypothesis that variation in CCR results from an enhanced ability to maintain metabolic homeostasis in the cold. Nuclear magnetic resonance spectroscopy showed that the metabolomes of tolerant and susceptible flies were strongly differentiated, with striking alterations to phosphorylcholine, tyrosine, ATP, and tryptophan metabolites. Tolerant flies experienced fewer alterations to metabolite concentrations during cold exposure, and didn't experience the marked drop in many metabolites during recovery that was seen in susceptible flies. Metabolic networks were more highly connected in tolerant flies. Thus, it appears that selection for cold tolerance alters the regulation of metabolic networks and enhances the maintenance of metabolic homeostasis during cold exposure.

Session 2/Environmental Physiology

Increasing genome size enhances desiccation tolerance in ectotherms and plants

Pacey, Evan K.
McMaster University

Desiccating environments are hazardous and often influence the physiology of organisms that experience them on a recurring basis. Desiccation tolerant species are known for having phenotypic adaptations to prevent water loss such as mucus secretion and curled leaves, yet fewer adaptations have been observed at the genomic level. On comparing the genome sizes of desiccation tolerant species it was observed that a common adaptation to desiccation is to increase genome size. As genome size increases cell size consequently increases leading to an overall larger cytoplasmic/water volume. Moreover, as overall cell size increases its surface area to volume ratio decreases giving the desiccation tolerant cell a lower rate of water loss than that of a smaller cell. Desiccation tolerant species such as salamanders, lungfish and resurrection plants will be discussed.

Genetics of short-term and long-term acclimation in *Drosophila melanogaster*

Egge, Alison R.[1]; Hahn, Daniel A.[2]; Morgan, Theodore J.[1]
[1]Kansas State University; [2]University of Florida

Daily and seasonal variation requires organisms to respond to thermal stresses on both the long- and short-term time scale. Capacities associated with long- and short-term thermal plasticity may evolve independently or may be constrained. To test the hypothesis of evolutionary genetic constraints of long- and short-term acclimation, we used the *Drosophila* Genetic Reference Panel to measure cold stress survivorship after chronic, acute, and rapid cold hardening pretreatments. Genetic analyses of single nucleotide polymorphisms indicate that unique gene sets are involved in long- and short-term acclimation, suggesting some evolutionary independence for long- and short-term acclimation. Correlation analyses indicate that basal thermal tolerance and plasticity have a significant trade-off (negative relationship) for long-term hardening alone and long-term + short-term hardening, suggestive of constraint in the evolvability of basal thermal tolerance and plasticity ability. These findings suggest that organismal response to thermal stresses may involve alternative genetic pathways but may be physiologically constrained.

➡ Lunch

Repeated low temperature stress elicits distinct responses at multiple levels of biological organization in the eastern spruce budworm *Choristoneura fumiferana*

Marshall, Katie E.; Sinclair, Brent J.
Department of Biology, Western University, London, ON, Canada

The eastern spruce budworm (*Choristoneura fumiferana*) is one of the most important natural pests of the Canadian boreal forest, and its cold hardiness has received significant attention. However, these studies only examined the effects of a single cold exposure. Since *C. fumiferana* overwinters in exposed habitats and experience repeated low temperatures, we investigated whether the number of cold exposures impacts the overwintering energetics and long-term success of *C. fumiferana*. Despite controlling for total exposure duration, we found that repeated low temperature exposures significantly decreased survival to eclosion. We also found this reduced survival was associated with increased cryoprotectant content at a cost to glycogen reserves. This response was mirrored at the transcriptome level, with budworm that received repeated cold exposures differentially regulating significantly more transcripts than a single cold exposure matched for time. Taken together, this suggests that responses to repeated cold exposure are distinct, and likely more ecologically-relevant.

Can aliens handle Ontario's coolness? - Overwintering biology of *Drosophila suzukii*

Jakobs, Ruth[1]; Gariepy, Tara D.[2]; Sinclair, Brent J.[1]
[1]Department of Biology, Western University, London, ON, Canada;
[2]Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, Canada

Spotted wing Drosophila (SWD, *Drosophila suzukii*) is native to Southeast Asia and was introduced to Ontario in 2010. SWD is a significant agricultural pest because it can lay eggs in healthy fruit. SWD overwinters as adults and their potential to establish in Ontario depends on its ability to survive the cold, desiccating, and energetic stresses of winter. I will use colonies of *D. suzukii* derived from individuals collected in Ontario and British Columbia to study stress tolerance in relation to overwintering of adults. I will measure cold tolerance and its plasticity over short and long timescales, as well as desiccation and starvation tolerance. My goal is to delimit thermal, desiccation, and starvation tolerance of adult flies to determine whether SWD has the potential to establish as a significant agricultural pest in Ontario, and inform pest management strategies for this species.

Phenotypic plasticity and evolution of *Drosophila* cold tolerance are associated with modulation of Na+ and K+ homeostasis

MacMillan, Heath A.[1]; Ferguson, Laura V.[1]; Nicolai, Annegret[1]; Staples, James F.[1]; Donini, Andrew[2]; Sinclair, Brent J.[1]
[1]Department of Biology, Western University, London, ON, Canada;
[2]Department of Biology, York University, Toronto, ON, Canada

The onset and recovery of chill-coma and progression of cold-induced injury in insects are associated with a loss of extracellular ion homeostasis. We use 24 species of *Drosophila* to test the hypothesis that phenotypic plasticity and species-level variation in cold tolerance are associated with modulation of hemolymph Na^+ and K^+ concentrations. Cold-acclimated *D. melanogaster* and cold tolerant species had reduced hemolymph [Na^+] and [K^+]. These changes in ion homeostasis are associated with low Na^+/K^+-ATPase activity on a whole-organism level. On the species level, the relationships between hemolymph ion concentrations and cold tolerance hold after phylogenetically-independent contrasts, and are consistent with data from crickets, suggesting that modulation of ion concentration may be generally associated with insect cold tolerance.

Session 3/Sinclair lab extravaganza

Does epithelial transport limit insects at low temperatures?

Des Marteaux, Lauren; Sinclair, Brent J.
Department of Biology, Western University, London, ON, Canada

In temperate regions, insect performance is limited by the critical thermal minimum (CT_{min}), below which chill coma (paralysis) occurs. Chill coma is accompanied by ionic disturbance across the gut and Malpighian tubules (sites of ion homeostasis), leading to altered haemolymph ion composition and muscle depolarization. Temperature-dependent failure of epithelial ion regulation is hypothesized to explain chill coma onset, with variation in CT_{min} driven by epithelial channel modification. To verify whether epithelial transport failure corresponds with the CT_{min}, I will measure ion transport and channel function in the gut and Malpighian tubules using an Ussing chamber and Ramsay assay, respectively. I will identify patterns of ion channel expression associated with variation in CT_{min} via gut and Malpighian tubule transcriptome sequencing and qRT-PCR. The crickets *Gryllus pennsylvanicus* (cold-susceptible) and *G. veletis* (cold-tolerant) will be used as models.

High-throughput RNA sequencing reveals candidate genes associated with freeze tolerance in *Eurosta solidaginis*

Udaka, Hiroko[1]; Dennis, Alice[2]; Sinclair, Brent J.[1]
[1]Department of Biology, Western University, London, ON, Canada; [2]Landcare Research, Auckland, New Zealand

Temperature affects many physiological processes in insects, and insects in temperate and polar zones have developed overwintering strategies to survive sub-zero winter temperatures. Some insects cannot survive internal ice formation and the other ones can tolerant internal ice formation. The goldenrod gall fly, *Eurosta solidaginis*, overwinters as a prepupa and can survive both extra- and intra-cellular ice formation. The molecular mechanisms underlying this freeze tolerance are not well-understood. We used high-throughput RNA sequencing to identify genes associated with the response to freezing stress. *E. solidaginis* prepupae were frozen at -12 ºC for 2 h and samples were collected after the cold treatment. Sequences obtained from all sampling points and control individuals were used for de novo transcriptome assembly, and transcription profiles were compared among control and cold treated samples. These comparisons will allow us to identify candidate genes associated with freeze tolerance, and to generate novel hypotheses about the physiological mechanisms underlying insect freeze tolerance.

Novel genes underlying freeze tolerance in *Eurosta solidaginis* that are expressed only after freeze treatment

Li, Daye; Udaka, Hiroko; Sinclair, Brent J.
Department of Biology, Western University, London, ON, Canada

Eurosta solidaginis can survive exposure to temperatures as low as -80 °C by withstanding internal ice formation. While some biochemical mechanisms underlying these extraordinary tolerances are known, such as the accumulation of cryoprotectants and acetylated lipids, little is known about the regulatory and molecular mechanisms underlying freeze tolerance in this species. High-throughput RNA sequencing was used to assemble a de novo transcriptome of *E. solidaginis* that was then probed to explore changes in gene expression associated with freeze tolerance. The focus of the candidate genes is on genes that are expressed only after internal ice formation. This method of identifying candidate genes will be efficient at identifying novel genes directly linked to freeze tolerance because they are expressed only after freeze treatment. qPCR was used to further explore and verify the patterns of gene expression. This will allow us to identify novel genes associated with freeze tolerance and to identify enzymes associated with accumulation of acetylated lipids.

Catching the cold bug: eco-immunology of cold-exposed and overwintering insects

Ferguson, Laura V.; Sinclair, Brent J.
Department of Biology, Western University, London, ON, Canada

Cold stress induces a variety of physiological changes in insects and appears to also affect immune function: genes related to immunity are up-regulated and resistance to fungal pathogens is increased. However, it is unclear whether immune function is also up-regulated following the prolonged cold experienced by temperate insects during overwintering and what the adaptive significance of this might be. I exposed nymphs of the spring field cricket, *Gryllus veletis*, to an artificial winter in the laboratory and compared immune function of overwintering and summer crickets. I measured various immune parameters, including hemocyte numbers, phenoloxidase activity, antibacterial activity, and survival following exposure to entomopathogenic fungi. Although numbers of circulating hemocytes increased in winter crickets, there was no additional indication of enhanced immune activity. Cold-induced immune activation may only result from stressful, as opposed to acclimated, exposures to low temperatures and damage to gut tissue during such exposures may be the trigger of immune activity.

Session 3/Sinclair lab extravaganza

Does cold exposure activate the insect immune system?

Salehipour-shirazi, Golnaz; Sinclair, Brent J.
Department of Biology, Western University, London, ON, Canada

Because cold tolerance and immune up-regulation are energetically expensive, but mechanistically different, an energetic trade-off is expected to reduce an insect's immune response after cold exposure. However, evidence of immune system activation after cold suggests a cold-immunity cross-talk instead of a trade-off. To investigate the potential cross-talk between cold and immune system, we determined whether the immune response of *Drosophila melanogaster* is activated by acute and chronic cold exposure, and which pathways are activated. We cold-exposed flies, and then used qPCR to examine up-regulation of immune-related genes in the Toll, IMD and JAK/STAT pathways, as well as counting hemocytes and measuring phenoloxidase activity and melanin formation. Cold exposure increased hemocyte count and melanin formation but, decreased phenoloxidase activity; qPCR studies of other pathways are in progress. Data so far suggest that cold exposure does not activate all immune pathways equally.

Strategy switch of cold tolerance in the land snail *Cepaea nemoralis*

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Terrestrial ectotherms use one of two broad strategies to tolerate sub-zero temperatures: they either keep their body fluids liquid at temperatures below the melting point (freeze avoidance), or withstand internal ice formation (freeze tolerance). Some species are able to switch strategy, and there is more speculation than conclusion about why one strategy or another is advantageous. I have found that the invasive snail *Cepaea nemoralis* switches from freeze tolerance in the fall to freeze avoidance in the winter. The strategy switch seems to be initiated by hypoxia and related to clearance of ice-nucleating agents from the gut and accumulation of cryoprotectants resulting from anaerobic metabolism. Although the acute low temperature tolerance of both strategies is similar, freeze avoidant snails have better survival of prolonged cold exposure, suggesting that freeze tolerance allows the snails to be active and survive freeze-thaw in the fall, but that long term survival in winter is achieved via freeze avoidance.

➡ Coffee break

Session 4/Brief Presentation

Heritability of SCP in *Microvelia reticulate*

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Low winter temperatures affect survival of most insects in temperate zone during overwintering. Cold hardiness is thus an important physiological trait. Selection for cold tolerance in insects has been demonstrated for several times, but the relative importance of genetic variation and phenotypic plasticity of cold hardiness is under debate. We chose supercooling point (SCP) as an indicator of cold hardiness of *Microvelia reticulata* (Heteroptera: Veliidae), a species with large SCP variation and one of several semiaquatic bug species that are known to be freeze-avoidant with tight correlation between SCP and lower lethal temperature. We provide a quantitative estimate of heritability of SCP, based on half-sib analysis. Other relevant studies are almost exclusively limited to drosophilid species; our data thus provide a broader perspective on the issue of heritability and individual variability of cold hardiness. The study was supported by the project P505/2010/0096 of Grant Agency of the Czech Republic.

Session 4/Brief Presentation

An integrated analysis of miRNA and mRNA related to immune response of *Plutella xylostella* to pesticide chlorantraniliprol

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This study first reports the integrated analysis of mRNA and miRNA analysis of chlorantraniliprole-resistant *P. xylostella* using Solexa sequencing in an attempt to gain insights into the immune response of *P. xylostella* against the pesticides. The dose of chlorantraniliprole had significant impacts on expression levels of 728 identified insect host transcripts. Gene ontology data illustrated that the majority of the differentially expressed genes are involved in binding, catalytic activity, and metabolic and cellular processes. In addition, the results show that transcription levels of most immune-related genes had different expressional profile in different chlorantraniliprole-resistance DGE libraries. Small RNA libraries of the *P. xylostella* were built from the whole body of all development stages, 65 miRNAs were significantly down-regulated and only 9 miRNAs were up-regulated with the increase of chlorantraniliprole-resistance. The results will provide insights into their possible involvement in insect immune response to chlorantraniliprole and offer an important resource for further studies.

Session 4/Brief Presentation

Physiological responses underpinning fast/slow life histories and species interactions.

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Multiple species of damselfly coexist in the same habitats accessing the same resources; therefore, to successfully coexist they require different life history strategies. Experiments will be conducted to evalutate the relationship between physiological responses (i.e. metabolic rate, thermal sensitivity, starvation tolerance and behaviour) and life history strategies. Species with fast life-styles are predicted to have faster rate processes, greater thermal sensitivity and reduced starvation tolerance. Species with slow life-styles are predicted to have a slower metabolic rate to take advantage of low productivity habitats, thus avoiding competition with fast life-style species.

Session 4/Brief Presentation

Characterization of antibacterial peptide genes from the diamond back moth and their expression in response to fungal and destruxin-A challenge

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Based on the unigenes sequence obtained from the transcriptome of *Plutella xylostella*, ten complete cDNAs encoding putative antibacterial peptides (ABPs) were identified and characterized in *P. xylostella*. These ABPs include four lysozyme like proteins, three cecropin proteins, one moricin, one gloverin and one defensin. Temporal and spatial expression analysis showed that ABPs were highly expressed in fat body, except for one lysozyme in midgut, ABPs were expressed in larval, pupa and adult stages, but little or no detectable expression in egg stage. When larvae were challenged with entomopathogenic fungi (*Isaria fumosorosea* Wize), the expression of all ten genes was up-regulated in the fatbodies. However, when larvae were challenged with destruxin-A, the expression of ten ABPs genes were inhibited. This study provides additional insights into the expression of antibacterial response genes in *P. xylostella* larvae and helps us better understand the immune defense response against fungal infection in *P. xylostella*.

Thermal reaction norms for development in ants: inter- vs. intraspecific variation

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Thermal requirements for development in ants have been analyzed based on literature data and own experiments. Variation of thermal reaction norms for development (TRND) among ant species is to a greater extent determined ecologically by the thermal conditions within species ranges. Both the coefficient of linear regression of development rate on temperature (CLR) and the temperature threshold for development (TTD) clearly tend to decrease from the south to the north. Geographic trends in intraspecific variation of thermal constants appeared to be opposite to that we observed at the interspecific level. Northern populations of ants exhibit faster development at relatively high temperatures; they are more thermophilic and stenothermic than southern ones. Latitudinal variation of TRND in *Myrmica* ants is more complicated: the values of TTD and CLR in larvae and pupae increased from south to north approximately as far as the Polar Circle and then decreased at the northern distribution boundary.

Soma vs. Germ line: protection of the offspring at all costs in warmer environment

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An organism has to allocate parsimoniously its energy toward reproduction (germ line) and maintenance (soma). The antioxidant processes which decrease the deleterious effects of Reactive Oxygen Species (ROS, by-products of metabolism) is a substential part of maintenance. Regarding ectotherm species, the metabolism is positively correlated to temperature, a higher production of ROS are thus expected in warmer environment. However, no study has focused yet on the possible differences in antioxidant capacities of the soma vs. the germ line cells in thermally contrasted environments. We hypothesized that germ cells were preferentially protected to increase individual fitness. To study these differences, we measured damages (TBARs methods) and defences (antioxidant enzyme activities) on muscles and gonads from *Oryzias latipes* individuals reared at two non-stressful temperatures. Our results confirmed that fish from the warmer environment have (i) a higher protection of gonads, (ii) a higher reproductive effort, but exhibit (iii) a shorter lifespan.

Oxygen exchange in galls of *Eurosta solidaginis* (Diptera: Tephritidae)

Long, Qian; Tattersall, Glenn
Brock University

Hypoxia in plant tissue should affect animals living within. Gallmakers stimulate their plant hosts to produce the gall they inhabit and feed on, and also influence the gall phenotype for other adaptations, such as defense against predators. The potential for hypoxia in galls of *Eurosta solidaginis* was studied in the context of potential adaptations to gall oxygen level. Mathematical modeling suggested the larva tolerates mild hypoxia in the gall for most of the growth season. One of the two years studied showed hypoxia more severe than expected, and coincided with adverse weather conditions and high larval mortality. The hypoxia may be related to host response to adverse weather. Whether hypoxia directly caused larval mortality requires further study.

Mild desiccation rapidly increases freeze tolerance of the goldenrod gall fly, *Eurosta solidaginis*: evidence for drought-induced RCH

Levis, Nicholas A[1,2].; Yi, Shu –Xia[2]; Lee, Richard E.[2]
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Both freezing and desiccation require cells to tolerate osmotic challenge and, not surprisingly, physiological responses to low temperature and desiccation share common features. To determine whether mild desiccation can improve freeze tolerance at organismal and cellular levels, we assessed survival, hemolymph osmolality and glycerol levels of control and desiccated larvae of the goldenrod gall fly, *Eurosta solidaginis*. Mild, rapid desiccation increased freeze tolerance at –15°C in September-collected larvae and at –20°C in October-collected larvae. Similarly, 6h of desiccation improved in vivo survival in fat body, Malpighian tubule, salivary gland and tracheal cells at –20°C. Desiccation also enhanced intrinsic levels of cold tolerance in midgut cells frozen ex vivo. Although hemolymph osmolality differed between groups, glycerol content did not. The rapidity with which a mild desiccation stress increased freeze tolerance closely resembles the rapid cold-hardening response, which occurs during brief sub-lethal chilling, and suggests that drought stress can induce rapid cold-hardening.

Adaptation to thermal stress in a Finnish butterfly threatened by climate change

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A consequence of climate change is that organisms living at high latitude may experience increased exposure to thermal extremes due to warmer summers and drier winters, which may adversely affect population persistence due to alteration of individual performance or reproduction. In a metapopulation of the butterfly *Melitaea cinxia* found on the Åland Islands of Finland, extirpation and re-colonization is linked to differences in dispersal that is dependent on differences in flight metabolic rate among phosphoglocuse isomerase (Pgi) genotypes. Here we found that thermal tolerance, flight performance and metabolic rate were highest for Pgi heterozygotes after mild heat stress, but after extreme stress, individuals homozygous for a Pgi allele common in Central Europe, but rare in Finland, fared best. Also, male mating activity was reduced after exposure to elevated temperatures regularly experienced in nature. Thus, Finnish populations may be vulnerable to conditions that may become more common as climate change proceeds.

➡ Coffee break

Ecophysiological plasticity assisting dry-season survival in the malarial mosquitoes *Anopheles gambiae* s.l.

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In dry savannahs of West-Africa, the mosquitoes *Anopheles gambiae* s.l., which represent the main vectors of malaria, must annually endure the desiccating conditions of the dry season. Yet, the mechanisms underlying desiccation tolerance in these species are still undefined, and even remain controversial. In this context, we investigated the degree to which the physiology of 1 h- and 24 h-old female mosquitoes is altered at the dry season. Therefore, we examined and compared the metabolic fingerprints (UPLC, GC-MS) and protein abundance (2D-DIGE) of specimens reared under conditions reproducing the rainy and dry season.

Ionic regulation in the Antarctic nematode, *Panagrolaimus davidi*

Wharton, David A.
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Panagrolaimus davidi is found in coastal sites of Ross Island and Victoria Land, associated with penguin colonies and a wide range of soil salinities. I have used energy-dispersive x-ray spectroscopy of pseudocoelomic fluid absorbed into Sephadex beads to determine the nematode's ability to regulate ions in response to external concentrations. Calibration curves were linear for Na, K and Cl, but Mg and Ca departed from linearity at higher concentrations. The nematodes maintain higher concentrations of Na, K and Cl in their pseudocoelomic fluid than in Antarctic soil water but lower concentrations of Mg and Ca. When external concentrations were elevated the nematodes were able to regulate the concentrations of Na, K, Mg and Cl but Ca was not transported into the pseudocoelomic fluid. Regulation of the K concentration was achieved within 4 h. The nematode has efficient ionic regulatory mechanisms that enable it to survive in its Antarctic environment.

Drought tolerance of Collembola revisited: are "hygrophilic" species really restricted to high humidity soils?

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Collembola are conventionally divided into different ecotypes on the basis of their vertical distribution in the soil profile and desiccation tolerance strategy. This classification proposes that surface dwelling Collembola have a relatively impermeable cuticle that prevents desiccation, whereas deeper dwelling species are often more drought sensitive than those species living on the soil surface. This view has been challenged in recent laboratory and field experiments, showing that euedaphic species are not as sensitive to drought as previously believed. The reason for this is probably that euedaphic Collembola can regulate their body fluid osmolality in order to maintain an inward-directed flux of water vapor, even in very dry soils, and hence avoid dehydration. Laboratory experiments simulating natural drought spells show that "hygrophilic" Collembola can stay active in very dry soils and adjust their body fluid osmolality at rates matching the decreases in relative humidity of soil pore air during drought spells.

Understanding the evolution of anhydrobiosis in the sleeping Chironomid, *Polypedilum vanderplanki*, by comparative genomics

Cornette, Richard[1]; Gusev, Oleg[1]; Suetsugu, Yoshitaka[1]; Okuda, Takashi[1]; Kawashima, Takeshi[2]; Sato, Noriyuki[2]; Nishiyama, Tomoaki[3]; Hasebe, Mitsuyasu[4]; Kikawada, Takahiro[1]
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The larvae of *Polypedilum vanderplanki* live in small rock pools in the semiarid regions of Africa. During the dry season, the rock pools dry up rapidly and *P. vanderplanki* larvae desiccate completely to reach an ametabolic state called anhydrobiosis. In this state, dry larvae can survive for months, before resuming normal activity within 1 hour after imbibition by the rain. In order to understand the evolution of anhydrobiosis in insects, we adopted a comparative genomic approach, focusing on the desiccation tolerant *P. vanderplanki* and a desiccation sensitive congeneric species, *Polypedilum nubifer*. Compared to *P. nubifer*, the genome of *P. vanderplanki* was clearly AT-rich. Oxidative stress during anhydrobiosis could be responsible for this bias. We also identified genomic regions, specific to *P. vanderplanki*, which contained clusters of highly duplicated genes. These battery of genes were highly up-regulated during anhydrobiosis and consequently, the corresponding genomic regions were named Anhydrobiosis Related Islands (ARId).

Insect Malpighian tubules: a comparative look at their structure, function and regulation

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Insects represent important yet often overlooked models for the study of water and ion homeostasis. This presentation will compare and contrast the different mechanisms for ion and water balance in the blood-feeding hemipteran *Rhodnius prolixus*, blood-feeding mosquito *Aedes aegypti*, and the fruitfly *Drosophila melanogaster*. Gaps in knowledge will be discussed.

➡ Lunch

Functional differentiation of small heat shock proteins in diapause embryos of *Artemia franciscana*

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Diapause-destined embryos of the crustacean *A. franciscana* produce three small heat shock proteins (sHsps) with p26 the most abundant in both mRNA and protein, followed by ArHsp21 and ArHsp22. Transcriptional and translational mechanisms appear to regulate sHsp synthesis during embryo development. As shown by RNA interference (RNAi) p26 contributes significantly to the stress tolerance of encysted *Artemia* embryos (cysts). Moreover, p26 influences embryo development and either diapause maintenance or termination. In contrast, ArHsp21 has a minor role in stress resistance and no impact on the development of diapause-destined *Artemia* embryos nor their release from diapause. The injection of females with dsRNA for ArHsp22 kills adult *Artemia* thereby preventing determination of ArHsp22 function in developing embryos and cysts by RNAi. The results demonstrate that sHsps synthesized in diapause-destined *Artemia* embryos have different functions and that these activities extend beyond a role in stress tolerance.

Chironomus riparius telomeric transcriptome and its response to heat shock

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Telomeres are nucleoprotein structures at the ends of eukaryotic chromosomes that preserve genome stability and function. *Chironomus riparius*, a midge, has telomeres with tandem arrays of 176 bp sequences classified in three subfamilies, named TsA, TsB and TsC. Previously, a telomeric RNA was reported and here we show that it is produced from one strand of TsA and both strand of TsB, while none can be detected from TsC. These RNAs respond singularly to heat shock and they are differently affected by transcription inhibitors, both in control and heat shock conditions. A computer search for transcription factor binding sites reveals some remarkable putative regulatory cis-elements, which could be related with the different transcription patterns observed. Altogether this suggests a dual regulation that could be reflecting specific functions for telomeric RNAs in the cell.

The effects of developmental thermal environment on the transcriptome and heat knock down tolerance phenotype in *Drosophila melanogaster*

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While effects on the thermal phenotype have been widely studied at constant temperatures, we lack information about the physiology and functional importance of thermal acclimation and hardening at ecologically relevant variable temperatures. Moreover, we have a limited knowledge of the molecular background of these plastic physiological adjustments. We investigated the impact of variation in mean and variance in temperature during development from egg to adult in *D. melanogaster* on heat resistance and gene expression in adult flies. We investigated 2 hypotheses, namely: Whether mean and variance in developmental temperatures effect heat tolerance in adults with higher and more variable developmental temperatures leading to increased thermal tolerance; and, whether mean and variance in developmental temperature elicit distinct transcriptional responses in adults when tested at benign as well as stressful temperatures. Results showed that mean and variance in developmental temperature impacts on different physiological and molecular mechanisms contributing to the thermal tolerance.

The role of Late Embryogenesis Abundant (LEA) proteins in a stress-tolerant crustacean

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Encysted embryos of the crustacean *Artemia francsicana* are excellent model organisms for the study of anhydrobiosis – life without water. The functions of Late Embryogenesis Abundant (LEA) proteins, which accumulate in stress-tolerant encysted *Artemia* embryos were examined herein. Group 1 LEA proteins were knocked down in encysted embryos using RNA interference (RNAi). Embryos lacking group 1 LEA proteins survived less well than control embryos after desiccation and freezing, suggesting a role for these proteins in dehydration tolerance. Embryos without group 1 LEA proteins were also more sensitive to hydrogen peroxide, a reactive oxygen species. This is the first in vivo functional study of group 1 LEA proteins in an animal. With improved understanding of the stress tolerance of *Artemia* it is possible to improve their use in aquaculture and perhaps advance methods of cell preservation.

Coffee break

Metabolomic and lipidomic characterization of diapause in the blue bottle fly *Calliphora vicina*

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At temperate latitudes virtually all insects utilize a specialized state of dormancy, termed diapause, as an overwintering strategy. Despite the importance of diapause, we know very little about the molecular mechanisms that underpin this environmentally adaptive dormancy. Many ecologically relevant species are yet to be sequenced, which limits our ability to characterize genetic changes associated with this phenotype. Metabolomics and lipidomics allow unbiased screening of metabolic changes associated with diapause, and have the advantage in representing an end-point measure of molecular adaptation downstream of any changes in gene expression. This facilitates the identification of mechanisms with a direct contribution to diapause associated phenotypes, such as enhanced cold tolerance. Here I describe the results of a detailed metabolomic and lipidomic analysis of diapause in the blue bottle fly *Calliphora vicina*, and discuss the potential role of specific metabolic and lipid changes.

Examining the genetic components implicated in maintaining inducible defences in *Daphnia lumholtzi*

McKinnon, Alexander H.
Mount Allison University

Introduction of a fish predator to *Daphnia lumholtzi*'s environment elicits the formation of elaborate head and tail spines. This study aimed to develop a method to elucidate the genetic components responsible for maintaining helmets in this exotic zooplankten during their first 96 hrs. Experimental individuals were reared in either the presence or absence of planktivorous fish kairomones. A substantial difference in helmet size was observed between the two conditions, with helmet size showing a two-fold increase in the populations exposed to fish kairomones. Differential display rt-PCR allowed for variation in genetic expression between the two conditions to be visualized. A general trend of down-regulation of gene expression was observed for individuals with substantial helmets. I identified 5 potential candidate sequences that may play a role in the *D. lumholtzi* response to fish predation including sequences that code for ribosomal proteins, cyclophilin, notch 2 and nitric oxide synthase 1.

Localisation of antifreeze proteins in *Rhagium mordax* by immunofluorescence.

Johnsen, Johannes L.
Roskilde University, Denmark

Larvae of the blackspotted pliers support beetle, *Rhagium mordax*, express antifreeze proteins (AFP) in their haemolymph during temperate climate winter. It is believed that they also express antifreeze proteins in their cuticle as a means of preventing inoculative freezing. Larvae of *Rhagium mordax* were collected during all seasons of 2012. Larvae were fixated, embedded in paraffin wax, sectioned on a microtome, incubated with custom made primary anti-AFP antibodies, stained with FITC secondary antibodies and visualised on a fluorescence microscope. The larvae of both winter and summer showed AFP activity in their cuticle, gut lumen and -epithelium. Due to the long synthesis process of AFPs, the larvae contain them all year round. The spatial distribution of these AFPs change during summer, possibly relocating to vesicles in the cuticle and gut lumen/epithelium.

Functional significance of post translational modifications to *Tenebrio* THP

McKellar, James; Marshall, Craig J.
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The larvae of the yellow mealworm beetle, *Tenebrio molitor* are freeze tolerant due, in part, to the present of thermal hysteresis proteins (THP). Purifying THP is difficult because of their small size and the existence of many isoforms. Most recent work on THP, particularly on their structure, has been done on proteins expressed in E. coli despite the difficulties of obtaining correct disulfide bond formation. We have developed a protocol for purification of individual THP from *Tenebrio* larvae and compared the properties of the native and recombinant forms. The native protein has more thermal hysteresis than the recombinant protein (per molecule) and forms complexes that are difficult to dissociate, even on SDS-PAGE. Specific cleavages in the peptide cause breaks that are ‘repaired’ by internal disulfide bonds. These cleavages may play a role in the somewhat different behaviours of native and expressed THP and may have some physiological significance.

Group photo
& Poster Session

Locomotor activity rhythm and circadian clock gene expression in the Antarctic midge *Belgica antarctica*

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Temporal coordination with day-night cycles is crucial for maintenance of organismal fitness, and thus most organisms have evolved a circadian clock enabling them to predict the time of day and to optimize timing of internal processes. In spite of our expanding knowledge on circadian clocks in species inhabiting temperate zones, information on circadian clocks in species inhabiting polar environments is still limited. It is of special interest to know how circadian clocks function during the polar summer with protracted light duration, a condition that leads to arrythmicity in some species from lower latitudes. In the present study, we investigated locomotor activity in the Antarctic midge *Belgica antarctica* under summer field conditions on the Antarctic Peninsula and under laboratory conditions. We also examined expression patterns of circadian clock genes and conclude that the circadian clock is not ticking in this midge.

In Synch: Genomic change associated with the evolution of seasonal synchrony via adaptation across the life cycle

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[1]University of Notre Dame, [2]University of Florida

Synchronization of complex life cycles with environmental fluctuations is a critical adaptation in seasonal environments. Insects typically regulate this synchrony through the modulation of the diapause response, timing exit from and entry into diapause with the beginning and end of the growing season, respectively. Any shift in seasonal timing, however, changes seasonal exposure of all life history stages, selecting for adaptations across the life cycle. Populations of the apple maggot fly, *Rhagoletis pomonella*, have recently shifted from a native hawthorn host to introduced apples, which fruit earlier in the year. These apple populations, which are in the early stages of speciation, have evolved differences in the timing of the termination of pupal diapause to adjust to earlier seasonal availability of their host fruit. As a consequence, diapause initiation also occurs earlier in the year when temperatures are warmer, exposing formerly covert variation for direct development (i.e., a second generation), which is a poor life history strategy. We have quantified genome-wide associations for traits associated with both diapause termination and initiation. We discuss how these associations relate to the process of rapid seasonal adaptation, genomic divergence and potential constraints limiting independent evolution across the life cycle.

Session 9/Diapause

Why the onion fly avoids daytime adult eclosion?

Tanaka, Kazuhiro
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Many dipteran insects emerge as adults at early morning. The timing might have evolved as an adaptation to daily cycle of both biotic and abiotic environments, but the selecting factor involved is not easily determined. Our hypothesis is that daytime high soil temperature inhibits emergence of newly eclosed adults from the soil. To test this hypothesis, newly eclosed onion fly adults were placed in the soil at different soil temperatures. Almost all flies escaped from the soil at 41 ºC, but none emerged at 43ºC. Because daytime soil temperature in agricultural habitats frequently exceeded 43ºC, it is expected that proportion of flies successfully emerged from the soil varies depending on time of day they eclose. Under the outdoor conditions, all flies placed in the soil at 0900 emerged from the soil, but none did so at 1300, thus confirming the hypothesis.

The long winter: the synergy between fluctuating thermal regime and quiescence physiology in the pollinator, *Megachile rotundata*

Yocum, George D.[1]; Rinehart, Joseph P.[1]; Torson, Alex S.[2]; Greenlee, Kendra J.[2]; Kemp, William P.[1]; Bowsher, Julia H.[2]

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The alfalfa leafcutting bee *Megachile rotundata* (F.) is the primary pollinator for alfalfa seed production. Developing a more thorough understanding of *M. rotundata* low-temperature and diapause physiology is essential for the sustainable management of this key pollinator. We previously demonstrated that a daily high-temperature pulse (fluctuating thermal regime, FTR) significantly increased the survival of *M. rotundata* exposed to low-temperature storage. Since the longest period of low-temperature storage of *M. rotundata* occurs during overwintering, we wanted to evaluate whether FTR would improve overwintering survival and overall quality of this alternative pollinator. Using a storage regime employing a base temperature of 6°C and daily pulse for 1 hour at 20°C, survival of post-diapausing *M. rotundata* was extended through the first post-larval growing season and into the second. Illumina sequencing was carried out to determine the underlying molecular physiology of FTR in this important solitary bee pollinator.

Repeated anoxia exposures during the immature stages have hormetic effects that extend into adulthood.

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Organisms experience multiple environmental stressors in their habitat during the course of their life cycle. While there are multiple strategies ranging from tolerance to avoidance, many soil-dwelling insects are confined in an immobile pupal stage. Additionally, organisms experience multiple bouts of the same stressors and the effects these bouts have on long-term organismal performance remain largely unknown, as stress physiology has largely focused on brief survival to single exposures. We studied the effects of repeated bouts of anoxia (3 hrs) during larval, pupal, and adult development in the Caribbean fruit fly, *Anastrepha suspensa*. We monitored metabolism, total antioxidant capacity, oxidative damage to lipids and proteins, energy reserve consumption, and multiple metrics of organismal performance. We found that multiple bouts of anoxia had a hormetic effect that led to increased adult emergence, flight ability, mating, and longevity. We recorded interesting correlative patterns between metabolism and oxidative damage.

➡ Coffee break

Can winter-active bumblebees survive the cold? Assessing *Bombus terrestris audax* cold tolerance and the effects of pollen feeding.

Owen, Emily L.; Bale, Jeffrey S.; Hayward, Scott A.L.
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There is now considerable evidence that climate change is disrupting the phenology of key pollinator species. The recently reported UK winter activity of the bumblebee *Bombus terrestris* brings a novel set of thermal challenges to bumblebee workers that would typically only be exposed to summer conditions. Here we assess the ability of workers to survive winter conditions by determining their lower lethal temperatures (LLTemp10, 50, 90), lower lethal times at 0°C (LLTime10, 50, 90) and the ability to rapidly cold harden (RCH). The influence of diet (pollen versus nectar consumption) on supercooling points (SCP) was also determined. Finally, comparisons were made between SCPs and the LLTime50 of worker and queen bumblebees. Results are discussed in the light of winter active bumblebees and climate change.

Cold adaptation of insects inhabiting East Siberia, Russia: from physiology to practical perspectives

Li, Natalia G.; Kershegoltz, Boris M.; Remigaijlo, Pavel A.
Institute for Biological Problems of Cryolithozone SD RAS

The insects inhabiting cold environments evolved a range of survival strategies, which can be defined into four main categories: freeze tolerance, freeze avoidance, vitrification and protective dehydration (Zachariassen, 1985; Wasyluk, 1988; Duman, 1991; Holmstrup, 2001; Sformo et al., 2009; Kostal, 2011). Using physiology approaches (measuring of SCP, ice-nucleating activity, specific ice nucleating activity, cold hardiness potential) we examine over-wintering strategies in 29 species of insects living in central Yakutia (Yakutsk, 62ºN; 120ºE). It is shown that most of these insects evolved freeze tolerance strategy. It is suggested that one of the main reasons of prevailing of the freeze-tolerant strategy is that cryoprotective effect of polyols is stronger in freeze tolerant insects than in freeze avoiding ones. Another reason is a shifting within species from freeze avoidance to freeze tolerance in extremely cold area, which is Yakutia. The evolution of physiological mechanisms of insect adaptation to ultra low environmental temperatures lead to production of highly effective cryoprotecive systems consisting of either polyols and ice nucleating proteins or polyols and antifreeze proteins. In this study we present data on using a multicomponent cryoprotective system produced by local highly freeze tolerant insects in cryopreservation of human blood lymphocytes.

Delayed freezing events: distribution in time and relationship between SCP and LLT

Nedvěd, Oldřich
University of South Bohemia and Institute of Entomology, České Budějovice, Czech Republic

Supercooled state of body liquids in an insect organism is unstable and freezing events spontaneously occur during prolonged exposure at constant temperature. We revealed the distribution of the timing of these events in a series of cold adapted and cold susceptible insect species. The frequency of freezing events gradually decreased from the start-point when the decreasing temperature reached the pre-set constant value – equal or above median SCP. We looked for the relationship of the distribution of these time values to the distribution of individual supercooling points in a large parallel sample. Finally, we tried to predict the number of frozen individuals after diverse periods of time at diverse constant temperatures and to explain the relationship between the mean value of supercooling point measured at constant cooling rate and lower lethal temperature measured after standard 24 hours exposure of insect sample at constant subzero temperature.

Life of a non-native midge in the maritime Antarctic

Everatt, Matthew J.[1]; Convey, Peter[2,3]; Bale, Jeffrey S.[1]; Worland, Michael, R.[2]; Hayward, Scott A.L.[1]

[1]School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK; [2]British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, UK; [3]National Antarctic Research Center, IPS Building, University Malaya, 50603 Kuala Lumpur, Malaysia,

The maritime Antarctic represents one of the most extreme environments on Earth. Microhabitat temperatures regularly fall below 0°C during the winter, and rarely rise above 5°C during the summer. Water availability is greatly restricted to terrestrial organisms, being locked up as snow and ice during the many months of winter. While in summer, meltwater can quickly evaporate resulting in drought. The midge, *Eretmoptera murphyi*, is one of the few insects to live in this environment. *Eretmoptera murphyi* was introduced from South Georgia (sub-Antarctic) onto Signy Island (maritime Antarctic) in the 1960s, and has since spread widely. The capacity and adaptation of this midge to survive under these extreme conditions will be discussed. In addition to low temperature and low water availability, climate warming is raising summer temperatures. How *E. murphyi* will cope with this change will also be explored.

Roles of carbohydrate reserves for local adaptation to low temperatures in the freeze tolerant *Enchytraeus albidus*

Fisker, Karina V.[1,2]; Overgaard, Johannes[1]; Sørensen, Jesper G.[2]; Slotsbo, Stine[2]; Holmstrup, Martin[2]

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Geographic variation in cold tolerance and associated physiological adaptations were investigated using a common garden approach on 7 populations of the freeze tolerant enchytraeid *Enchytraeus albidus* (Oligochaeta). Cold tolerance varied between populations in relation to the environmental temperature of their location. Populations from the coldest climatic regions were able to tolerate freezing below -15°C and endured being frozen at -5°C for more than 4 weeks and populations from milder climates had a lower freeze tolerance (about -9°C) and typically endured -5°C for less than 2 weeks. The amount of glucose accumulation and glycogen reserves as well as the metabolic rate varied between populations but neither of these metrics could be related directly to cold tolerance. The metabolic depression due to freezing of these populations was relatively small (<50%) suggesting that the large carbohydrate accumulations may be important as fuel to sustain long term freezing at moderately low temperatures. The differences in freeze induced metabolic depression may in part explain the population specific variance in cold tolerance.

➡ Lunch

Geographic variation and environmental plasticity of growth and development in the colorado potato beetle

Kutcherov, Dmitry A.
Department of Entomology, Saint Petersburg State University, Saint Petersburg, Russia

We have compared responses to temperature and photoperiod in two populations of the Colorado potato beetle. One of them originated from southwest Russia where the species usually has two generations per season. The second population occupies northernmost habitats of the beetle's European range near St. Petersburg. Main traits under study were developmental time and body weight under various temperature-photoperiod combinations. Responses to these factors had both similarities and specificities in the two populations. E.g., short day accelerated development in both under all temperatures; body weight in southern beetles did not depend on temperature but depended on photoperiod while the opposite was observed in the northern population; beetles from the north tended to develop faster at all stages and all experimental regimens. We have also analyzed a number of morphometric parameters as a preliminary attempt to reveal mechanisms underlying geographic and environmentally induced differences in body weight.

The thermal biology and thresholds of the spider mite predator *Phytoseiulus macropilis*

Coombs, Megan R.; Bale, Jeff S.
University of Birmingham

Biological control is an increasingly important component of pest control in commercially grown crops, however there is an inherent risk that use of an exotic organism could harm the native ecosystem. All potential biocontrol agents are subject to thorough investigation in order to prevent the introduction of a species that has the capacity to become invasive. The safety and suitability of a candidate glasshouse biocontrol agent, *Phytoseiulus macropilis* (Acari: Phytoseiidae), which predates the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), was assessed through a combination of laboratory and winter field trials. Efficacy of the predator was compared with the leading market agent, *Phytoseiulus persimilis* (Acari: Phytoseiidae). The results demonstrate that it is unlikely that *P. macropilis* will survive a typical UK winter, and the lower thermal activity threshold data indicate that *P. macropilis* will make an effective biological control agent in temperate climates.

Effects of some internal and external factors on growth of the linden bug *Pyrrhocoris apterus*

Balashov, Sergey V.
Saint-Petersburg State University, Saint-Petersburg, Russia

Thermal conditions experienced during the first part of nymphal development have an effect on the trajectory of subsequent growth. Nymphs grew more rapidly after their exposure to slightly hotter conditions as compared with the control group kept under the constant (reference) temperature. On the contrary, rearing nymphs in a slightly cooler environment resulted in deceleration of both processes once the insects had been transferred to the reference temperature regimen. Day length also influences growth intensity in the linden bug. Nymphs were less resistant to starvation at a day length promoting rapid growth than under a photoperiodic regimen where growth was slower. Nymphs reared at threshold combinations of temperature and day length showed differences in their growth rate depending on whether they were to reproduce or undergo diapause after final molting. Under the same conditions, females tentatively identified as active had grown more rapidly as nymphs than females showing symptoms of diapause.

How to measure *Drosophila* cold tolerance: Chill coma temperature predicts distribution and tolerance limits

Andersen, Jonas L.; Manenti, Tommaso; Sørensen, Jesper G.; Loeschcke, Volker; Overgaard, Johannes
Institute for Bioscience, Aarhus University

Cold tolerance is an important factor determining the distribution of ectothermic species. Several measures have been used to characterize insect cold tolerance, including: chill coma temperature (CT_{min}), chill coma recovery time (CCRT), lethal temperature (LTe50), lethal time at low temperature (LTi50) and the supercooling point (SCP). Whether these measures describe the natural variance in cold tolerance that determines species distribution is, however, unclear. Similarly, it is unknown to what extend these measures are associated with common physiological mechanisms. To investigate this we measured the 5 different cold tolerance traits in 13 *Drosophila* species with variable latitudinal distribution. Drosophilids are chill sensitive and accordingly, the SCP points showed poor correlations to all environmental and phenotypic variables. In contrast, CT_{min} correlated well to environmental variables suggesting that this is a superior proxy to evaluate cold adaptation in drosophilids. Chill coma temperature also correlated well with LTi50 and LTe50 but interestingly not to CCRT which is thought to involve a reversal of the processes leading to chill coma.

Coffee break

The importance of sex, developmental stage, acclimation and using different indices of cold tolerance: a case study with *Tetranychus urticae*

White, Nicola; Bale, Jeff; Hayward, Scott
University of Birmingham

The cold tolerance of many insects has been classified over the past 100 years, and there are a number of experimental procedures for this purpose. Early years of cold tolerance classification relied only on the supercooling point until the discovery of chill injury occurring at temperatures higher than that of the supercooling point. The diversity of insect physiological responses means that no single laboratory indices can accurately characterise the cold tolerance of a species. Using *Tetranychus urticae* as a study system, we investigate how sex and life stage influences physiological responses to cold stress. We also employ different indices of cold tolerance measurement to determine cold tolerance classification. The study shows that if only a subset of experimental methods are used, there is a risk of misinterpreting the thermal tolerance of a species.

Why do insects enter and recover from chill coma? Low temperature and high extracellular potassium compromises muscle function in *Locusta migratoria*

Findsen, Anders; Pedersen, Thomas H.; Nielsen, Ole B.; Overgaard, Johannes
Aarhus University

Many insect species enter a comatose state when their body temperature is lowered to a critical limit. Several studies have demonstrated that the transition to chill-coma involves a disruption of neuro-muscular performance and ion homeostasis is also often disrupted during chill coma. Chill coma could therefore be associated directly with the depolarisation caused by loss of ion-homeostasis or alternatively by the effect of temperature on neuro-muscular function. Using isolated tibial muscle of the chill susceptible locust we investigated the relationship between tetanic force production, temperature and extracellular [K^+]. Maximum tetanic force decreased approximately 75% when temperature was reduced from 23°C to 0.5°C and increasing extracellular [K^+] from a control value of 10 mM to 30 mM also caused a severe (50%) reduction of force. The ability to produce muscle force was almost abolished when the muscles were simultaneously cooled and exposed to high [K^+] as force was reduced to 1% of the control. These results suggest that both temperature and ion disruption are of importance when insects enter and recover from chill coma.

On the roles of so far neglected structural components of biological membranes–lyso-phospholipids, sterols and tocopherols–in insect overwintering.

Koštál, Vladimír[1,2]; Urban, Tomáš[2]; Řimnáčová, Lucie[1]; Berková, Petra[1]; Šimek, Petr[1]

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Ectotherm animals including insects are known to undergo seasonal restructuring of the cell membranes in order to keep their functionality and/or protect their structural integrity at low body temperatures. Though the lipidic composition of biological membranes is highly complex, most studies so far focused either on fatty acids or on major phospholipid classes only. We will present the results on seasonal changes in neglected phospholipid classes, lysophosopholipids, free fatty acids, phytosterols and tocopherols in heteropteran insect, *Pyrrhocoris apterus*. Possible adaptive meanings of such changes will be discussed including: preventing the unregulated transition of membrane lipids from functional liquid crystalline phase to non-functional gel phase; decreasing the rates of ion/solute leakage; silencing the activities of membrane bound enzymes and receptors; and counteracting the higher risk of oxidative damage to PUFA in winter membranes.

Cold tolerances of drosophilds correlate with membrane phospholipid fatty acid composition

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Drosophilid species are found in tropical, subtropical and temperate environments and are accordingly exposed to different environmental temperatures. According to the hypothesis of homeoviscous adaptation different species should display characteristics of their membrane lipid composition that reflect the environmental conditions they experience in the field. Thus cold adapted species are hypothesized to have phospholipid fatty acids (PLFA) with 1) Shorter chain length, 2) Higher proportion of unsaturated fatty acid and 3) Lower average melting point. The present study reports PLFA composition of 56 species of drosophilids that have previously been characterized with respect to cold tolerance and geographical distribution. All species were reared under common garden conditions and interspecific variance in cold tolerance and minimal environmental temperature of their habitat was correlated to the metrics of membrane PLFA composition. The data support a strong positive correlation between cold tolerance and the proportion of unsaturated fatty acid and a negative correlation with PLFA melting point. However no significant correlation was found between cold resistance and average PLFA length. Overall these data support the hypothesis that interspecific differences in PLFA composition conform to the idea of homeoviscous adaptation.

Posters

Tuesday, August 13th

#	Presenting author/ Title
P1	**Michael Elnitsky** The impacts of climate change on the overwintering energetics and microenvironmental conditions of the goldenrod gall fly, *Eurosta solidaginis*
P2	**Dennis Friis** Remarkable thermal properties of an insect antifreeze protein
P3	**Josiah Gantz** Investigating the sensitivity and mechanisms of drought-induced rapid cold-hardening in *Eurosta solidaginis* larvae.
P4	**Behnaz Ghaedi** Thermal tolerance of aphid: what is being assessed?
P5	**Katrin Jõgar** Influence of neem-based insecticide on hibernation physiology in *Pieris brassicae* L. pupae
P6	**Johannes Johnsen** Localisation of antifreeze proteins in *Rhagium mordax* by immunofluorescence.
P7	**Indrikis Krams** Predation promotes survival of beetles with lower resting metabolic rates
P8	**Richard Lee Jr.** Rapid, mild desiccation improves the RCH response in the flesh fly, *Sarcophaga bullata*
P9	**Natalia Li** Some features of a seasonal cold hardiness of *Upis ceramboides* beetles inhabiting East Siberia, Russia.
P10	**Heath MacMillan** A high-throughput method of hemolymph extraction from adult *Drosophila* without anesthesia
P11	**Craig Marshall** Cold tolerance in a winter-active New Zealand chafer
P12	**José-Luis Martínez-Guitarte** Multi-biomarker responses in natural populations of *Chironomus riparius* larvae exposed to environmental pollutants
P13	**Megan Meuti** The role of circadian clock genes in the overwintering diapause of the Northern House Mosquito, *Culex pipiens*

#	Presenting author/ Title
P14	**Timothy Muir** Characterizing the cold-conditioning response in a vertebrate ectotherm
P15	**Oldřich Nedvěd** Contrasting supercooling ability in lowland and mountain European *Colias* butterflies
P16	**Oldřich Nedvěd** Clinal variation in ecophysiological traits in drosophilids of the Indian subcontinent
P17	**Johannes Overgaard** Metabolic cold adaptation in *Drosophila*: Fact or fiction?
P18	**Dominik Paschke** The role of ice active proteins in the freezing tolerance mechanisms of *Hemideina maori*
P19	**Benjamin Philip** Seasonal stability of supercooling points in spiders living in a temperate ecosystem
P20	**Alice Reynolds** Membrane adaptation in phospholipids and cholesterol in the widely distributed, freeze-tolerant wood frog, *Rana sylvatica*
P21	**Andrew Rosendale** Importance of a glucose transporter in the cryoprotectant system of the freeze-tolerant wood frog, *Rana sylvatica*
P22	**Konrad Schöttner** Shedding light on thermoperiodism in a drosophilid fly, *Chymomyza costata*
P23	**Petra Simunkova** The response of circadian clock neurons in the brain of *Chymomyza costata* to photoperiodic and thermoperiodic signals
P24	**Tomáš Štětina** Funcional analysis of the expression of HSPs complex in response to cold in *Drosophila melanogaster*
P25	**Glenn Tattersall** Evaporative cooling augments prey detection in the rattlesnake, *Crotalus durissus*
P26	**Dragana Vukasinovic** Overwintering energetics of *Harmonia axyridis*: The effects of cold and mild winters

Poster

[P1] **The impacts of climate change on the overwintering energetics and microenvironmental conditions of the goldenrod gall fly, *Eurosta solidaginis***

Spacht, Drew; Elnitsky, Michael A.
Mercyhurst University

The winter temperatures experienced by goldenrod gall fly (*Eurosta solidaginis*) larvae directly influence the potential fecundity of the adults, as the energy reserves remaining at the end of winter determine body size and are used for gamete production in the flies. The purpose of this study was to assess the current and future impacts of climate change on *E. solidaginis*. Based upon historical temperature data and the relationship between CO_2 production and temperature, the estimated energy utilization during winter (November through March) has increased by ~30% over the last 50 years. Each additional 1°C rise in temperature is predicted to increase overwintering energy consumption by ~13%. Continued climate change will also have significant impacts on the winter thermal microenvironment of *E. solidaginis*. Elevated winter temperatures are predicted to significantly decrease the number of freeze-thaw cycles, the average time spent frozen per cycle, and the total time spent frozen during winter.

Poster

[P2] **Remarkable thermal properties of an insect antifreeze protein**

Friis, Dennis; Loerup, Johannes; Kristiansen, Erlend; Westh, Peter; Ramlov, Hans
Roskilde University

Antifreeze proteins (AFPs) are found in many cold-living ectothermic species, where they function as a protective measure against lethal internal ice formation. Despite the fact that antifreeze proteins function at sub-zero temperatures, very high protein melting temperatures have been observed for some insect AFPs. These high melting temperatures are probably caused by the high number of disulphide bonds seen in these proteins. The AFPs from the beetle *Rhagium mordax* have only one disulphide bond. The present study focuses on the thermal stability of one of these proteins, RmAFP1. This protein was found to have an extremely low melting temperature at neutral pH. Furthermore, it was observed that an unusual large fraction of the protein refolds into an active form, albeit slowly, after being heated well above its melting temperature. Thus, compared to other insect antifreeze proteins, RmAFP1 seems to have unusual physical/chemical properties.

Poster

[P3] **Investigating the sensitivity and mechanisms of drought-induced rapid cold-hardening in *Eurosta solidaginis* larvae.**

Gantz, Josiah D.; Lee, Richard E. Jr.
Miami University

Rapid cold-hardening (RCH) is a highly conserved response in insects that induces physiological changes within minutes to hours of exposure to low temperature and provides protection from chilling injury. Recently, an acclimatory response termed drought-induced RCH was described as a result of acute desiccation stress in *Eurosta solidaginis* larvae. In this study, we investigated the sensitivity and mechanisms of drought-induced RCH. Tolerance to low temperature increased markedly with as little as 2 h of desiccation and a 2% loss of fresh mass, as organismal survival increased from 32% to 56%. At the cellular level, the low-temperature survival rate of mid gut tissue increased following desiccation (3.5 h, 0% RH) from <12% (control) to >90%. Fat body also showed an increase in survival with desiccation; however, the difference was not as pronounced (43.5% control versus 67.7%). These results indicate that drought-induced RCH is an important component of low-temperature survival in insects.

Poster

[P4] **Thermal tolerance of aphid: what is being assessed?**

Ghaedi, Behnaz; Nigel, Andrew R.
Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale, NSW, 2351, Australia.

Here we will assess the current state of published research on aphid responses to thermal extremes. We synthesise and compare findings of aphid thermal tolerance based on 30 papers published from 1970 to 2012. The current assessment of literature indicate that aphid cold tolerance has been relatively well studied, but the vast majority of work has focused on the effects of a single cold exposure on specific metrics (such as SCP), with consideration to abiotic factors in scarce and limited to north-hemisphere in Europe. There is a knowledge gap surrounding the impacts of thermal tolerance on responses of aphids at the molecular level. There is a clear need to design future experiments around multiple exposures to extreme temperatures with attention to other abiotic and biotic factors, at the same time.

Keywords: aphid, thermal tolerance, repeated stress, cold tolerance, super cooling point

Poster

[P5] **Influence of neem-based insecticide on hibernation physiology in *Pieris brassica*e L. pupae**

Jõgar, Katrin; Kuusik, Aare; Metspalu, Luule; Hiiesaar, Külli; Ploomi, Angela; Tasa, Tea; Luik, Anne; Mänd, Marika

Estonian University of Life Sciences, Institute Agricultural and Environmental Sciences, Department of Plant Protection, Kreutzwaldi 1, Tartu, 51014, Estonia

The Large White Butterfly (LWB), *Pieris brassicae* L., is a cosmopolitan insect, and is found wherever cruciferous plants are grown. Synthetic insecticide application against the LWB larvae is the primary method of control. The neem tree, *Azadirachta indica*, is a plant that can potentially control insects with the advantage of being food and environmental safe. The aim was to determine the effect sublethal dosage of bioinsecticide, NeemAzal T/S, on diapause intensity of *P. brassicae* pupae. Larvae were reared on leaves treated with two neem concentrations: 0.01%; 0.1% or distilled water (control). Laboratory experiments showed that there was a significant effect of treatment on pupal mass, with the performance being lowest on 0,1% neem variant. Pupae of neem treatments had higher standard metabolic rate and lost greater mass per day than those reared on control variant. The results suggest that sublethal dosages of neem had an influence on diapause intensity.

Poster

[P7] **Predation promotes survival of beetles with lower resting metabolic rates**

Krams, Indrikis; Krama, Tatjana
University of Daugavpils

In this study, we investigated whether female and male yellow mealworm beetles, *Tenebrio molitor*, differ in their hiding behaviour, individual response latency time, and duration of immobility to treatments mimicking an approaching predation threat. We experimentally tested whether consistently repeatable anti-predatory responses and resting metabolic rates (RMR) correlated with survival rates of individuals exposed to a nocturnal predator, the brown rat, *Rattus norvegicus*. Resting metabolic rate was part of a syndrome involving anti-predator behaviour. Individuals with lower RMR concealed themselves against predators in substrate more successfully than individuals with higher RMR, and hiding was associated with longer periods of immobility. Ultimately, mortality was higher in the high-RMR beetles compared to the low-RMR beetles. Our results provide direct evidence of natural selection against mobility, i.e., for reduced RMR in *T. molitor* beetles.

Poster

[P8] **Rapid, mild desiccation improves the RCH response in the flesh fly, *Sarcophaga bullata***

Yi, Shuxia; Gantz, Josiah D.; Lee, Richard E. Jr.
Department of Zoology, Miami University, Oxford, OH 45056, USA

Rapid cold-hardening (RCH) increases cold tolerance of insects by protecting against non-freezing cold-shock injury. In a study of the interaction between desiccation and cold tolerance, we demonstrated that acute desiccation, in which both larval and adult *Sarcophaga bullata* lost only 2-3% of their fresh mass, enhanced protection from low temperature afforded by RCH. At the organismal level, desiccation coupled with RCH increased the pupation rate of larvae by 26% relative to the RCH treatment alone. The same treatment applied to adults improved the speed of RCH recovery, as restoration of the righting reflex was increased by 46% within 20 min and by 53% within 30 min, after exposure to low temperature. At the cellular level, desiccation increased survival by 13% in fat body cells of RCH-treated larvae and by 19% in gut epithelial cells of RCH-treated adults. These results indicate that desiccation significantly improves the effectiveness of the RCH response.

Poster

[P9] **Some features of a seasonal cold hardiness of *Upis ceramboides* beetles inhabiting East Siberia, Russia**

Li, Natalia G[1]; Osakovsky, Vladimir L.[2]

[1]Institute for Biological Problems of Cryolithozone SD RAS, [2]North-East Federal University after name Ammosov Institute of Health

Upis ceramboides is a highly cold hardy insect (Miller, 1978; Walters, 2009; Li, 2006; 2011). Despite sensitivity to a rate of freezing, Yakutian population of *Upis ceramboides* is tolerant to both prolonged period of extremely low temperatures and ultra low temperatures as low as -85°C. The basis of high resistance to ultra low temperatures is freeze tolerant strategy that is associated with production of moderate concentrations of polyols and highly effective ice nucleating proteins. Specific activity of ice nucleators significantly changes depending on seasons: it is highest in winter and lowest in summer. The specific ice nucleating activity depends on polyol concentration and total protein concentrations. According to it, the new parameter of evaluating of cold hardiness of freeze tolerant insects is offered. In summer, cold hardiness of beetles is based on a deep cleaning of body from accident nucleators and de-masking endogenous ice nucleating agents that enable the beetles a tolerance to the temperatures below -17°C.

Poster

[P10] **A high-throughput method of hemolymph extraction from adult *Drosophila* without anesthesia**

MacMillan, Heath A.[1]; Hughson, Bryon N.[2]

[1]Department of Biology, The University of Western Ontario, London, Canada; [2]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada

A rapid and cost-effective method of sampling hemolymph from the model insect *Drosophila melanogaster* is needed for studies in several fields, including ionoregulatory physiology, metabolism, immunology and toxicology. Here, we describe the construction and use of a device that uses airflow and pressure to manipulate adult flies and extract high-volume hemolymph samples. This method is rapid and inexpensive, and does not require cold or CO_2 anesthesia at any point in the sampling process. This allows us to avoid the confounding effects of these treatments on the biochemical properties of the hemolymph sampled. To demonstrate one of the many potential uses for this method, we measure active concentrations of Na^+ and K^+ in isolated hemolymph droplets from male and female *D. melanogaster* using ion-selective microelectrodes.

Poster

[P11] **Cold tolerance in a winter-active New Zealand chafer**

Lee, Brendon; Marshall, Craig J.
Department of Biochemistry and Genetics Otago, University of Otago

The striped chafer, *Odontria striata*, is a common pasture pest in sub-alpine regions of the South Island of New Zealand. The larvae feed in the soil of grasslands and may cause substantial economic losses by damaging the root of pasture grasses. Adult *Odontria* are active during the cooler period from March to October. Winter night temperatures in subalpine regions fall –8 °C. Adults appear to burrow into the soil to avoid the cold, will feed at 2 °C and sometimes reach 20 °C. Our studies show that adult *Odontria* supercool by about 5 °C, do not survive freezing, and that beetle extracts contain a potent non-protein antifreeze with no evidence of thermal hysteresis. There is also evidence that the supercooling point rises during the winter. We have characterised the small molecules present in beetle extracts that might be responsible for antifreeze activity.

Poster

[P12] **Multi-biomarker responses in natural populations of *Chironomus riparius* larvae exposed to environmental pollutants**

Planelló, Rosario[1]; Herrero, Oscar[1]; Gómez-Sande, Pablo[2,3]; Servia, María-José[4]; Martínez-Guitarte, José-Luis[1]; Cobo, Fernando[2,3]; Morcillo, Gloria[1]

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Biomarkers are an important tool in laboratory assays of specific toxicants, but they have not been widely used in invertebrate populations exposed to complex mixtures of contaminants in their natural habitats. This study focused on a battery of biomarkers and their comparative analysis in natural populations of *Chironomus riparius* larvae (Diptera), sampled in three differentially polluted rivers in Galicia (Spain). Some parameters, such as hsp70 gene activity, GST enzymatic activity and mouthpart deformities, showed significant differences among populations from the three rivers that differed in the levels and types of pollutants. In contrast, other parameters showed no significant differences (hsc70 gene, EcR gene or total protein content). Our results provide new data about the biological responses to multiple-stressor environments and add new molecular endpoints as suitable tools that may help to improve the methodologies of freshwater monitoring, suggesting hsc70/hsp70 ratio as a potential indicator of polluted environments.

Poster

[P13] **The role of circadian clock genes in the overwintering diapause of the northern house mosquito, *Culex pipiens***

Meuti, Megan E.; Denlinger, David L.
The Ohio State University, Department of Entomology

Diapause is an arrested state of development that allows insects to survive adverse seasonal conditions. Temperate insects enter diapause in response to the short day lengths of late summer and early fall. However the molecular mechanisms by which insects measure day length are unknown. The circadian clock, which provides insects with information on the time of day, might also be involved in measuring day length. To determine whether the circadian clock initiates the overwintering diapause of the Northern House Mosquito, *Culex pipiens*, we measured the expression profile of several clock genes in diapausing and non-diapausing mosquitoes. While the clock genes cycle robustly in non-diapausing mosquitoes, we found that the circadian clock genes show dampened oscillations in 1-week old non-diapausing mosquitoes. We anticipate that clock genes will cease to cycle as diapause progresses. These results suggest that the circadian clock is involved in initiating the overwintering diapause of *Cx. pipiens* mosquitoes.

Poster

[P14] **Characterizing the cold-conditioning response in a vertebrate ectotherm**

Kumar, Manisha; Wiebler, James M.; Muir, Timothy J.
Augustana College

Ectothermic animals rely on seasonal increases in cold hardiness to survive thermally variable environments, but a more rapid and supplementary increase in cold hardiness is often necessary for surviving short periods of extreme cold. Here, we further characterize the cold-conditioning response, a quickly induced supplementary increase in cold hardiness previously described in hatchling turtles, by briefly chilling winter-acclimated hatchlings (*Chrysemys picta*) to -3.5, -7, or -10.5°C and monitoring their recovery from a subsequent exposure to -12.7°C. Our results suggest that cold conditioning to -3.5 or -7°C were equally protective, whereas cold conditioning to -10.5°C did not increase cold hardiness. Cold conditioning to all three temperatures increased lactate in blood plasma, brain, and liver, but the lactate levels did not correlate well with increased cold hardiness. However, plasma and brain glucose concentrations correlated positively with survival, suggesting that increased glucose levels may have a mechanistic role in the cold-conditioning response.

Poster

[P15] **Contrasting supercooling ability in lowland and mountain European *Colias* butterflies**

Vrba, Pavel; Nedvěd, Oldřich; Konvička, Martin
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We report different values of supercooling points (SCP) of overwintering larvae of four European species belonging to the globally distributed butterfly genus *Colias*. The selected species represent diverse habitat and altitude preferences. The Mediterranean migrant *C. crocea* did not survive the acclimation temperature of 5°C. All four remaining species were freeze-susceptible. *Colias palaeno*, a peat bogs species, showed a high degree of cold hardiness (mean SCP: –24.8°C). It was followed by the steppe grassland specialist *C. alfacariensis* (–18.6°C). Alpine *C. phicomone* (–13.8°C) and lowland generalist *C. hyale* (–14.5°C) exhibited similar high values. We argue that besides ambient temperature, the specific microclimate at overwintering sites, and continentality influencing snow cover influence the diversity of cold hardiness in *Colias* butterflies. Two populations of *C. palaeno* (one mountain and one alpine) differed in accumulated concentrations of glycerol and sugars (trehalose, glucose).

Poster

[P16] **Clinal variation in ecophysiological traits in drosophilids of the Indian subcontinent**

Rajpurohit, Subhash[1,2,3]; Nedvěd, Oldřich[4,5]

[1]Maharshi Dayanand University, Rohtak, India; [2] The University of Haifa, Haifa, Israel; [3] University of Nevada, Las Vegas, USA; [4]University of South Bohemia; [5]Institute of Entomology, České Budějovice, Czech Republic

In a review and meta-analysis, we examined spatial heterogeneity across the Indian subcontinent in many morphological, life history and ecophysiological traits previously investigated (body weight, desiccation tolerance, pigmentation, copulation duration, fecundity, ovariole number, wing length, alcohol dehydrogenase fast allele frequency, rate of water loss and starvation resistance) of a dozen species of wild drosophilid fruit flies. We found a linear correlation between trait variation and latitude. Our data suggest that a single climatic component, the coefficient of variance of monthly temperature averages, which is strongly correlated with latitude, explains a large proportion of variation in the traits investigated.

Poster

[P17] **Metabolic cold adaptation in *Drosophila*: Fact or fiction?**

Messamah, Branwen; Malte, Hans; Loeschcke, Volker; Overgaard, Johannes

Department of Bioscience, Aarhus University

Metabolic Cold Adaptation (MCA) is the phenomenon whereby cold climate species display an elevated metabolic rate (MR) relative to their warm climate sister species at the same trial temperature. The presence and relevance of MCA has received mixed support in the literature, but appears to be present as a general trend among insect species across a wide range of orders. The present study examines the presence of MCA within a narrower phylogenetic context, using 67 species from the Drosophilid family representing a wide range of environmental conditions spanning temperate and tropical habitats. All species are reared under identical conditions and MR is subsequently measured at 10 and 20 ℃ using intermittent closed-system respirometry. This system allows several independent measurements of oxygen consumption rate and activity at each trial temperature, providing reliable measurements of resting MR. Preliminary analysis suggests a negative correlation between mass-specific MR and latitude (opposite the MCA hypothesis). This correlation is, however, driven largely by a size effect such that no correlation is present when controlling for the effects of size on MR.

Poster

[P18] **The role of ice active proteins in the freezing tolerance mechanisms of *Hemideina maori***

Paschke, Dominik
University of Otago

Ectotherms exposed to temperatures below their body fluids' melting point employ two main strategies: freeze tolerance and freeze avoidance. Freezing at relatively high sub-zero temperatures and surviving 82% of its body water freezing, the New Zealand alpine weta, *Hemideina maori*, is the world's largest freezing tolerant insect. However, little is known about the role of ice active proteins in the cold tolerance of *H. maori*. This study aimed to investigate *H. maori*'s tissues for thermal hysteresis, using nanolitre osmometry, and recrystallization inhibition (RI) activity, using optical recrystallometry and splat freezing assay. Varying degrees of RI were found, but very little thermal hysteresis. Thus RI is probably important for *H. maori*'s cold tolerance strategy, perhaps by controlling the shape and/or size of ice crystals. More research is needed to further investigate the RI factor and its importance to *H. maori*'s freezing tolerance and determine the importance of low molecular weight cryoprotectants.

Poster

[P19] **Seasonal stability of supercooling points in spiders living in a temperate ecosystem**

Philip, Benjamin N.; Whitney, Thomas D.; Harwood, James D.
Rivier University; University of Kentucky; University of Kentucky

Although many studies have examined the physiological responses of ectotherms to cold, little of this research has focused on spiders inhabiting temperate regions. Spiders are predators that can often be found actively hunting in a winter landscape. Understanding the relationship between these active winter predators and low temperature tolerance was the focus of this nine month study. Two species of wolf spiders (*Schizocosa ocreata* and *Schizocosa stridulans*) were collected monthly in a deciduous forest in Kentucky and exposed to supercooling point (SCP) determination assays. Our results show that the SCP did not significantly change from August 2012 to April 2013. Interestingly, temperature monitoring of the leaf litter determined that the daily minimum temperature dropped below the average SCP of the spiders on multiple occasions during the winter, however we determined that these spiders were freeze-intolerant and their lower lethal temperature appears to be their SCP. To determine if the relatively high SCPs were a result of the spiders feeding during the winter, trials in which spiders were fed before testing did not result in significantly different average SCPs. The results from this study suggest that despite the seasonal changes in temperatures experienced by the spiders, their SCPs do not change, which therefore places them at a high risk of freezing in the winter. This increased freezing risk associated with relatively high SCPs may be a tradeoff with an increase in foraging level in the winter that provides these spiders an advantage over others in the spring.

Poster

[P20] **Membrane adaptation in phospholipids and cholesterol in the widely distributed, freeze-tolerant wood frog, *Rana sylvatica***

Reynolds, Alice M.; Lee, Richard E.; Costanzo, Jon P.
Miami University

Maintaining proper membrane phase and fluidity is important for preserving membrane structure and function in the face of environmental stress. By altering membrane composition, organisms can adapt to changing conditions. We investigated the phospholipid and cholesterol composition of liver and brain plasma membranes of *Rana sylvatica* seasonally in populations from Ohio and Alaska, after thermal acclimation, and during acute freezing/thawing exposure. Cholesterol levels remained constant in all experiments except in the brain after thermal acclimation, where there was less cholesterol at lower temperature, which is a common response to increase fluidity. Accordingly, liver membranes contained less cholesterol in Alaskan than Ohioan frogs. Whereas brain phospholipid composition did not change in any experiment, liver phospholipids changed in all but the freeze/thaw experiment. Predictably, phosphatidylethanolamine increased with lower acclimation temperature. From summer to winter, Ohioan frogs decreased phosphatidylcholine and increased sphingomyelin, while Alaskan frogs decreased phosphatidylserine, indicating different strategies for membrane adaptation.

Poster

[P21] **Importance of a glucose transporter in the cryoprotectant system of the freeze-tolerant wood frog, *Rana sylvatica***

Rosendale, Andrew J.; Lee, Richard E.Jr.; Costanzo, Jon P.
Laboratory for Ecophysiological Cryobiology, Department of Zoology, Miami University, Oxford, OH 45056 USA

Accumulation of the cryoprotectant glucose, mobilized from liver glycogen, promotes freezing survival of the wood frog, *Rana sylvatica*. We investigated the hepatic glucose transporter (GLUT2) in relation to freezing-induced mobilization of glucose and freeze tolerance. Transport kinetics of GLUT2, which was inhibited by the cryoprotective solute urea, did not differ between Alaskan and Ohioan *R. sylvatica*, but GLUT2 abundance was 3.5-fold greater in the northern frogs. Organismal exposure to various winter-related stresses that cause glucose accumulation in frogs, including freezing, anoxia, and dehydration, all resulted in a two-fold increase in liver GLUT2 abundance. Seasonal variation and freezing response of GLUT2 was compared between northern and southern phenotypes. Our results suggest that GLUT2 is important in freezing survival of *R. sylvatica*, and that greater GLUT2 abundance enhances the glucosic cryoprotectant system, potentially contributing to the evolution of extreme freeze tolerance in northern wood frogs.

Poster

[P22] **Shedding light on thermoperiodism in a drosophilid fly, *Chymomyza costata***

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Larvae of the drosophilid fly, *Chymomyza costata* have been proven to be a remarkable and unique model to study cold acclimation and freeze tolerance. Survival of extreme coldness requires entering diapause, which can be induced by fluctuations of reliable environmental signals such as light and temperature. Whereas the understanding of photoperiodically induced diapause is relatively enhanced, much is unknown about the mechanisms of thermoperiodism. We exposed larvae of *C. costata* to various light and temperature regimens to estimate the influence of the thermoperiodic signal, either separately or combined with photoperiodism. Both zeitgebers were sufficient to induce diapause solely but when combined in a “conflicting” manner, it has been shown that light/dark cycles dominate the thermoperiodic information. In the ongoing study, we investigate the ways the photo- and thermoperiodic signals affect core elements of the fly's central circadian clock.

Poster

[P23] **The response of circadian clock neurons in the brain of *Chymomyza costata* to photoperiodic and thermoperiodic signals**

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While the circadian clock system helps the insects adapting to daily light/dark cycle, the photoperiodic calendar allows them coping with annual cycles in their environment. Both systems likely cooperate. The nature of such cooperation, however, remains largely unknown. We expose the larvae of drosophilid fly, *C. costata* to different photoperiodic and thermoperiodic conditions and study the expression of circadian clock proteins such as PER, TIM, and PDF in the brain neurons using confocal and fluorescence microscopy. Responses of wild-type (Sapporo) and non-photoperiodic (NPD) strains will be compared in order to subtract all possible responses that are not reflected in photo- and thermoperiodically driven phenotypic change– induction of larval diapause.

Poster

[P24] **Funcional analysis of the expression of HSPs complex in response to cold in *Drosophila melanogaster***

Štětina, Tomáš [1,2]; Korbelová, Jaroslava[2]: Koštál, Vladimír [1,2]

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The rapid expression of Heat Shock Proteins (HSPs, dominated with HSP70) in response to high temperature was proven as important mechanism of survival in *Drosophila melanogaster*. Is the expression of HSP70 vital also for survival at low temperature? We compared responses to cold in the larvae of a wild -type strain (Oregon) and a mutant strain (w1118) that lacks all 7 copies of hsp70 gene. Survival after chronic exposure to 0°C did not differ in two strains. While the wild-type strain showed remarkable up-regulation of hsp70 gene at transcript (250-fold increase) and protein levels (3.5 fold increase), similar response was not possible in the mutant strain. Moreover, we detected no compensatory up-regulation at transcript level for missing hsp70 in another 23 different genes of the HSPs complex in the mutant strain. At protein level, surprisingly, we have seen a 2.5-fold up-regulation of so far unidentified protein of approximate size 70 kDa.

Poster

[P25] **Evaporative cooling augments prey detection in the rattlesnake, *Crotalus durissus***

Tattersall, Glenn J[1]; Cadena, Viviana[1]; Bovo, Raphael[2], Andrade, Denis V.[2]

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Snakes possess a profound degree of respiratory evaporation, which significantly cools the face and head. As pit vipers, rattlesnakes also utilize their forward-facing facial pit organs for sensing thermal fluctuations associated with their prey. The greater the difference in temperature between the prey and the pit organ, the greater the thermal flux; cooler pit organs, therefore, are hypothesized to provide greater thermal detection. We examined the potential for respiratory cooling to enhance the rattlesnake's ability to sense their endothermic prey by exposing two group of snakes to different humidities to manipulate the degree of respiratory cooling. The latency for snakes to find their prey was significantly longer at high than at low humidity. These differences were accompanied by significant respiratory and facial cooling at low humidity compared to high humidity. This cooling imparts greater capacity to detect endothermic prey suggesting that snakes may exhibit some degree of respiratory control over thermal detection.

Poster

[P26] **Overwintering energetics of *Harmonia axyridis*: The effects of cold and mild winters**

Vukasinovic, Dragana[1]; Overgaard, Johannes[2]; Holmstrup, Martin[3]; Esbjerg, Peter[1]

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In North–West Europe, including Denmark, it is predicted that annual temperatures will increase especially during winter. Overwintering conditions play an important role for the fitness of insects and warmer winters may cause loss of energy reserves compared to cold winters. *Harmonia axyridis* (Pallas), (Coleoptera: Coccinelidae) overwinters as an adult. After winter diapause, the rate of population growth depends on the overwintering survivors. Survived coccinellids, after diapause, feed on aphids to fuel up their subsequent reproduction and oviposition. Here, we investigated the energetic costs and reproductive output of overwintering on *H. axyridis* exposed to two contrasting temperature regimes representing cold and mild winters in Denmark (3 months at -5˚C or +5˚C, respectively). Metabolic rate at 5˚C was 6 fold higher than at -5˚C, and maintenance at constant -5˚C did not ellict a metabolic compensation. Accordingly warm winters exhausted energy reserves such that both dry mass and lipid content was significantly lower in animals overwintering at high temperature. Feeding and mating behavior of the emerging adults was, however, only marginally affected by winter conditions suggesting that the stored energy reserves were sufficient to ensure onset of population growth.

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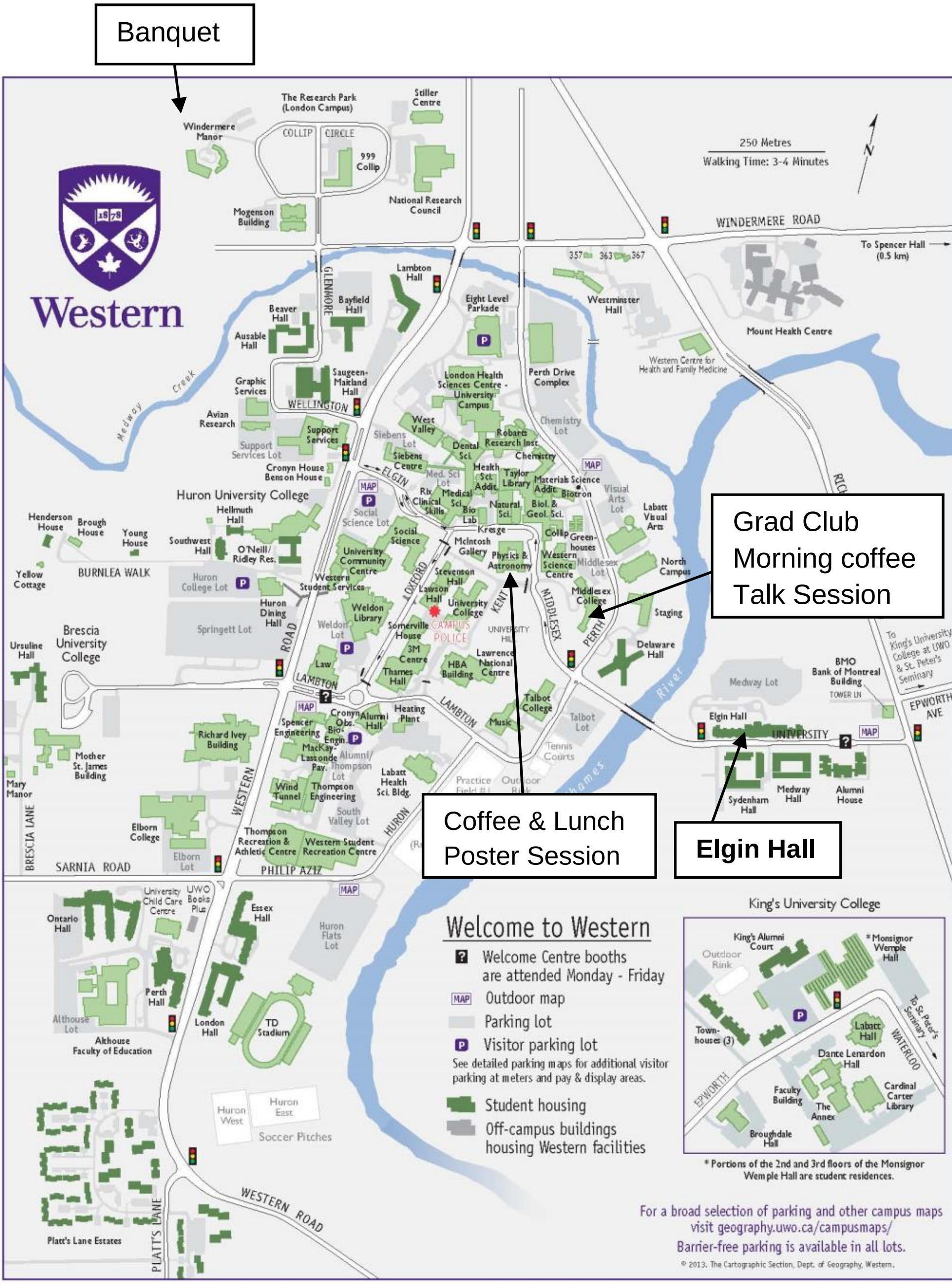
	Monday	Tuesday	Wednesday	Thursday	Friday
09:00	Coffee	Coffee	Excursion all day	Coffee	
09:30	Welcome	Insect Environmental Physiology		Diapause	Coffee
09:45					
10:00	Plenary Talk				Business meeting & awards
10:15					
10:30					
10:45		Coffee		Coffee	Plenary Talk
11:00	Coffee	Water & Ion Balance		Insect Cold Tolerance	
11:15					
11:30	Evolutionary Physiology				
11:45					Farewell
12:00					Lunch
12:15					
12:30	Lunch	Lunch		Lunch	
14:00	Sinclair Lab Extravaganza	Molecular Physiology I		Temperature & Performance	
14:15					
14:30					
14:45					
15:00		Coffee		Coffee	
15:15	Coffee	Molecular Physiology II		Mechanisms Underlying Cold Tolerance	
15:30					
15:45	Brief Presentations				
16:00					
16:15					
16:30		Group Photo			
Evening	Reception [Grad Club]	Poster Session [P & A Atrium]	Free	Banquet [Windermere Manor]	

-Morning Coffee (Middlesex College, TA106)
-Talks (Middlesex College, 110)
-Lunch & Coffee (Physics & Astronomy Atrium)



Banquet
Grad Club
Morning coffee
Talk Session
Coffee & Lunch
Poster Session
Elgin Hall
Western
Windermere Manor
The Research Park (London Campus)
Stiller Centre
COLLIP CIRCLE
999 Collip
National Research Council
Mogenson Building
250 Metres
Walking Time: 3-4 Minutes
WINDERMERE ROAD
To Spencer Hall (0.5 km)
Lambton Hall
Eight Level Parkade
Westminster Hall
Mount Health Centre
Western Centre for Health and Family Medicine
Bayfield Hall
Beaver Hall
Ausable Hall
GLENMORE
Saugeen-Maitland Hall
Graphic Services
London Health Sciences Centre - University Campus
Perth Drive Complex
Avian Research
WELLINGTON
Support Services
Support Services Lot
West Valley
Chemistry Lot
Medway Creek
Cronyn House
Benson House
Siebens Lot
Siebens Centre
Dental Sci.
Robarts Research Inst.
Chemistry
Huron University College
Hellmuth Hall
Henderson House
Brough House
Young House
Southwest Hall
O'Neill/ Ridley Res.
Yellow Cottage
BURNLEA WALK
Huron College Lot
Social Science Lot
Social Science
University Community Centre
Western Student Services
Med. Sci Lot
Health Sci. Addit
Taylor Library
Materials Science Addit
Biotron
Rix Clinical Skills
Medical Sci.
Bio Lab
Natural Sci.
Biol. & Geol. Sci.
Visual Arts Lot
Labatt Visual Arts
Kresge
McIntosh Gallery
Physics & Astronomy
Collip
Green-houses
Western Science Centre
Middlesex Lot
North Campus
Middlesex College
Staging
Stevenson Hall
Lawson Hall
University College
CAMPUS POLICE
UNIVERSITY HILL
Huron Dining Hall
Springett Lot
Weldon Library
Weldon Lot
Somerville House
Delaware Hall
Brescia University College
Ursuline Hall
ROAD
Law
3M Centre
Thames Hall
HBA Building
Lawrence National Centre
Medway Lot
BMO Bank of Montreal Building
TOWER LN
To King's University College & St. Peter's Seminary
EPWORTH AVE
LAMBTON
Spencer Engineering
Cronyn Obs.
Alumni Hall
Heating Plant
Talbot College
Talbot Lot
Elgin Hall
UNIVERSITY
Richard Ivey Building
Bio-Engin.
MacKay-Lassonde Pav.
Alumni/ Thompson Lot
Music
Tennis Courts
River
Thames
Mother St. James Building
Mary Manor
Labatt Health Sci. Bldg.
Practice Field #1
Outdoor Rink
Sydenham Hall
Medway Hall
Alumni House
Wind Tunnel
Thompson Engineering
South Valley Lot
BRESCIA LANE
Elborn College
WESTERN
HURON
Thompson Recreation & Athletic Centre
Western Student Recreation Centre
SARNIA ROAD
Elborn Lot
PHILIP AZIZ
Ontario Hall
University Child Care Centre
UWO Books Plus
Essex Hall
Huron Flats Lot
King's University College
Welcome to Western
Welcome Centre booths are attended Monday - Friday
Outdoor map
Parking lot
Visitor parking lot
See detailed parking maps for additional visitor parking at meters and pay & display areas.
Student housing
Off-campus buildings housing Western facilities
Perth Hall
Althouse Lot
Althouse Faculty of Education
London Hall
TD Stadium
King's Alumni Court
Outdoor Rink
*Monsignor Wemple Hall
Town-houses (3)
Labatt Hall
Dante Lenardon Hall
To St. Peter's Seminary
WATERLOO
EPWORTH
Faculty Building
The Annex
Cardinal Carter Library
Broughdale Hall
* Portions of the 2nd and 3rd floors of the Monsignor Wemple Hall are student residences.
Huron West
Huron East
Soccer Pitches
Platt's Lane Estates
PLATT'S LANE
WESTERN ROAD
For a broad selection of parking and other campus maps visit geography.uwo.ca/campusmaps/
Barrier-free parking is available in all lots.
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