Welcome to

ISEPEP

Sponsored by

CARLSBERG FOUNDATION

SABLE SYSTEMS INTERNATIONAL

SOLIDUM PETIT IN PROFUNDIS · UNIVERSITAS ARHUSIENSIS ·

Aarhus, Denmark, August 2015

ISEPEP6

Important places for ISEPEP6:

Below is a map of Aarhus University showing the three important places relevant for the conference.

Sunday evening early attendees are welcome at an **Icebreaker event at Zoophysiology,** which is in building 1131 (close to the steno museum at the campus) – This will also be the meeting place for the welcome reception on Monday after the sessions.

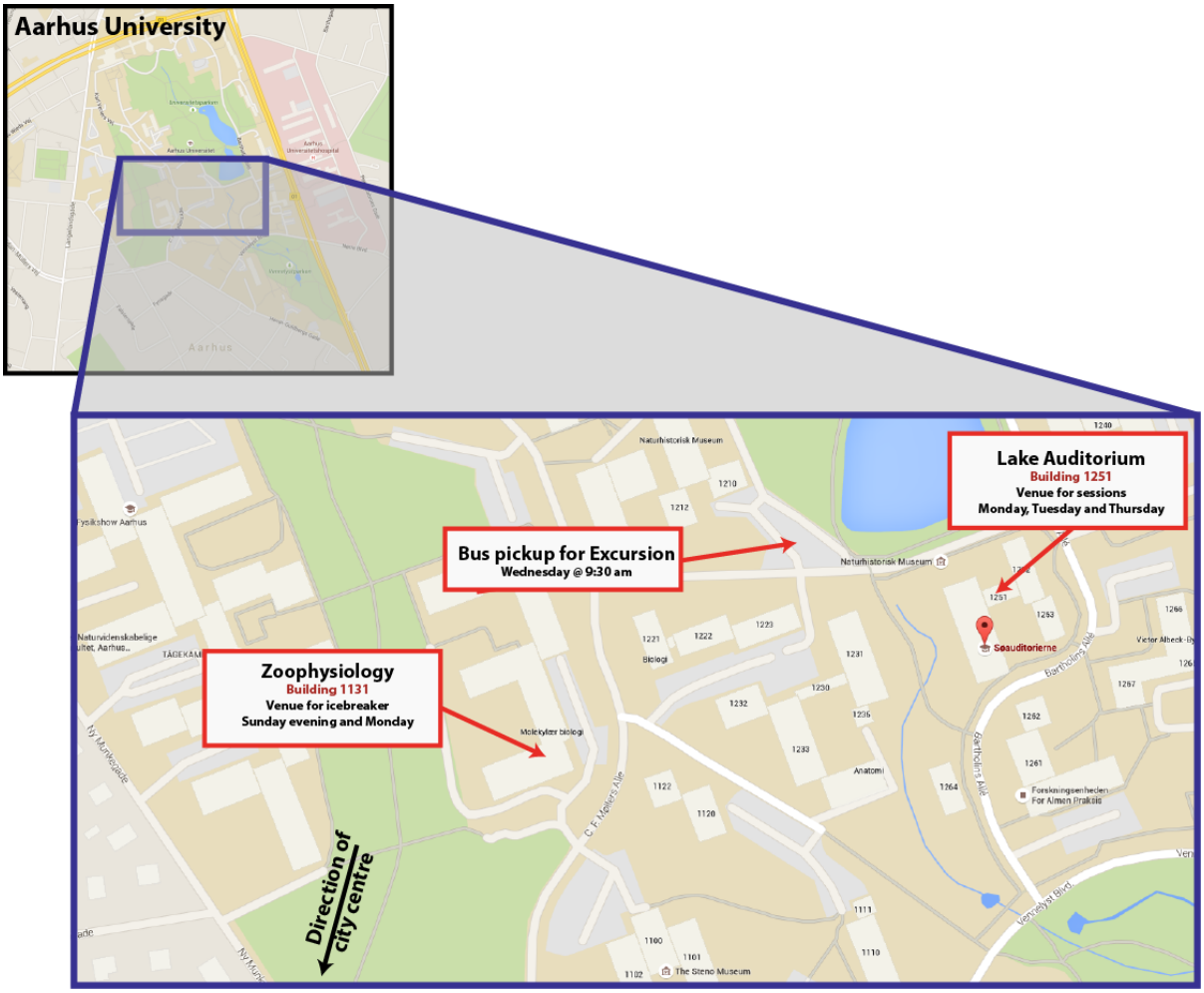
Monday, Tuesday and Thursday the **sessions** will be held at the lake auditoria (building 1251).

Wednesday we have **an excursion** where the bus will pick you up at 9.30 AM at the parking lot in front of the natural history museum.

All three places are within a few hundred meters so it should all be easy to find.

If you want to explore where the campus is relative to your hotel we suggest you use google maps and zoom in from this link that shows the lake auditoria:

https://goo.gl/maps/4NHZp



Program

Sunday 2nd August 2015

18.00-20.00 **Registration, drinks and snacks, Department of Bioscience, Zoophysiology**

Program Monday 3rd August 2015

9.00-9.15 Welcome and practical information

9.15-10.00 Keynote by Joel Kingsolver: Organismal and evolutionary responses of insects to variable and changing climates

10.00-10.30 **Break for coffee**

Session: Behaviour and abiotic environmental factors – chair: Johannes Overgaard

10.30-10.50 Tanya Dann: The dance of the damselflies: Does starvation affect movement behaviour?

10.50-11.10 Indrikis Krams: Life-history, anti-predator responses, metabolism and non-repeatable personality across contexts differing in the risk of predation in a cricket

11.10-11.30 Toke T. Høye: Early spring activity of Marsh fritillary in relation to abiotic conditions assessed with time lapse cameras

11.30-11.50 Simon Bahrndorff: Light cues are of fundamental importance for behavioral responses to temperature changes

11.50-13.00 **Lunch**

Session: Multiple stresses and multiple stressors – chair: Scott Hayward

13.00-13.20 Jose-Luis Martínez-Guitarte: Identification and analysis of stress and endocrine related genes in *Chironomus riparius*

13.20-13.40 Michael Ørsted: Investigating fitness consequences of single and multiple stresses experienced during development in *Drosophila melanogaster* and *Drosophila hydei*

13.40-14.00 Gregory Ragland: Running the transcriptome through the gauntlet: a comparative analysis of core gene expression responses to multiple stressors

14.00-14.20 Behnaz Ghaedi: The impacts of repeated high temperature exposure on green peach aphids

14.20-14.40 Brent Sinclair: Thermal biology of insect immunity

14.40-15.10 Break for coffee

Session: Diapause – chair: Gregory Ragland

15.10-15.30 Thomas MacRae: Diapause and Stress Tolerance in the Crustacean, *Artemia franciscana*

15.30-15.50 Vladimir Kostal: Global transcriptomic analysis of diapause induction in the drosophilid fly, *Chymomyza costata*

15.50-16.10 Julian Mensch: Temperate but not tropical *Drosophila* species maintain high fertility after long-term cold exposure: evidence for reproductive diapause?

16.10-16.30 Scott Hayward: Diet and diapause influence rapid cold hardening and activity thresholds in *Calliphora vicina*

17.00-19.00 **Ice breaker, drinks and snacks (Zoophysiology canteen)**

Program Tuesday 4th August 2015

9.00-9.15 Welcome and practical information

9.15-10.00 Keynote by Meldrum Robertson: Coping with environmental stress: regulation of potassium ion homeostasis in the insect CNS

10.00-10.30 **Break for coffee**

Session: Heat tolerance (and a little cold) – chair: Meldrum Robertson

10.30-10.50 Tommaso Manenti: Mechanisms underlying the heat resistance of flies selected in fluctuating thermal regimes

10.50-11.10 Heidi MacLean: Geographic divergence in upper thermal limits across insect life stages: Does behavior matter?

11.10-11.30 James O'Sullivan: Hot locusts experience loss of ion balance: An exploration of its causes and effects

11.30-11.50 Lauren Des Marteaux: How does cold acclimation affect ion transport, gene expression, and ultrastructure of the insect hindgut?

11.50-13.00 **Lunch**

Session: Chill tolerance – chair: Brent Sinclair

13.00-13.20 Ruth Jakobs: Chillin' in Ontario – Plasticity of cold tolerance in adult *Drosophila suzukii*

13.20-13.40 Johannes Overgaard: The physiology of chill tolerance – Linking phenotype to mechanism

13.40-14.00 Jonas Andersen: Cold tolerant drosophilids preserve muscle resting membrane potential at low temperatures

14.00-14.20 Anders Findsen: Loss of muscle function during acute cooling in locust is caused by reduced Ca^{2+} transients as a result of altered muscle fiber excitability

14.20-14.40 Heath Macmillan: Concurrent effects of cold and hyperkalemia cause insect chilling injury

14.40-15.00 **Break for coffee**

15.00 – 18.00 **Poster session – (now with beer)**

Wednesday 5th August 2015

9.30-15.00 **Excursion**

Program Thursday, August 6, 2015

9.00-9.15 Welcome and practical information

9.15-10.00 Keynote by Kai Finster: The role of microorganisms in atmospheric processes and its implication for life detection on exoplanets

10.00-10.30 **Break for coffee**

Session: Cold tolerance – Chair: Martin Holmstrup

10.30-10.50 Tomáš Ditrich: SCP value in insects - is it useful index of lower lethal temperature?

10.50-11.10 Herve Colinet: Complex regulatory processes underlie the physiological response of *Drosophila melanogaster* to cold acclimation

11.10-11.30 J.D. Gantz: The limits of drought-induced rapid cold-hardening: Extremely brief, mild desiccation triggers enhanced freeze-tolerance in *Eurosta solidaginis* larvae

11.30-11.50 Kate Mitchell: Understanding costs and benefits of plastic physiological performance: insights from the integration of laboratory, semi-field and field assessments

11.50-13.00 Lunch

Session: Overcoming winters and droughts – Chair: Joel Kingsolver

13.00-13.20 Peter Convey: Invasions in the Antarctic

13.20-13.40 Martin Schebeck: Development and overwintering of the larch bark beetle, *Ips cembrae* (Coleoptera, Curculionidae)

13.40-14.00 David Renault: Comparative physiological plasticity to desiccation among distinct populations of the malarial mosquito, *Anopheles coluzzii*

14.00-14.20 Mark Bayley: Amino acids as compatible osmolytes during dehydration in soil invertebrates

14.20-14.40 Elizabeth P. Dahlhoff: Adaptation to thermal stress in a Finnish butterfly threatened by climate change.

14.40-15.10 Break for coffee

Session: Thermal biology, developmental optima and plasticity – Chair: Johannes Overgaard

15.10-15.30 Stine Slotsbo: Reversibility of developmental acclimation in *D. melanogaster*

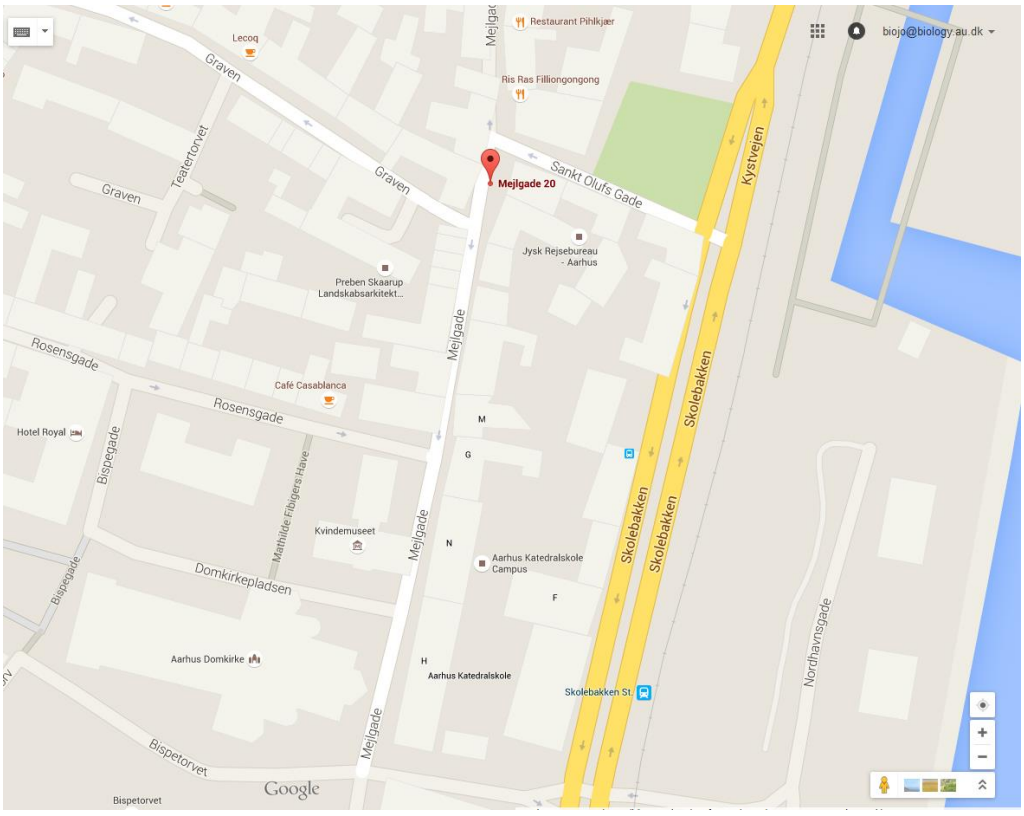
15.30-15.50 Maxim Timofeyev: Determination of Lake Baikal endemic and Palearctic amphipods thermal optima limits by changes in its stress markers

15.50-16.10 Elena Lopatina: Environmental plasticity of thermal reaction norms for development in true bugs: intergenerational and interpopulational differences

16.10-16.30 Dmitry Kutcherov: Thermal reaction norms for development in leaf beetles (Chrysomelidae): variation across taxa and latitudes

16.30-16.50 Nathan E. Rank: Mitonuclear interactions influence cold and heat tolerance along elevation gradients in a montane insect

19.30 Conference dinner at "Den Rustikke" Mejlgade 20, 8000 Aarhus C (see map below)



Keynote lecture

Organismal and evolutionary responses of insects to variable and changing climates

Joel Kingsolver

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Abstract:

Insects and other terrestrial organisms experience variation in temperature, radiation and other environmental factors at a variety of spatial and temporal scales. I will describe two case studies with insects that consider variation across two temporal scales—diurnal fluctuations in temperature, and decadal changes in recent climate—and their consequences for performance, fitness, selection and evolution. First, experimental studies with Manduca (hornworm) larvae show that diurnal temperature fluctuations alter rates of growth, development and survival, due both to the non-linear effects of temperature on performance (thermal performance curves) and to the time-dependent effects of stress and physiological acclimation. We develop a simple mathematical model that extends thermal performance curves to incorporate time-dependent effects of heat stress, and predicts growth rates in fluctuating environments. Second, we use historical data, recent experiments, and modeling to evaluate potential evolutionary responses of Colias butterflies to recent climate changes. Our empirical studies detect evolutionary shifts in phenotypic traits for some life stages but not others. Our models for the fitness and evolutionary effects of climate on Colias adults suggest that weather variability among years causes fluctuations in the fitness landscapes for key thermoregulatory traits, strongly limiting evolutionary responses to overall climate warming.

The dance of the damselflies: Does starvation affect movement behaviour?

Tanya Dann*, Grant Humphries and Gerry Closs

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Abstract:

An organism's activity is shown that fast-slow life history strategy dichotomies exist between multiple organisms and within communities of Odonate assemblages. Slow species display a sit and wait behaviour, waiting for prey to come to them, while fast species actively hunt. It is expected that during periods of starvation, the movement behaviour of slow species will decrease to allow survival through the lean times until food becomes available. On the other hand, when fast species are starved, individuals are expected to increase their movements with the aim of increasing their rate of encounters with sparsely distributed prey as they have higher net energetic demand that cannot be reduced.

Larvae of two species of damselflies (Odonata: Zygoptera) were collected from a single site in rural Otago, New Zealand. Movement behaviour was recorded for each individual as they starved. Xanthocnemis zealandica (a slow species) took longer to starve and moved less than Austrolestes colensonis (a fast species). Differences in starvation tolerance and movement behaviour between the two species are consistent for species exhibiting a fast-slow life history dichotomy. However, contrary to expectations, as starvation occurred Xanthocnemis movement increased and Austrolestes decreased. The increase in movement of Xanthocnemis is likely to be due to plasticity in behavioural responses. It becomes advantageous for individuals to abandon the sit and wait behaviour and increase their chances of encountering prey. The higher movement rate of Austrolestes uses up valuable resources foraging for non-existent prey, once energy is used up, there is no more available and movement decreases before death occurs.

Life-history, anti-predator responses, metabolism and non-repeatable personality across contexts differing in the risk of predation in a cricket

Indrikis Krams*, Tatjana Krama, Sanita Kecko, Petri Niemelä, Marika Mänd, Raine Kortet

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Abstract:

Repeatability of behavioral responses is essential for animal personality, while the mere presence of predators may cause a gradient of predation risk across environments affecting decisions of prey individuals. In this study we measured, as a proxy for boldness, the time spent immobile after handling in familiar and unfamiliar environments, and resting metabolic rate (RMR) in western stutter-trilling crickets, *Gryllus integer*. The crickets were selected for their life-historical developmental time for two generations. Under conditions of the familiar environment of their housing-boxes, after being handled slowly developing, often shy, crickets resumed body movements significantly later than rapidly developing, often bold, crickets and control individuals. We also found that slowly developing crickets resumed active movements significantly sooner under conditions of unfamiliar environment of the insect chamber where they were placed to measure RMR. All the measurements of behavioral reactions and RMR were highly repeatable. However, repeatability of behavioral responses was significant only within the same environment, while the responses were not repeatable across contexts. Surprisingly, slowly developing individuals had higher RMR than control and rapidly developing crickets, suggesting that slowly developing individuals resume their movements in unfamiliar environment because they are under higher physiological stress than control and rapidly developing crickets. This shows the importance of environmental stress on individual decisions of prey individuals. Thus, stress reactivity of animals of different life-history and personality types may make detection of repeatability of anti-predator responses impossible especially when environments differ in the levels of risk. We also found that the lowest rates of CO2 emission differed in crickets in relation to their personality. While bold individuals reached their RMR within 1 h, shy crickets needed 3 h to reach the lowest levels of CO2 emissions. This should be taken into account when measuring metabolism in animals of different personality.

Early spring activity of Marsh fritillary in relation to abiotic conditions assessed with time lapse cameras

Toke T. Høye*, Giedre Belousova, Nanna R. Lauridsen

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Abstract:

Larvae of the Marsh fritillary (*Euphydryas aurinia*) become active very early in the season. This species, which is critically endangered in Denmark, overwinters in family groups consisting of several hundred larvae. The early activity is thought to be an adaptation to avoid parasitism by reaching the pupal stage before parasitoids appear. The larvae are black and are often seen basking in groups during sunny days already in February in Denmark. However, little is known about the abiotic controls of this behaviour as well as the transition towards solitary movement which has been observed before pupation. We studied early spring behaviour of Marsh fritillary larvae using several hundred thousand images recorded with six time lapse cameras during the spring 2015. We are able to demonstrate that sunny weather is much more important than changes in air temperature for the activity and aggregative behaviour of Marsh fritillary larvae. We estimate the period during spring and the abiotic conditions, where aggregative behaviour was observed and discuss the possibilities of estimating overwinter survival from revisits to larval web groups marked during the previous growing season. Our results demonstrate that time lapse recordings can be a suitable tool to quantify movement behaviour of butterfly larvae under field conditions.

Light cues are of fundamental importance for behavioral responses to temperature changes

Simon Bahrndorff*, Niels Hansen, Thomas, Primdahl, Morten Hansen, Thøger Nielsen, Tina Trolle, Torsten Nygaard Kristensen and Anders Kjærsgaard

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Abstract:

Organisms can respond to and cope with stressful environments in a number of ways including behavioural, morphological and physiological adjustments. Still, the importance of behavioural adjustments to thermal stress is poorly understood. Studies have shown inter- and intraspecific differences in behavioural responses at benign and stressful high temperatures. However, it is unclear if behavioural responses are simply a consequence of differences in thermal resistance or if insects actively use behavioural adjustment to avoid stressful conditions. To address this question, we tested if the fruit fly Drosophila melanogaster uses light cues for behavioural adjustments to temperature stress. In this experiment we exposed flies to increasing temperatures while allowing them to choose between light and dark areas and while monitoring their activity in respectively the light and dark area. Results show that at moderate temperatures flies prefer to stay in the light, whereas at stressfully high temperatures flies show a preference for the dark area. The results suggest that light cues are of fundamental importance for behavioral responses to temperature changes and that flies seek dark habitats when it becomes too warm. We thus suggest that behavioural adjustments to high temperatures are of evolutionary importance and flies actively use them to avoid stressful thermal conditions.

Identification and analysis of stress and endocrine related genes in *Chironomus riparius*

Mónica Aquilino, Irene Ozáez, Pedro-José Martínez-Paz, Raquel Martín-Folgar, Óscar Herrero, Rosario Planelló, Mónica Morales, Estrella Cortés, José-Luis Martínez-Guitarte*, Gloria Morcillo

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Abstract:

Chironomids are a widely distributed group of dipterans and some species, like *Chironomus riparius*, *Chironomus tentans*, and *Chironomus tepperi*, have a relevant role in ecotoxicology and ecological risk assessment of chemicals. They are distributed ubiquitously and can be found in such diverse places as Antarctica, freshwater streams worldwide, sea, and dry environments. Although several species have been extensively used in a diverse number of research areas they are still very poorly known at molecular level. Stress and hormonal genes are key players in the adaptation of an organism to the environment. The identification of genes that are involved in the endocrine system and the stress response is a first step to improve our knowledge about the physiology of these organisms and to understand how they have adapted to this great diversity of environments. Recently it has been carried out a great effort to perform transcriptome projects in some chironomids like *Belgica antarctica*, *Polypedilum vanderplankii*, *Chironomus riparius*, and other related species. Taking advantage of the data uploaded to the databases we have searched *Chironomus riparius* genes related with the endocrine system and with stress. Several transcripts belonging to the heat shock protein family have been found. On the other hand, sequences from genes involved in hormone synthesis and response pathway have been identified. All of them show high homology with Drosophila and other Diptera genera. These transcripts have been characterized and analyzed in fourth instar larvae by retrotranscription and Real-Time PCR. Additionally a study to analyze how they response to different chemicals has been carried out and the obtained results show a complex expression profile that varies depending on the substance tested. Globally, our results improve the knowledge about the endocrine system and the stress response in this species and will help to a better understanding of the physiology of this group of insects. Furthermore, these results provide the most comprehensive picture to date of the *Chironomus riparius* endocrine system opening new possibilities to analyze its development.

This work has been funded by the Ministerio de Economía y Competitividad, CICYT (SPAIN), CTM2012-37547.

Investigating fitness consequences of single and multiple stresses experienced during development in *Drosophila melanogaster* and *Drosophila hydei*

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Abstract:

Environmental conditions play a major role in the ecological and evolutionary dynamics of natural populations. During the last century, many plant and animal population have experienced rapid deterioration of their natural habitat e.g. due to climate change and anthropogenic impacts, which have led to an increased level of environmental stress perceived by individuals. By definition exposure to abiotic and biotic stresses decrease fitness of populations relative to benign conditions. However, in nature environmental stresses seldom occur alone, but populations will experience a multitude of different stresses simultaneously, e.g. stressful temperatures, malnutrition and exposure to toxicants. An important question is whether decreases in fitness are additive when stresses are combined, or if fitness is decreased more (or less) than expected under the assumption that each stress act independently. When the simultaneous effects of the stressful environmental conditions are not additive there is an environment (E) by E interaction. The fitness consequences of interactions between environmental stresses (E x E, and possibly E x E x E interactions) are not yet fully understood. In this study we aim at investigating the effects of thermal, chemical and competition stress experienced during development of *Drosophila melanogaster* and *D. hydei* on fitness components, including viability, thermal resistance and behavioural traits. Specifically, we examine the effects of developing at cold, benign and hot temperatures and two levels of chemical stress (i.e. as different concentrations of the insecticide, dimethoate) on both Drosophila species separately and in competition. We investigate each stressor individually and in every possible combination to elucidate the complex nature of effects of multiple stresses. Preliminary results suggest an interaction between the effects of dimethoate and thermal stress and that the fitness consequence of stresses are highly trait and species specific.

Running the transcriptome through the gauntlet: a comparative analysis of core gene expression responses to multiple stressors

Gregory Ragland

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Abstract:

With the advent of microarray, and more recently next-generation sequencing technology, we are rapidly accumulating massive amounts of information about how genes are expressed in a variety of tissues and in response to a variety of environmental factors. One basic conclusion from transcriptome-level work is that the transcriptome is highly plastic, with a large percentage of genes often displaying differential regulation across environments. This is particularly true when organisms are exposed to stressful environments at or near their tolerance limits. In yeasts, there appears to be a core 'cassette' of genes, termed the Environmental Stress Response (ESR), that are nearly universally up- or down- regulated in response to a variety of stressors. But, the regulatory mechanisms that mediate the ESR are not conserved across animals, and similar core responses have not been identified in other taxa. Using published transcript profiling data sets from Drosophila and Daphnia, I will present evidence for a core response in non-yeast systems, including tests for any generality of this set across Drosophila, Daphnia, and *Saccharomyces cerevisiae*.

The impacts of repeated high temperature exposure on green peach aphids

Behnaz Ghaedi*, Nigel R. Andrew

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Abstract:

Up to now, the key assessment of environmental stress tolerance in the aphid, *Myzus persicae* has been at low temperatures. In these cases, and in the rare cases of high temperature tolerance being assessed, all of them expose the aphid to a single stress event. In nature, *Myzus persicae* is exposed to repeated bouts of high temperature stress punctuated by periods of recovery. In the present study, we examined the physiological consequences of repeated high temperature with recovery between them in *Myzus persicae*. We subjected individuals to either a single prolonged three hour heating event, or three one hour heating events with 24 hours recovery time between bouts. We observed more cost and benefits in multiple heating events: aphids exposed to multiple bouts of high temperatures had a higher thermal tolerance, more glucose and higher expression of proteins and osmolytes compounds such as glycerol compared to the prolonged exposure group. But the repeated high temperature group have reduced sources of energy such as trehalose and triglyceride compounds and lower active metabolic rate than the prolonged exposure group. In conclusion, recovery time has more costs (based on production of more protein and increasing metabolic rate and consumption more trehalose and triglyceride) and benefits (based on production of more osmolytes and higher thermal tolerance) in repeated high temperature experiment. In addition, because aphids are known to respond differently to constant versus more 'natural' fluctuating temperature regimes, conclusions drawn from constant temperature data may be problematic, and we suggest future experiments incorporate a repeated stress and recovery pattern into their methodologies.

Thermal biology of insect immunity

Brent J. Sinclair, Golnaz Salehipour-shirazi, and Laura V. Ferguson

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Abstract:

Research on overwintering insects often focuses on their tolerance to abiotic stress. However, animals are also part of complex ecological webs, including a range of pathogens that can threaten survival. Previous work hinted at an upregulation of insect immunity after cold exposure, so we explored the thermal biology of insect immunity more closely by determining 1) which components of the immune system are upregulated by cold; 2) whether acclimation affects the shape of the thermal performance curve for immune responses; and 3) how the phenotypic plasticity of the host and the pathogen interact to determine the outcome of infection in a complex thermal landscape. We show that while cold exposure activates potential immunity in *Drosophila*, it does not appear to affect the fly's ability to fight off pathogens. We found that low temperature acclimation leads to a paradoxical narrowing of the thermal performance curve of some measures of immunity, and that phenotypic plasticity by a pathogen can negate any inherent advantages a host might gain through acclimation. Together, this demonstrates that the temperature-immune interaction is complex, and often counter-intuitive, but that the outcome of that complexity may not undermine attempts to predict responses to climate change based on thermal biology.

Diapause and Stress Tolerance in the Crustacean, *Artemia franciscana*

Thomas MacRae

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Abstract:

In anticipation of unfavorable environmental conditions, the brine shrimp, *Artemia franciscana*, produces gastrula stage embryos enclosed in a rigid chitinous shell. Upon release from females, these cysts, as they are known, enter diapause, a state of dormancy characterized by profound reduction in metabolic activity and extreme tolerance to stressors such as desiccation, heat, cold and anoxia. Termination of diapause depends on the exposure of cysts to desiccation and/or freezing, thus their survival depends on being tolerant of such stresses. Several proteins are up-regulated in diapause-destined embryos and cysts of *A. franciscana* including three small heat shock proteins (sHSPs), a ferritin homologue termed artemin, and group 1 Late Embryogenesis Abundant (LEA) proteins. Some of these diapause-specific proteins were shown by RNA interference (RNAi) to contribute to the stress tolerance of *A. franciscana* cysts. The sHSPs and artemin function as ATP-independent molecular chaperones in vitro and in vivo, most likely acting as platforms to which partially denatured proteins attach and avoid irreversible denaturation. LEA proteins enhance the resistance of cysts to desiccation and freezing by influencing vitrification, functioning as molecular shields and/or acting as chaperones. HSP70 and Hsp90, ATP-dependent molecular chaperones, contribute to the tolerance of heat, cold and bacterial infection in *A. franciscana*. Experiments are in progress to more fully characterize chaperone function in diapausing *A. franciscana*, to identify client proteins of the sHSP p26, and to determine if/how the transcription factors p8 and HSF1 regulate stress protein synthesis in diapause-destined *A. franciscana* embryos. This work on stress-related proteins, which contributes to our fundamental understanding of diapause, may have practical applications for aquaculture and the control of pest organisms such as insects able to enter diapause as a means to survive efforts directed at their eradication.

Supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to THM.

Global transcriptomic analysis of diapause induction in the drosophilid fly, *Chymomyza costata*

Vladimír Koštál*, Rodolphe Poupardin, Jaroslava Korbelová

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Abstract:

Diapause is a developmental alternative to direct ontogeny in many invertebrates. Its primary adaptive meaning is to secure survival over unfavourable seasons in a state of developmental arrest, which is usually accompanied by metabolic suppression and enhanced tolerance to environmental stressors. During photoperiodically triggered diapause of insects, the direct ontogeny is centrally turned off under hormonal control but the molecular details of this transition are poorly understood. By using RNAseq technology, we characterized gene expression profiles associated with photoperiodic diapause induction in the larvae of the drosophilid fly *Chymomyza costata*. We will present data on differential expression in several strong candidate genes coding for upstream regulators of a complex phenotypic switch. The candidate genes include the regulators of biosynthesis of developmental hormones, hormonal receptors and members of their signalling cascades. Other factors with broad influence on phenotypic expression, such as histone marking by methylation, transcription factors, alternative mRNA splicing, and protein translation, likely participate in regulation of the blockade of direct development and deep restructuring of metabolic pathways for diapause.

Temperate but not tropical *Drosophila* species maintain high fertility after long-term cold exposure: evidence for reproductive diapause?

Julián Mensch*, Juan Hurtado, Paula Zermoglio, Gerardo de la Vega, Carmen Rolandi, Pablo Schilman, Therese Markow and, Esteban Hasson

University of Buenos Aires, Argentina

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Abstract:

Diapause consists of a distinct physiological state characterized by arrested development, enhanced stress resistance and metabolic changes in response to unfavorable environmental conditions. Although a wide variety of animals exhibit such adaptive phenotypic plasticity across seasons it is not clear to what extent the genetic and environmental mechanisms underlying diapause are conserved across insect taxa. We initially determined and compared the environmental conditions for cold-induced arrest of reproduction in four closely related fly species, two temperate and two tropical cactophilic South American Drosophila. Subsequently, we established that only the two temperate species appear to experience winter conditions require for arrest of maturation. Based on these findings, we predicted that only temperate species would show adaptations in terms of energy metabolism, cold tolerance and fitness after long-term cold exposure, as they are the only ones that evolved the capacity to undergo reproductive diapause. Our comparative study revealed both shared and distinct features between tropical and temperate species subjected to long-term cold exposure. On one hand, we found similar energy metabolism across species during cold exposure. On the other hand, not only were temperate species more fertile and fecund after cold exposure, but their cold tolerance also was superior. These findings highlight the role of thermal-induced reproductive plasticity as an integrated mechanism of cold adaptation in temperate South American Drosophila.

Diet and diapause influence rapid cold hardening and activity thresholds in *Calliphora vicina*

Scott A. L. Hayward, Paul C. Coleman

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Abstract:

Virtually all temperate insects have evolved to survive the winter by entering a physiological state of reduced metabolic activity termed diapause. There is increasing evidence that climate change is disrupting the diapause response, however, resulting in non-diapause life stages encountering periods of winter cold. This is a significant problem for adult life stages in particular, as they must remain mobile, periodically feed, and potentially initiate reproductive development at a time when resources should be diverted to enhance stress tolerance. Here we show how meat (but not sugar) feeding completely restricts rapid cold hardening (RCH), and increases low temperature activity thresholds in adult female blow flies (*Calliphora vicina* Robineau-Desvoidy). We present evidence that this is unlikely to be the result of altered extracellular K^+, as activity thresholds of alanine-fed adults were not significantly different from flies fed sugar and water. Climate data indicate there would be significantly different exposure to chill injury depending on whether adults had access to meat or not. Finally, we also investigated the RCH capacity of diapausing larvae and discuss how disruption of diapause and/or climate variability could impact on winter survival.

Keynote lecture

Coping with environmental stress: regulation of potassium ion homeostasis in the insect CNS

Meldrum Robertson

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Abstract:

Survival in harsh environments depends critically on preserving neural function so that appropriate behaviours and physiological control mechanisms can still be carried out. Nevertheless, neural activity in the CNS is arrested under extreme conditions of temperature or oxygen availability at which point insects enter a protective coma. This is associated with a surge in potassium ion concentration in the CNS extracellular space and, when conditions improve, recovery is dependent on restoration of normal ion gradients. Preconditioning by prior exposure can alter the threshold conditions that trigger the potassium surge and the rate of recovery. The mechanisms responsible for this phenotypic plasticity in the CNS are of considerable interest for predicting the ecological consequences of global climate change, and in the context of human health. In larval *Drosophila*, the stress protein HSP70 can protect locomotion at high temperatures by stabilizing presynaptic Ca^{++} dynamics. In adult flies, expression of HSP70 in glia can delay the loss of K^+ homeostasis associated with repeated anoxia. In locusts, however, stress proteins do not appear to have a role but prior heat shock causes trafficking of the Na^+/K^+-ATPase into neuronal membranes, modulates neuronal K^+ conductances and modifies axonal excitability. In addition, activation of second messenger pathways involving cGMP-dependent protein kinase or AMP-activated protein kinase is also able to modify the dynamics of the potassium surge and thus the timing of neural arrest. Future experiments using tissue-specific targeting in Drosophila will enable genetic dissection of the mechanisms regulating potassium ion homeostasis in the CNS in response to environmental stress and the relative roles of neurons and glia.

Mechanisms underlying the heat resistance of flies selected in fluctuating thermal regimes

Tommaso Manenti*, Volker Loeschcke and Jesper Givskov Sørensen

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Abstract:

Temperature varies on a yearly, seasonal, and daily scale, challenging organisms on multiple temporal scales, thereby affecting thermal adaptation. This presentation will show the underlying mechanisms of thermal adaptation to fluctuating temperatures, with two different approaches. First, we investigated if the experience of predictable daily fluctuation of temperatures affects the daily pattern of heat resistance in flies. Furthermore, we studied if a long term exposure to a consistently fluctuating temperature regime led to a circadian controlled adjustment of heat resistance in flies. Second, we investigated the transcriptome of for 20 generations laboratory natural selected *Drosophila simulans* in constant, predictable and unpredictable thermal regimes during a thermal challenge to identify the adaptive strategy of these flies. While daily variation in heat resistance was found, this seemed to be related with the light cycle rather than the temperature in the thermal regimes. The results suggested that there was no apparent continuous acclimation response induced by experiencing fluctuating temperatures. In the second experiment we showed that flies selected in fluctuating regimes displaced the stress response to heat to a higher temperature, as a consequence of the better performance in heat resistance of these flies compared to flies selected in the constant regime.

Geographic divergence in upper thermal limits across insect life stages: Does behavior matter?

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Abstract:

Insects with complex life cycles vary in size, mobility, and thermal ecology across life stages. We examine how differences in the capacity for thermoregulatory movement influence geographic differences in heat tolerance among eggs and adult *Colias* butterflies. *Colias* adults exhibit differences in morphology (wing melanin and thoracic setal length) along spatial gradients, whereas eggs are morphologically indistinguishable. Here we compare *Colias eriphyle* eggs and adults from two elevations and Colias meadii from a high elevation. We find that hatching success and egg development time of *C. eriphyle* eggs did not differ with the elevation of origin. Egg survival declined in response to heat-shocks above 38-40°C and development time was shortest at intermediate heat-shock temperatures of 33-38°C. Laboratory experiments showed that *Colias* adults from higher elevation sites better survived heat shocks than those from lower elevations. Common-garden experiments at the low-elevation field site showed that *C. meadii* adults initiated heat-avoidance and over-heating behaviors significantly earlier in the day than *C. eriphyle*. Our study demonstrates the importance of examining thermal tolerances across life stages. Our findings are inconsistent with thermoregulatory behavior buffering selection differences among mobile stages in different environments, but suggest that sessile stages may evolve similar heat tolerances in different environments due to microclimate similarity or evolutionary constraints.

Hot locusts experience loss of ion balance: An exploration of its causes and effects

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Abstract:

Thermal tolerance is an important trait in defining the spatial and temporal distributions of terrestrial insects, and models of distribution can be particularly informative when based on the limits of thermal performance. However, relatively little is known of the physiological mechanisms that underlie the arthropod critical thermal maximum (CTmax), and there is little support for the OCLTT model in terrestrial insects. In some arthropods, increased haemolymph potassium is associated with heat death, and high [K+]ext is known to have detrimental effects on neuromuscular excitability. Disruption of ion balance may therefore form a promising alternative line of investigation into the physiological mechanisms that set CTmax in the terrestrial insects. To address this we quantified changes in ion and water balance in haemolymph and muscle tissue of migratory locusts during acute exposures to two static ambient temperatures clustered around their CTmax (48oC and 50oC). At both temperatures we found that [K+]ext more than doubled during acute hyperthermia which likely resulted in a depolarisation of muscle resting potential. Part of the observed hyperkalaemia was associated with a decrease in haemolymph volume which may have acted to concentrate K+. These findings suggest that disruption of water and ion balance is an important factor leading to loss of whole organism function during acute hyperthermic exposure, at least in some terrestrial insects, and may constitute a promising alternative model to OCLTT for these animals.

How does cold acclimation affect ion transport, gene expression, and ultrastructure of the insect hindgut?

Lauren E. Des Marteaux and Brent J. Sinclair

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Abstract:

Insects in chill coma lose ion and water homeostasis, which implies that epithelial transport function is lost at low temperatures. Cold-acclimated insects enter chill coma and maintain homeostasis at lower temperatures than warm-acclimated conspecifics, but little is known about the mechanisms underlying loss of transport function in the cold, or how cold acclimation alters transport function. The insect hindgut (a major site of ionoregulation) is a likely target for modification during cold acclimation. To generate hypotheses about the mechanisms underlying cold-tolerance plasticity we investigated the effects of cold acclimation on the hindguts of adult *G. pennsylvanicus* crickets. We used an Ussing chamber to measure active ion transport across the rectum, and quantified hindgut Na^+/K^+ ATPase activity by spectrophotometric assay. We quantified differential gene expression in the hindgut using RNASeq, and used histology to identify hindgut ultrastructural changes.

Chillin' in Ontario – Plasticity of cold tolerance in adult *Drosophila suzukii*

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Abstract:

The worldwide emerging crop-pest *Drosophila suzukii* has been found in Ontario since 2010, but its cold tolerance and therefore potential to successfully establish has not been thoroughly explored. We determined the effect of low temperatures on reproductive output, activity and survival in both male and female adult *D. suzukii*. We reared flies under control conditions (21 °C, long days) and induces plasticity by rapid cold-hardening, constant cold acclimation or acclimation under fluctuating temperatures. Cold shock lowers the reproductive output of mated females, while remating after cold exposure increased the reproductive output, indicating that males and females have to survive winter conditions. The chill coma onset at relatively mild temperatures sets activity boundaries for *D. suzukii* in fall and spring. Low-temperature survival was highly limited in this chill-susceptible species, suggesting that it might not be able to survive winter conditions outside. Adult phenotypic plasticity seems insufficient to allow overwintering of *D. suzukii* in Ontario, which indicates that these flies could overwinter associated with built structures or that there is in an increase in cold tolerance through developmental acclimation.

The physiology of chill susceptibility in insects

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Abstract:

Studies of the physiology of insect cold tolerance have classically focused on adaptation related to avoiding or tolerating ice formation. We therefore have a good understanding of the physiological adaptations associated with freeze tolerance, cryoprotective dehydration and freeze avoidance. However, a large group of insects do not tolerate low temperature *per se*, and for these species the physiological challenge at low temperature is unrelated to the water/ice transition. Instead, these insects are challenged to maintain homeostasis when temperature is reduced, and in this talk we will review the present understanding of "chill tolerance" and highlight how the integrative physiology of osmoregulation and neuromuscular function is a central prerequisite of cold tolerance. When exposed to low temperature most insects lose neuromuscular function and enter a reversible comatose state (termed chill coma). Following brief or mild cold exposures the insect can recover from this comatose state upon return to benign temperature. However, long or severe cold exposures results in prolonged recovery time, chill injury and ultimately in mortality. In a series of studies we and others have demonstrated how all these phenotypes are associated with the insect's inability to preserve and recover resting membrane potential of excitable tissues. The cause of cold induced membrane depolarization varies depending on the duration of cold exposure. Initially membrane potential is lost due to a marked reduction in the electrogenic potential as a result of reduced activity of electrogenic ion-motive pumps. A secondary effect of reduced ion-pumping capacity at low temperature is the progressive dissipation of ion gradients (particularly potassium) which leads to further cell depolarization, cellular chill injury and death. Accordingly the ability to maintain ion homeostasis at low temperature is likely to be one of the defining characteristics of chill tolerance of insects.

Cold tolerant drosophilids preserve muscle resting membrane potential at low temperatures

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Abstract:

Chill susceptible insects enter a reversible paralytic state, termed chill coma, at mild low temperatures. Chill coma is caused by neuromuscular impairment, allegedly triggered by cold-induced depolarization of muscle resting membrane potential (V_m). We used five *Drosophila* species that vary in cold tolerance (chill coma temperature spanning approx. 11°C) and repeatedly measured muscle V_m during a downward temperature ramp (20 to -3°C). Cold tolerant species were able to defend their V_m down to lower temperatures. An ability not explained by species specific differences in initial V_m at 20°C, but by cold tolerant drosophilids defending V_m across a broad range of temperatures. We found support for a previously suggested "critical threshold" of $V_{m,}$ related to chill coma, in three of the five species, interestingly, the cold tolerant Drosophila species may enter coma due to processes unrelated to muscle depolarization as their V_m was not significantly depolarized at their chill coma temperatures.

Loss of muscle function during acute cooling in locusts is caused by reduced Ca^{2+} transients as a result of altered muscle fiber excitability

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Abstract:

Many insect species enter a state of neuromuscular paralysis when their body temperature is lowered to a critical limit, but the physiological and cellular processes underlying this chill coma are largely unknown. Previous studies on locusts show that muscle force production is highly depressed at low temperature implicating impairment in cellular mechanism in the muscle per se. Aiming to determine these mechanisms we examined the thermal sensitivity of several events in the excitation-contraction-coupling process including: i) Ca^{2+} currents in the sarcolemma membrane ii) Excitability of the muscle; iii) Intracellular Ca^{2+} release during muscle stimulation iv) Maximum force of the contractile apparatus and the effects of changes in $[Ca^{2+}]$.Using skinned muscles fibers we show that similar maximum force can be obtained regardless of temperature at both high and low $[Ca^{2+}]$, suggesting that maximum force is not altered by temperature and sensitivity to Ca^{2+} remains high at both temperatures. Membrane potential responses were markedly variable at both temperatures, and therefore we categorized different muscle fibers into four groups: Fibers that only generated an excitatory postsynaptic potential (EPSP) that is characterized by opening of ligand (glutamate) mediated channels and is not associated with an activation of voltage-sensitive channels (i.e. no indication of an action potential). The three other groups of fibers generated action potentials with different waveform morphologies. They were characterized as single action potential, action potential with plateau or action potentials with two peaks. Excitability were largely affected when temperature was lowered from 20 °C to 5 °C, hence the number of muscle fibers producing AP with two peaks or AP with a plateau giving rise to high contractions upon nervous stimulation were largely abolished. Instead the occurrence of EPSPs, characterized by low or no contractions increased with lowering of temperature. Ca^{2+} transients following nerve stimulation correlated with action potential morphology: Hence the change in excitability from 20 °C to 5 °C changes the release of calcium and thus lower force production. The changes in excitability reflects reduced function of the L-Type voltage gated channels, as the currents are 5x smaller and right-shifted at low temperature. In conclusion the cold-induced drop in force observed when insects go into chill-coma is explained by lowered Ca^{2+} transients, as a result of the altered muscle fiber excitability caused by reduced function of L-Type Ca^{2+}-channels in the muscle fiber membrane.

Concurrent effects of cold and hyperkalemia cause insect chilling injury

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Abstract:

Chilling injury and death are the ultimate consequence of low temperature exposure for chill susceptible insects, and low temperature tolerance is considered one of the most important factors determining insect distribution patterns. The physiological mechanisms that cause chilling injury are unknown, but chronic cold exposure that causes injury is consistently associated with elevated extracellular [K+], and cold tolerant insects possess a greater capacity to maintain ion balance at low temperatures. Here, we use the muscle tissue of the migratory locust (*Locusta migratoria*) to examine whether chill injury occurs during cold exposure or following return to benign temperature and we specifically examine if elevated extracellular [K+], low temperature, or a combination thereof causes cell death. We find that in vivo chill injury occurs during the cold exposure (when extracellular [K+] is high) and that there is limited capacity for repair of immediately following the cold stress. Further, we demonstrate that that high extracellular [K+] causes cell death in situ, but only when experienced at low temperatures. These findings strongly suggest that that the ability to maintain ion (particularly K+) balance is critical to insect low temperature survival, and highlight novel routes of study in the mechanisms regulating cell death in insects exposed to environmental stress.

Keynote lecture

The role of microorganisms in atmospheric processes and its implication for life detection on exoplanets

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Abstract:

The atmosphere is an extreme and stressful environment with high UV fluxes, low water potential and step temperature gradients. Nevertheless, the atmosphere is full of microorganism including fungi, bacteria, archaea and virus. Pasteur was the first to demonstrate the presence of microorganisms in air in the midst 18hundreds. Since then the atmosphere has been shown not only to serve as routes of microbial dispersal but microorganisms have been upgraded to being important players that are involved in atmospheric processes by e.g. acting as ice nuclei (IN), which influence the formation of clouds and by degrading atmosphere organic constituents in competition with photochemistry. IN-positive bacteria can have significant implications for the global distribution of clouds and precipitation, and consequently for Earth's weather patterns and climate. In addition, viable airborne bacteria have the physiological potential to both withstand atmospheric stress and grow on atmospheric organic compounds. About 20 years ago the first planet, a Jupiter-size body in close orbit around its central star, was observed outside our solar system - a so-called exoplanet. Since then the number of detected exoplanets has exceeded 4800, among them an increasing number of Earth-like planets. Similar to Earth, exoplanetary atmospheres may provide information on the presence of life. I will present some adaptations that allow microbes surviving in the atmosphere and shaping it and discuss how these properties may allow studying life light years away from Earth.

SCP value in insects - is it useful index of lower lethal temperature?

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Abstract:

Determination of a supercooling point (SCP) has been used in research of arthropod cold tolerance for many years. Nevertheless, relevance of using the SCP value as an estimate of lower lethal temperature in freeze-avoiding species has been often discussed, questioning either stochastic or deterministic nature of the SCP. The main problem is that whereas SCP of homogenous liquid droplets can be measured repeatedly, SCP of the whole arthropod body can be determined just once. Hawes (2006) tried to test the relevance of SCP by the repetitive freezing of the Antarctic collembolan and showed that the temperature of crystallization (SCP) and re-crystallization (of the dead body) do not usually change much. Here I present the results of cooling a groups of *Pyrrhocoris apterus* (Hemiptera: Heteroptera) to the population median SCP (the temperature when half of the sample freeze), recovery and subsequent repetitive cooling to this temperature of the survived individuals. In the case of stochastic nature of the SCP, significant part of the survived specimens would freeze during each of the subsequent cooling to the median SCP. The results show that most of the individuals survived repetitive cooling. The value of SCP at individual level was thus relatively stable, and did not change over time. The value of SCP can be (at least in some freeze-avoiding species) used as a relevant estimate of lower lethal temperature.

Complex regulatory processes underlie the physiological response of *Drosophila melanogaster* to cold acclimation

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Abstract:

We investigated the physiological response of adult *Drosophila melanogaster* to rapid cold hardening (RCH). A gradual reduction of temperature (-0.1°C min^{-1}) prior cold stress (-6°C) markedly improved cold tolerance compared to control, and hence, we expected this plastic response to be detectable at the proteomic level. Using 2D-DIGE proteomics, we detected a very weak proteomic signal with only a few proteins that changed in abundance. Of these, we identified a set of four proteins that corresponded to two different variants of glycogen phosphorylase (GlyP) whose levels changed in opposite directions. We then focused on this prime candidate by looking at events upstream of translation but did not confirm the proteomic signal at the transcriptional level. Downstream of translation, we also found no effect of RCH on the activity of GlyP, but we found a small increase in glucose level following RCH. By looking at consequences of a RCH across five levels of biological organization (organism tolerance, metabolite concentration, enzyme activity, protein abundance and mRNA level), we found that the relationship among these levels were far from "linear". The different forms of GlyP that we detected represented different charge variants changing in opposite direction. Such a complex regulation could be due to posttranslational regulation (e.g. phosphorylation). A previous proteomic study on cold acclimation also suggested that acquired cold tolerance may involve regulatory processes such as posttranslational regulation rather than deep changes of protein abundance. Overall these results suggest that regulatory processes downstream the transcriptional machinery (e.g. phosphorylation) might be sufficient to carry out cold acclimation. We have expanded upon this line of inquiry by conducting a large-scale comparative phosphoproteomic analysis. First results from LC–MS/MS methods coupled with phosphopeptide enrichments reveal that cold-acclimated phenotype seems hyperphosphorylated compared to control.

The limits of drought-induced rapid cold-hardening: Extremely brief, mild desiccation triggers enhanced freeze-tolerance in *Eurosta solidaginis* larvae

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Abstract:

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, a similar response, termed drought-induced RCH, was described following as little as 6 h of desiccation, producing a loss of less than 10% of fresh mass. In this study, we investigated the limits and mechanisms of this response in larvae of the goldenrod gall fly *Eurosta solidaginis* (Diptera, Tephritidae). The cold-hardiness of larvae increased markedly after as few as 2 h of desiccation and a loss of less than 1% fresh mass, as organismal survival increased from 8% to 41% following exposure to -18 °C. Tissue-level effects of desiccation were observed within 1 h, as 87% of midgut cells from desiccated larvae remained viable following freezing compared to 57% of controls. We also demonstrated that drought-induced RCH occurs independently of neuroendocrine input, as midgut tissue desiccated ex vivo displayed improved freeze-tolerance relative to control tissue (78–11% survival, respectively). Finally, though there was an increase in hemolymph osmolality beyond the expected effects of the osmoconcentration of solutes during dehydration, we determined that this increase was not due to the synthesis of glycerol, glucose, sorbitol, or trehalose. Our results indicate that *E. solidaginis* larvae are extremely sensitive to desiccation, which is a triggering mechanism for one or more physiological pathways that confer enhanced freeze-tolerance. From Journal of Insect Physiology, volume 73, February 2015, pages 30-36.

Understanding costs and benefits of plastic physiological performance: Insights from the integration of laboratory, semi-field and field assessments

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Abstract:

Phenotypic plasticity is likely to influence survival by providing a rapid tolerance response in variable environments; however, this may be costly if plastic responses fail to match the future environment. To date, laboratory and field estimates of the costs and benefits of plastic physiological responses have found disparate results. Using laboratory, semi-field (greenhouse) and field estimates of performance, we examined the influence of different thermal acclimation regimes on thermal tolerance and flight performance (mark-release-recapture, MRR) using a model agricultural pest species system, the Mediterranean fruit fly, *Ceratitis capitata*. Laboratory estimates provided evidence of beneficial acclimation, with cooler-acclimated individuals performing best under lower thermal limit assays (chill coma recovery time and CTmin) but worse for upper thermal limits (heat knockdown time and CTmax) and vice versa for warm-acclimated flies. Field MRR assays matched these results, with cold-acclimated individuals being recaptured more frequently than those that were warm-acclimated, under predominately cool environmental conditions. However, semi-field MRR estimates showed no evidence of performance costs, with both cool- and warm-acclimated flies being recaptured more frequently than control flies. The benefits of matching plastic responses to the environment experienced during stress are most evident when the examined under more extreme conditions, with thermal tolerance estimates being a better predictor of field fitness. Less extreme conditions and less complex operational environments, i.e. semi-field conditions, allow effects to be examined under more defined/controlled environmental conditions and provide a method of comparison for field-based MRR experiments, thereby allowing identification of the factors influencing a given laboratory-field performance relationship.

Invasions in the Antarctic

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Abstract:

Most ice-free habitats on land in Antarctica and the sub-Antarctic are effectively islands surrounded by hostile ice and ocean. Terrestrial ecosystems are extremely isolated, and have developed unique and striking features. True terrestrial vertebrates are generally absent, meaning that most foodwebs consist only of invertebrates. Ecosystem structure is generally simplified, with few true native herbivores or predators present, and the predators in particular having very limited impact on their prey species. Over the last two centuries human activities have led to the accidental introduction and establishment on land of many non-indigenous species of vertebrate, invertebrate and plant, particularly to the ecosystems of the sub-Antarctic islands. These introductions have encompassed a range of trophic functions, some of which are poorly or not represented in indigenous ecosystems, in some cases leading to drastic alterations in ecosystem structure and function. A smaller number of introductions are already apparent in parts of the Antarctic Peninsula, and the sub-Antarctic provides a direct warning of the likely trajectories of these and any future establishment events. This presentation will give an overview of the impacts of non-indigenous biota in Antarctic ecosystems to date, and their implications in a future where these ecosystems are also faced by some of the most rapid rates of environmental change on the planet.

Development and overwintering of the larch bark beetle, *Ips cembrae* (Coleoptera, Curculionidae)

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Abstract:

The larch bark beetle, *Ips cembrae*, is an important forest pest that mainly attacks larch stands in the Palaearctic. Heavy infestations occur in years with hot and dry summers or after storm and snow events. The aim of this study was to elaborate data on the development and overwintering of this species. Comparing environmental conditions of the habitat, e.g. in high elevations, similar life history traits of *I. cembrae* and the European spruce bark beetle, *I. typographus*, could be expected. *I. typographus* enters an adult diapause in winter and has an univoltine life cycle in subalpine regions. However, in *I. cembrae* there is a big lack of knowledge about the presence of a diapause and thermal values affecting phenology. These data would be important for monitoring or phenological models and can help to elucidate a currently unknown chapter of the biology of this species.

The lower developmental threshold was determined by observing the development time at 15 °C, 20 °C, and 25 °C under long day conditions (L:D 16:8) using logs and the sandwich method. The mean development of one generation in logs comprised 143, 110 or 47 days, respectively. The lower developmental threshold was 10.8 °C. Applying the sandwich method, the development lasted 120, 64 or 37 days, respectively and we assessed a lower threshold for the development of the beetle of 11.2 °C.

Overwintering studies on larvae, pupae, and adults comprised the determination of the frost tolerance and of chilling injuries. Monthly mean supercooling points of imagos from October 2012 to April 2013 were between -10.9 °C and -13.5 °C, no significant differences and thus no variation during winter was observed. Larvae were highly susceptible to chilling injuries (exposure to -4 °C and -10 °C for one and two weeks), showing a mortality of 100% under artificial conditions, whereas only 25-50% of pupae died. A crucial point for overwinter survival may be the evacuation of the gut before cold periods.

Data on emergence rates and ovary maturation under long and short day conditions, as well as frost tolerance and respiration rates do not indicate the presence of an adult diapause in this species.

Comparative physiological plasticity to desiccation among distinct populations of the malarial mosquito, Anopheles coluzzii

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Abstract:

In the dry savannahs of West Africa, the population dynamics of the malarial mosquito *Anopheles coluzzii* closely follows the pace of surface water availability: the species pullulates during the rainy season and disappears during the hot and dry season. The ability of *An. coluzii* to endure a large panel of temperature and relative humidity conditions in their geographical range likely results from its great capacity to express many distinct phenotypes. Consistently, specimens from temporary water collections were described as "strong aestivators" programmed to engage into a dormant state at the onset of the dry season, while those from permanent water collections are described as "weak aestivators". In this study, we compared the physiological plasticity to desiccation in temporary and permanent populations of female *An. coluzzii*, sampled from the northern and south-western parts of Burkina-Faso (West Africa). We experimentally exposed female mosquitoes to contrasting conditions mimicking the climatic conditions they are experiencing in their natural habitats, during both rainy and onset of the dry seasons. We expected that the physiological responses would differ among the different mosquito populations grown under common garden conditions, thus reflecting local adaptations. We hypothesised that exposure to dry season conditions would trigger changes in (i) the levels of triglycerids and proteins, and in the expression levels of glycogen synthase and phosphorylase genes, (ii) circulating metabolites with osmoprotectant or energetic roles, and (iii) changes in the expression levels of adipokinetic hormone (AKH) genes which regulates energetic metabolism. We expect that mosquitoes programmed to engage into "strong" aestivation would exhibit overexpression of mRNA transcripts of glycogen phosphorylase and AKH when exposed to dry conditions. They should exhibit high amounts of lipids and osmoprotectant compounds as already know for overwintering diapause strategy. Our data suggest that important elements regulating bioenergetics differ between the two phenotypes.

Amino acids as compatible osmolytes during dehydration in soil invertebrates

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Abstract:

It has long been known that free amino acids can occur at elevated concentrations during periods of low environmental water activity in a wide variety of taxa. They are ascribed a variety of roles including anaerobic metabolytes, compatible osmolytes and cryoptotectants. Recent studies have shown that in soil invertebrates exposed to summer drought, free amino acids can reach concentrations exceeding 200 mOsmolal where they can be argued to play a major role in water retention or even water vapour uptake. In some of these soil invertebrate examples their concentrations exceed those of the more commonly measured sugars and polyols. Alanine is commonly the free amino acid that is most unregulated during water shortage and can be argued to play a double role of simple osmolyte, but also as a compatible sink for nitrogen during periods where urine production may be suppressed or even eliminated. Data supporting these various roles will be presented from earthworms and collembolans.

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

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Abstract:

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. These environmental factors may adversely affect population persistence due to alteration of individual performance or effects of stress on reproductive success. In a metapopulation of the Glanville fritillary butterfly *Melitaea cinxia* found on the Åland Islands of Finland, extirpation and re-colonization is linked to differences in dispersal abilities, which vary as a function of genetic variation at the glycolytic enzyme locus phosphoglocuse isomerase (Pgi). Generally, Pgi heterozygotes have the most robust performance and fitness characters. Here we found that thermal tolerance, flight performance, metabolic rate and mating success were highest for Pgi heterozygotes after mild heat stress, consistent with earlier findings; however, after exposure to more extreme thermal stress, individuals homozygous for a Pgi allele common in Central Europe, but rare in Finland, fared best. Male fitness (mating duration, spermatophore size, fecundity of mating partner) was highest for males homozygous for this rare, southern Pgi allele after exposure to extreme thermal stress, suggesting that natural selection might cause an increase in frequency of the southern allele as climate change proceeds. However, there is also evidence that low frequency of the southern Pgi allele in isolated Finnish populations is due to linkage to lethal genes not present elsewhere. Thus, allele frequency shifts in response to climate change may be limited, leaving Finnish populations of the Glanville fritillary especially vulnerable to a warmer, drier climate.

Reversibility of developmental acclimation in *D. melanogaster*

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Abstract:

Insect species are found under most environmental conditions. A key aspect of the success of insects is their adaptive plasticity in high and low temperature tolerance, which has allowed different species to thrive under different thermal conditions, including some of the harshest environments on earth. Adaptive plasticity in thermal tolerance is also found within Drosophilids, which evolutionarily have a tropical origin, but have since colonised subtropical and temperate environments. Reviews on thermal acclimation often distinguish between developmental and adult acclimation. Developmental acclimation, such as body size, is largely regarded as fixed (nonreversible). Adult acclimation, on the other hand, is regarded as reversible responses to environmental changes. In Drosophilids several studies have found short-time acclimation to a given stress, to enhance an organism's survival in both juvenile and adult life stages to subsequent stresses. However, the overlap in the mechanisms activated, the interaction between developmental and adult acclimation and the dynamics of reversibility for thermal tolerance is yet to be fully explored. Our aim is to investigate the reversibility of developmental acclimation effects in the adult stage and study potential carry-over effects from the developmental acclimation.

We found that both high and low temperature acclimation led to beneficial acclimation benefits on critical thermal limits, and that the developmental acclimation effects were only partly and non-linearly reversible. The dynamics of the reversibility were very different for high and low temperature acclimation. Finally, we observed a tendency for flies from developmental acclimation at low temperature to maintain a long lasting benefit on traits related to senescence. The non-linear reversibility coupled with the differences between cold and heat acclimation suggest that the trajectory of the phenotypic plasticity for organisms thriving in variable thermal environments is highly complex and a result of different delays in phenotypic adjustments across traits.

Determination of Lake Baikal endemic and Palearctic amphipods thermal optima limits by changes in its stress markers

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Abstract:

Temperature stress provokes energy-demanding responses on a cellular level, which eventually may reduce the organism's competition and reproduction abilities. Hence, temperature stress is a significant physiological and ecological factor. In evolution, those species apparently are more successful that better cope with the physiological effects of stress, i.e. respond with less expense of energy. In the present study metabolic stress markers were comparatively monitored in an endemic amphipods species *Eulimnogammarus verrucosus* (Gerstf., 1858) and *Ommatogammarus flavus* (Dyb., 1874) from Lake Baikal and in the Palearctic amphipod *Gammarus lacustris* (Sars, 1863) (Amphipoda, Crustacea) exposed to a wide range of ambient temperatures. These metabolic data were compared with thermal preferendum data obtained for the same species in behavioral experiments. It was found that exposure of amphipods under increased temperatures resulted in increase of HSP70 content and lactate, activating of antioxidant enzymes (catalase and peroxidase), as well as reduction of lactate dehydrogenase and glutathione S-transferase activities. Exposure of amphipods at low temperatures resulted in decrease of HSP70 content, increase of lactate level, peroxidase and lactate dehydrogenase activation and reduction of glutathione S-transferase activity. It was noted that the most expressed changes of metabolism markers and the area of stability of cellular metabolic markers in all amphipods correlated with a preferred temperature limits obtained in behavioral experiments. Thus, for a first time with Lake Baikal amphipods we showed that the zone of stability of cellular metabolism closely related to their behavioral thermal preference zones and is likely to reflect thermal optima limits of each species.

Environmental plasticity of thermal reaction norms for development in true bugs: intergenerational and interpopulational differences

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Abstract:

The insect developmental time is mostly regulated by direct influence of temperature. Other seasonal cues, such as photoperiod, food etc. have a modifying effect. We found out that photoperiod can modify thermal reaction norms for development in various ways and that this effect is widespread among insects. Assuming a linear relationship between developmental rate and temperature we have shown that photoperiod affected the temperature-sensitivity of development in some insects, that is, altered the slope of the regression line and the thermal threshold for development. In *Pyrrhocoris apterus* bugs from the northern population (Belgorod, Russia) reared at lower temperatures, short-day photoperiods accelerated larval development as compared to long-day photoperiods, but the reverse was true at higher temperatures. As a result, the slope of the regression line was shallower and the thermal threshold was lower (larval development was less temperature-dependent) under short-day conditions. So the bugs from early-summer and late-summer generations demonstrated different thermal sensitivity of development. In *P. apterus* from the southern population (Israel), the long-day photoperiod accelerated larval development at all temperature regimes. The regression lines (i.e. the thermal sensitivities of development) under long- and short-day conditions ran almost in parallel to each other, but the thermal threshold was lower under the former photoperiod. Similar results were obtained for the Israel and the Cyprus populations of the bug Scantius aegyptius, but, in this case, the thermal sensitivity of development was slightly higher under short-day conditions. The adaptive significance of seasonal and geographical changes in the thermal sensitivity of development will be discussed. The work was supported by the RBRF grant no. 14-04-01156-a.

Thermal reaction norms for development in leaf beetles (Chrysomelidae): variation across taxa and latitudes

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Abstract:

Thermal reaction norms are usually compared in terms of the maximal trait value and the "optimal temperature" at which this maximum is achieved. Although this approach may be successful with certain traits, this is arguably not so for developmental rate. Development is often fastest at unnaturally high temperatures that are well above the permissible range and impair survival and fecundity. Therefore, selection is unlikely to affect this portion of the reaction norm directly, and the implications based on this "thermal optimum" may be misleading. Instead, it is suggested to adopt a century-old linear approach for comparing thermal reaction norms for development. Despite its shortcomings, linear regression is more justifiable ecologically and evolutionarily than various non-linear models, although the latter are best at exploring the underlying biophysical machinery. Variation of linear thermal reaction norms falls into four principal patterns, and one pattern may gradually transform into the other in the course of evolution. This is exemplified by the interspecific variation of thermal reaction norms for immature development in the leaf-beetle family. Analysis of this variation shows, among other things, that caution should be taken in calling species "cold-adapted" or "warm-adapted" after the shape of their thermal reaction norms. The extent to which thermal sensitivity and lower temperature threshold for development are adaptive has yet to be investigated. In particular, leaf beetles inhabiting colder climates are rather "cold-exapted", because their close relatives from warmer climates have similar thermal reaction norms. The work was supported by the Russian Foundation for Basic Research (project no. 14-04-01156-a).

Mitonuclear interactions influence cold and heat tolerance along elevation gradients in a montane insect

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Abstract:

Organisms in montane habitats often live in small fragmented populations that are expected to change dramatically with climate change induced by global warming. The ability of populations to persist in montane habitats depends partly on whether they possess genetic variation in their physiological capacity to respond and adapt to changes in climate. In the Eastern Sierra Nevada mountains of California, the willow leaf beetle *Chrysomela aeneicollis* occurs at high elevations just below tree line. Variation at genetic marker loci [5 allozymes, 5 microsatellites, and a 550 bp region of mitochondrial cytochrome II oxidase (COII)] shows significant variability among montane drainages along a 75 km transect from Taboose Pass in the south to Rock Creek in the north. Geographic variation along this transect is much greater for the allozyme locus phosphoglucose isomerase (PGI) than for other nuclear marker loci. In prior studies, we have described functional, physiological, and reproductive differences among PGI genotypes that correspond to their changes in frequency over a latitudinal temperature transect. Here, we show that mitochondrial genotype also mediates response to cold and heat. Performance and fitness characters at high elevation show that the best combination of mitonuclear genotypes corresponds to the natural distribution of PGI and COII alleles (northern mitochondrial genotypes perform best when paired with northern PGI genotypes). In contrast, at lower elevations performance is best when a mismatch is present between mitochondrial and nuclear genotypes (northern mitochondrial genotype performs best in combination with the southern PGI genotype). Natural selection may thus act jointly on COII and PGI and this genetic variability may contribute to population persistence in the face of anticipated rapid environmental change.

Poster session

Heat Shock Factor 1 (HSF1) and HSP90 as Potential Mediators of Stress Tolerance in Diapausing Embryos of Artemia franciscana

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Abstract:

Diapause is a physiological situation characterized by arrested development, reduced metabolism and enhanced stress tolerance. The crustacean, *Artemia franciscana*, commonly known as brine shrimp, undergoes alternate developmental pathways resulting in release from the female of either swimming larvae or gastrula-stage embryos enclosed in a chitinous shell known as cysts. The cysts experience a profound reduction in metabolism and greatly enhanced stress tolerance as they enter diapause. Molecular chaperones play an important role in *Artemia* diapause with small heat shock proteins (sHSPs) essential to stress tolerance and diapause maintenance. Heat shock factor one (HSF-1) is an important transcriptional regulator of heat shock protein (HSP) genes in all organisms so far examined. The purpose of my research is to determine the role of HSF1 in the regulation of sHSP synthesis and stress tolerance in *A. franciscana*. HSF-1 cDNA was cloned from *A. franciscana* and shown to contain a typical DNA binding domain (DBD) and conserved HR-A and HR-B domains indicating that HSF-1 from *A. franciscana* is a transcriptional regulator. Antibodies are being used to probe Western blots to detect HSF1 and sHSPs such as p26 in developing embryos. RNA interference (RNAi), using long double-stranded RNA (dsRNA), is being employed to study the role of HSF1 in *Artemia* development with emphasis on the synthesis of sHSPs. The molecular chaperone, HSP90, may interact with HSF-1 and inhibit its DNA binding activity. Consequently, HSP90 cDNA was cloned from *A. franciscana* and compared to HSP90 from other organisms by multiple sequence alignment. An antibody was prepared to *A. franciscana* Hsp90 and it is in use to study HSP90 synthesis during embryo development and the interaction between HSF1 and Hsp90. It is anticipated that this work will lead to a better understanding of HSF1 and HSP90 during diapause of *A. franciscana* and other organisms.

Supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to THM.

Population Genomics of Diapause Phenotypes in European Ips typographus (Coleoptera, Curculionidae) Using High-Throughput RADSeq)

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Abstract:

The European spruce bark beetle, *Ips typographus*, is an economically important species in Norway spruce (*Picea abies*) stands. Due to its aggregation by pheromones, polygyny, voltinism, fecundity and its ability to produce sister broods, I. typographus is a highly efficient pest. Moreover, it transmits blue stain fungi which might help to overcome the host tree's defence system.

In winter, *I. typographus* enters a reproductive diapause in the adult stage when the critical daylength falls below 14.7 hours. Diapause is terminated in December/January and is followed by a temperature-dependent quiescence. In Europe we find populations with facultative (multivoltine) and obligate (univoltine) diapausing beetles. Diapause phenotypes can be distinguished by physiological traits, i.e. gonad development, emergence rates, and respiration rates under different photoperiodic and thermal conditions.

Diapause phenotypes of Central European (low elevations and high elevations in Austria) and Scandinavian (northern Sweden) populations have previously been determined in physiological studies. We will apply ddRADSeq to identify loci connected to diapause phenotypes by comparing facultative and obligate diapausing individuals across several populations.

Subsequently, we will determine ratios of multivoltine and univoltine individuals in European populations to estimate phenology patterns for risk assessment.

The Combined Effect of Freeze-thaw Events and Heavy Metal Pollution Leads to Distinct Lethal Synergy in Enchytraeus albidus

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Abstract:

Many anthropogenic activities negatively affect the environment and stress the organisms living here in various ways. Due to global warming it is likely that freeze-thaw events will replace permanent freezing of soils in arctic regions. Metals are some of the most common contaminants in soil in Europe, and recent interest in increasing mineral deposit mining activity in arctic regions further emphasizes the need for focus on the environmental impact in these areas. In the present study it was investigated how the combination of freeze-thaw events and copper contamination of soil affected the Icelandic, freeze tolerant annelid worm, *Enchytraeus albidus*.

Worms were exposed to one of three temperature treatments (constant +1.5^{o}C, constant -6^{o}C, or daily cycles between +1.5 and -6^{o}C) in combination with one of several different copper ($CuCl_2$) concentrations in soil. The results showed a distinct synergy between freeze-thaw cycles and copper stress, leading to an elevated mortality of enchytraeids subjected to these conditions. Enchytraeids subjected to conditions of constant freezing and copper stress were not as severely affected.

To examine this synergy an array of analyses were conducted. Glucose and glycogen content in the worms were measured to assess the degree of cryoprotectant accumulation. It was hypothesized that cryoprotective glucose will have formed in worms exposed to constant freezing of -6^{o}C, while the formation may be inhibited in worms exposed to freeze-thaw cycles. Bioaccumulation of copper was also quantified to expose any increase in body burden in freeze-thaw treated worms.

Regardless of the physiological responses, it is evident that arctic organisms are negatively affected by the environmental impact of global warming and exploitation of mineral deposits through mining.

Impact of multiple stressors on risk of xenobiotics to soil fauna

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Abstract:

Effects of five xenobiotics (α-cypermethrin, pyrimethanil, ivermectin, phenanthrene and copper) on soil fauna, naturally occurring in agricultural soils, were investigated in relation to ecological stressors such as predation, competition and feed status. Stressors may decrease soil fauna performance and tolerance towards toxics just as toxicants have been shown to decrease e.g. heat and cold tolerance. The contaminants are present in the agricultural environment through application as insecticide, fungicide, veterinary medicine, and through fertilization from sewage sludge and animal manure. In order to examine the effect of feed status on springtail tolerance towards agricultural chemicals, five single-species laboratory tests were performed. Prior to toxicity testing, test animals (*Folsomia candida*) were starved during a period of 6 weeks, and subsequently exposed to contaminants during a period of three weeks. Preliminary results show that starvation affects the toxicity of xenobiotics to *F. candida*. Furthermore, results indicated that growth and reproductive development in starved individuals cease due to long term starvation. Hence, these results suggest that natural stressors such as starvation should not be neglected in the assessment of risk of hazardous substances.

Heat stress disrupts ion balance in the European green crab (*Carcinus maenas*)

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Abstract:

Acute exposure of ectotherms to critically high temperatures causes injury and death and the mortality observed at these extreme temperatures has been, in part, associated with impaired oxygen transport capacity. However, other physiological malfunctions are also known to occur at high temperature extremes. Exposure to acute heat stress does, for example, lead to loss of ion and water homeostasis in both freshwater crustaceans and terrestrial insects. Loss of ion balance can impair neuromuscular function (including cardiac function), and conversely impaired oxygen transport can reduce ion transport capacity through reduced ATP supply. For these reasons it may be difficult to discern which of these factors, if any, are the proximate cause of heat injury. In the present study we investigated if heat stress causes a similar failure of ion regulation in marine crabs and examined whether such failure precedes or follows failure of heart function. We held marine crabs (*Carcinus maenas*) at temperatures immediately below their critical thermal maximum and measured extracellular (hemolymph) and intracellular (muscle) ion concentrations. As hypothesised, heat stress depolarized the equilibrium potentials of both K+ and Na+, which may drive a subsequent loss of function in excitable tissues. In a second set of experiments we exposed the crabs to the same temperatures, but this time measured ion concentrations at the time of paralysis (from which the crabs do not recover) and also at the time of heart failure, to explore whether this loss of ion balance causes, or is caused by, cessation of the heart.

Reproductive diapause alters stress tolerance in Drosophila suzukii

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Abstract:

Drosophila suzukii (Diptera: Drosophilidae) is an invasive agricultural pest in southern Canada. Its ability to overwinter and therefore establish in this country could have severe implications for fruit crop industries. We demonstrate here that laboratory populations of Ontario *D. suzukii* larvae reared under short-day, low temperature, conditions develop into dark 'winter morph' adults similar to those reported globally from field captures, and observed by us on Pelee Island in Ontario. These adults have delayed reproductive maturity, enhanced cold tolerance, and can remain active at lower temperatures. Female D. suzukii have underdeveloped ovaries and altered transcript levels of genes associated with reproduction and stress. All of these traits are consistent with an adult reproductive diapause. The traits of this 'winter morph' likely facilitate overwintering in Ontario, and have probably contributed to the global success of this fly as an invasive species.

Oleic acid elevation in diapausing larvae of pomegranate fruit moth

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Abstract:

Pomegranate fruit moth *Apomyelois ceratoniae* Zeller, a worldwide pest of fruits and nuts in many tropical and subtropical countries encounters below zero temperatures in pomegranate orchards of Iran. Even though overwintering inside pomegranate can partially buffer these chill-susceptible diapausing larvae from low ambient temperature, improving mechanisms to withstand cold would be critical to survive. Analysis of the whole body fatty acids of the larvae showed that cold-induced membrane reinstruction caused a significant increase in overall degree of unstauration from 0.5 in nondiapausing larvae to 1.7 in diapausing ones and the ability to harden in the cold. Among detected fatty acids in the larvae (Palmitic, palmitoleic, stearic, linoleic, oleic and myristic acid), the proportion of oleic acid reached from 26% of the total fatty acid pool in non diapausing larvae to 52% in diapausing ones and was responsible for increased unsaturation. Oleic acid can promote membrane fluidity at low temperatures, provides the best environment for the critical membrane proteins such as membrane ATPases and allows the cell membrane to maintain a liquid crystalline state when temperature increases during winter.

Adaptive seasonality and cold tolerance interact to allow a native bark beetle to invade novel habitat in Canada

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Abstract:

Mountain pine beetle (MPB), a tree-killer at high population densities, recently expanded its range in western Canada when beetles from an epidemic in British Columbia were carried east across the Rocky Mountains on upper atmospheric winds and deposited in northern Alberta. The Rockies had previously served as a biogeoclimatic barrier, but MPB now threatens to spread east across Canada's boreal forest. Climate is a major factor limiting MPB. The new range in Alberta generally has colder winters, but much warmer growing seasons, than the historic range in British Columbia. This on-going study has found that MPB's life stages vary in their cold tolerance and only late instar larvae can survive winters in the new range. Insect development during the growing season determines the life stage that overwinters and thus survival. At some field sites in the new range, developing insects received 40% more degree days than needed for a one-year life cycle yet most of the population maintained an adaptive seasonality and entered the winter as late instar larvae; only a small percentage of the population pupated before winter despite accumulated degree days. A previously unreported delay in development associated with cold experience was discovered. The developmental delay, which could be a facultative cold diapause, may be critical for MPB to maintain its adaptive seasonality in habitats with warm growing seasons, but cold winters, like Canada's boreal forest.

Plasticity of the thermal reaction norms for development in the European Peacock butterfly *Inachis io* (Nymphalidae) from two populations

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Abstract:

The thermal reaction norms for development, which are described by the regression coefficient (or the thermal sensitivity coefficient), the lower developmental threshold, and the sum of degree-days, were studied in *Inachis io* immatures. There was a photoperiodic effect on the thermal reaction norms for larval development. In the Bryansk population, there was a gradual increase of the lower threshold from 10.7 °C at 18L:6D to 11.3 °C at 16L:8D and 12.4 °C at 12L:12D, and the slope of the regression line of development rate on temperature became steeper with decreasing day length. In the Saint Petersburg population, the reaction to short-day (12L:12D) and long-day (22L:2D) conditions was weaker, but the tendency was the same: under short day, the caterpillars developed a little faster than under long day. The developmental thresholds and the slopes of regression lines in these two cases did not differ. At an intermediate day length of 18L:6D, development of caterpillars from Saint Petersburg was less temperature-sensitive and characterized by a lower threshold than under shorter and longer days. In both populations, the influence of short-day photoperiod on larval development manifested itself most distinctly in the changes of pupal weight: in all the temperature regimens, the pupae were lighter under short-day than under long-day conditions and consistently heavier with rising temperature. The development of caterpillars was characterized by a lower thermal sensitivity than the development of pupae. Individual rearing led to a longer duration and lower thermal sensitivity of larval and pupal development as well as to a reduced weight of the pupae. The work was supported by the RBRF grant no. 14-04-01156-a.

The mechanisms of drought-induced rapid cold-hardening

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Abstract:

Rapid cold-hardening (RCH) is a physiological process by which insects “instantaneously” enhance their cold-tolerance in response to acute exposure to abiotic stressors. While a variety of stressors induce RCH, most research has focused on chilling as the trigger, thus, much of what is known about the mechanisms of RCH is specific to chilling. However, recent evidence suggests that the mechanisms of RCH triggered by brief desiccation (drought-induced RCH) may be distinct from those of chilling-induced RCH. In this study, we will investigate the cellular processes triggered by brief desiccation in larvae of the goldenrod gall fly, *Eurosta solidaginis* (Diptera, Tephritidae). Previous studies found that brief desiccation causes larger-than-expected increases in hemolymph osmolality; however, the metabolytes responsible for this increase were not identified. We will use a metabolomic approach to identify these accumulated compound(s). Additionally, autophagy, a process used to sustain cells through periods of stress, is upregulated during long-term dehydration in insects. Here, we found evidence that autophagy is an important process in drought-induced RCH as well: 78% of midgut cells dehydrated ex vivo for 2 h survived subsequent freezing; however, when the autophagy-blocking drug chloroquine was added to the dehydration media, midgut cell survival of freezing was reduced to control levels (33 and 26%, respectively). Further, treatment with chloroquine had no effect on survival in chilling-induced RCH treatments, suggesting that desiccation and chilling induce RCH by different mechanisms. Finally, p38 MAP kinase activation is an important part of the signaling cascade that elicits chilling-induced RCH. Thus, we will investigate the activation of stress-responsive MAP kinases during brief desiccation to identify the particular MAP kinases involved in drought-induced RCH. Results from this study will provide important insights into the diversity of mechanisms of RCH responses in insects.

Targeted transcriptomic analysis of diapause development in larvae of the drosophilid fly, *Chymomyza costata*

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Abstract:

Insect diapause development consists of a sequence of several ecophysiological phases, which are poorly characterized in biochemical and molecular terms. In an effort to describe the phases in more detail, we used larvae of malt fly, *Chymomyza costata*, for which the environmental regulation of diapause development was established in our previous work (Koštál et al., 2000, J. Insect Physiol. 46, 417-428). We exposed the larvae to specific photoperiodic and temperature conditions and sampled them during the course of diapause induction, initiation, maintenance, termination, quiescence and resumption of development. Total RNA was isolated from larval samples, converted to cDNA and subjected to microarray analysis using our own custom DNA microchips containing 1047 oligonucleotide probes for candidate genes. Candidate genes were selected to cover a broad range of processes likely involved in the regulation of developmental arrest, cell division cycle, hormonal signaling, diapause progression throughout the ecophysiological phases, cold acclimation and cold tolerance, metabolic suppression and long-term maintenance of homeostasis including ionic balance, protection against aging, apoptosis and oxidative damage. We will present a preliminary analysis of global transcriptomic patterns characterizing individual phases of diapause development in *C. costata* as well as selected data showing temporal changes in gene expression in specific candidate sequences (gene clusters) associated with major dichotomy between diapause maintenance vs. diapause termination.

Comprehensive study of phenoloxidase activity in *Tribolium castaneum* across temperature regimes

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Abstract:

The host immune response is one of the main targets of selection, as it is locked in a constant arms race with rapidly evolving pathogens but is also considered costly for organisms, especially in sub-optimal environmental conditions. In insects, phenoloxidase activity is one of the most prominent elements of the immune systems due to its involvement in processes like the encapsulation of pathogens. To date, though, only a few studies have investigated the immunological response across a range of temperatures, especially with consideration to differences between sexes and using quantitative genetics techniques, and even fewer have specifically examined the effects of temperature on phenoloxidase. We exposed isofamilies (progeny of diallel crosses between inbred lines) of *Tribolium castaneum* to three temperature regimes (24°, 30°, and 36°C) during all stages of development and measured phenoloxidase activity, body mass, and protein content in adult haemolymph of both sexes.

Phenoloxidase activity per minute per unit of body mass was significantly influenced by temperature ($p<0.001$), with values at 24° and 36°C higher than at the more-optimal 30°C. Sex was not a significant factor, but its interaction with temperature was ($p=0.044$), with activity in males lower at 30°C and higher at 36°C. However, this trend changed when obtained values were standardised by the amount of protein in the sample: temperature was still a significant factor ($p<0.001$), but in this case activity increased with temperature. Additionally, sex was now a significant factor ($p=0.012$), with male phenoloxidase activity being generally higher in not-optimal conditions.

Our data provide interesting insight into the influence of environmental cues on insects' immunological activity. We also emphasize the importance of appropriate and universal data standardisation as this had a clear impact on our obtained results.

Transcriptional changes induced by the plasticizer benzyl butyl phthalate (BBP) in *Chironomus riparius* (Diptera), a model species for ecotoxicity studies

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Abstract:

Butyl benzyl phthalate (BBP) has been extensively used worldwide as a plasticizer in the polyvinyl chloride (PVC) industry and the manufacturing of many other products, and its presence in the aquatic environment is expected for decades. BBP is included in the list of Substances of Very High Concern (SVHC) by the European Chemicals Agency (ECHA), and in the Substance Priority List (SPL) by the Agency for Toxic Substances and Disease Registry (ATSDR). The compound is classified as a reprotoxic substance in the CLP Regulation 1272/2008, and its use is prohibited in the production of toys and childcare articles, cosmetics, and materials intended to come into contact with food. In the present study, the toxicity of BBP was investigated in *Chironomus riparius* aquatic larvae. The effects of acute 24-h and 48-h exposures to a range of BBP doses were evaluated at the molecular level by analysing changes in genes related to the ribosomal machinery, the stress response, the endocrine system, the energy metabolism, and detoxication pathways. Furthermore, early cytotoxic effects on the transcriptional activity of larvae were immunocytochemically evaluated in polytene chromosomes from salivary gland cells after BBP treatments. BBP caused a dose and time-dependent toxicity in most of the selected biomarkers. Although no significant effects were detected in 24-h acute exposures, longer treatments triggered a general repression of transcription in all the analysed genes. Moreover, delayed toxicity studies were specially relevant, since they allowed us to detect unpredictable toxic effects, not immediately manifested after contact with the phthalate. Polytene chromosomes showed significant effects of BBP on the puff formation in Balbiani rings, demonstrating the ability of this compound to alter the transcriptional pattern in exposed larvae. This study provides novel and interesting results on the toxic effects of BBP in C. riparius and highlights the suitability of this organism for ecotoxicological risk assessment, especially in aquatic ecosystems. This research was supported by Ministerio de Economía y Competitividad (Spain) contract Grant No. CTM-2012-37547.

Effect of temperature rates on upper and lower thermal tolerance limits in *Drosophila melanogaster* and *Arabidopsis thaliana* – common responses across kingdoms?

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Abstract:

There is evidence that climate change has increased the variability of temperatures within and across seasons, and the frequency and severity of erratic temperatures events is projected to increase further over the 21st century. This implies that natural populations of insects and plants are exposed to increasingly variable and rapidly changing temperatures. Similarly to ectothermic insects, plants cannot, or only to a very limited extent, metabolically regulate their temperature. Both organisms have therefore evolved various mechanisms to tolerate changes in temperature. Physiological data on insect and plant model organisms such as *Drosophila melanogaster* and *Arabidopsis thaliana* are often used to provide information of general relevance across kingdoms. But to what extent do plant studies provide relevant information for insects and vice versa? And are insects and plants equally sensitive to rapid temperature changes or has plants developed greater tolerance to temperature fluctuations than insects due to their sessile lifestyle? Here we test the consequences on upper and lower thermal limits of exposing *D. melanogaster* and *A. thaliana* to temperatures that gradually increase or decrease with different rates. Preliminary studies have shown that fast ramped flies have higher upper thermal limits compared to flies ramped to high temperatures at a slower rate, suggesting that more cellular damage is induced the slower the ramping. In on-going studies we test whether this is also observed in A. thaliana and determine the effects of ramping rate on lower thermal limits when temperatures are ramped down in both species. Comparative thermal studies of insects and plants may help to elucidate whether organisms, which primarily regulate their temperature through the environment, have evolved common adaptations to temperature fluctuations across kingdoms and provide new insights into the ability of two of the most commonly used model organisms, D. melanogaster and A. thaliana, to cope with increasingly unstable temperature regimes.

Multiple biological proxies reveal the chronosequence of acclimation processes in insects

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Abstract:

Low temperature is a widespread cause of death in insects during winter. As a result, the level of cold tolerance is enhanced (acclimation) in many insect species before the onset of winter. Several studies have theorised and demonstrated the positive effects of acclimation on the survival, onset and recovery from chill coma in insects. The exact mechanisms of cold acclimation in insects appear to be more complex than initially thought, and temporal changes in their physiology may gradually take place over the course of acclimation. In this study, we thus examined the effects of seven acclimation durations, ranging from 0 to 11 days, on different biological end proxies (critical thermal minimum, chronic cold survival, antioxidant capacity and metabolic phenotypes) in adults of the tenebrionid beetle, *Alphitobius diaperinus*. The critical thermal minimum quickly decreased and reached a plateau after the adults were cold acclimated for 3 days. A similar pattern was observed when assessing the total antioxidant activity. Interestingly, it required a longer acclimation period (ca. 7 days) to observe an enhanced cold survival and elevated amounts in cholesterol. These data suggest that the accumulation of cholesterol was used for the progressive adjustments of membrane fluidity. Altogether, our results reveal the existence of distinct sequences in the acclimation process of *A. diaperinus* adults, suggesting different physiological pathways associated to the elicitation of cold tolerance.

Joint influence of temperature and nutrition on immunological response, drought tolerance, and body composition of Arion vulgaris

Paulina Kramarz*, Joanna Homa, Daniel Kübler, Karolina Naumiec, Stine Slotsbo, Elzbieta Szulinska, Jesus Mari Txurruka, Grazyna Wilczek, Kamila Zajac, Szymon Drobniak

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Abstract:

Temperature and food are two of the most important abiotic factors influencing all aspects of an animal's biology. In the food, carbohydrates are a source of energy, whilst proteins are important mainly for body growth. As temperature influences the speed of biochemical processes, it also affects the rate of digestion and absorption of food, probably in different ways for each macronutrient. Exposure to sub-optimal temperatures, together with an unbalanced diet which lacks a sufficient concentration of a given macronutrient, may therefore influence an animal's response to other environmental factors.

Our study explored the interaction of thermal conditions and the nutritional composition of food (different protein:carbohydrate ratios) on ecologically relevant aspects of the physiology of the slug *Arion vulgaris*. First, we studied the effects on phenoloxidase activity, a main component of the immunological response, as this animal is faced with a wide range of pathogens in its natural environment. Additionally, as slugs are sensitive to humidity, while also extremely resistant to water loss, we characterized slugs' response to drought conditions. Finally, we measured the growth rate and body composition (proteins, carbohydrates, and fat in body wall and hepatopancreas) of slugs from all treatments.

Slugs were raised in optimal (15°C) or one of two not-optimal temperature regimes (10°C or 20°C). Animals were provided one of five different diets with the following protein:carbohydrate ratios: 1:5, 2:4, 3:3, 4:2, 5:1.

Phenoloxidase activity was affected only by temperature – the activity of this enzyme was enhanced in both not-optimal conditions. Drought resistance was most affected by the highest-temperature treatment and the lowest-protein diet, but there was no interaction between factors. Growth rate was highest in the optimal temperature regime (15°C) and in the balanced diet treatment (3:3 protein:carbohydrate ratio), but again, no interaction was observed. Body composition was only affected by changes in macronutrients ratio – most interestingly, we observed an increase in glycogen concentration in the body wall of slugs fed a high-carbohydrate diet.

As could be expected, the response of the slugs to the studied factors was rather complex. However, such studies are necessary for the extrapolation of laboratory experiments to field conditions. They also serve as a basis for further, more detailed investigation of the response of the invasive slug Arion vulgaris to the novel habitats in which it spreads so successfully.

Lipophilic contaminants influence cold tolerance of invertebrates through changes in cell membrane fluidity

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Abstract:

Contaminants taken up by living organisms in the environment as a result of anthropogenic contamination can reduce the tolerance of natural stressors (e.g. low temperatures), but the physiological mechanisms behind these interactions of effects are poorly understood. The tolerance to low temperatures of organisms that cannot regulate their body temperature (ectotherms) depends on their ability to increase the fluidity of their cellular membranes at low temperature. Our study shows that contaminants accumulating in lipids of organisms alter the physical state of their membranes simply by being present. Contaminants of varying chemical structure can alter the membrane fluidity in either direction and correspondingly modulate the cold tolerance of intact animals. Here, we provide evidence that the membrane partitioning of two lipophilic compounds, namely phenanthrene (representing the family of aromatic hydrocarbons), and 4-nonylphenol (an amphiphilic, yet hydrophobic, organic compound, which in our case is a mixture of branched isomers of 4-nonylphenol), translates into oppositely directed changes of model membrane fluidity and bending rigidity, and that this phenomenon is congruent with the cold tolerance of intact invertebrates exposed to these contaminants. Partitioning of sub-lethal concentrations of phenanthrene (PHE) causes membranes to become more fluid in vitro, and this effect was congruent with an increased cold tolerance of the freeze-tolerant oligochaete, *Enchytraeus albidus*, and the chill-sensitive collembolan, *Folsomia candida*. In contrast to this, membrane partitioning of 4-nonylphenol (NP) reduced the fluidity of model membranes in vitro and also reduced the cold tolerance of these two animals. Our study links the partitioning of contaminants into membranes with phenotypic responses to cold, and hence fundamentally improves our understanding of combined exposure to contaminants and natural stressors.

Cold acclimation allows *Drosophila* flies to maintain mitochondrial functions

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Abstract:

Environmental stress generally disturbs cellular homeostasis. Heat shock, for example, disrupts the ability of mitochondria to phosphorylate ATP's diphosphate precursor, resulting in ATP depletion. During cold exposures, it has been hypothesized that chilling injury is linked to a shortage of ATP reserves. However, previous studies conducted on insect models are conflicting: some detected a depletion while others found increased levels of ATP. These incongruities may result from the fact that ATP measurements reflect the net result of production and consumption, and hence, modification of ATP levels could result from various mismatches among various metabolic pathways. Here we focused on the production site by measuring respiratory activity and ATP synthesis directly in isolated mitochondria. Organelle responses were evaluated and compared between cold-acclimated and control *Drosophila melanogaster* adult flies. Temporal assessment of mitochondrial functions during prolonged cold stress (4°C) reveals that mitochondrial ATP synthesis and respiration decline with cold exposure duration. Parameters used to quantify the functioning of mitochondria indicated that cold-acclimated phenotype can better maintain mitochondrial functions than control flies under cold stress.