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# Review of PhD thesis

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Title: **Low-temperature injury in insect tissues and mechanisms of its repair**

Reviewer: **Ing. Miloš Faltus, Ph.D.**
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General comments:

The reviewed doctoral thesis is written in English and it is arranged into nine parts: 1. Introduction; 2. Insects at low temperature; 3. Physiological mechanisms of insect cold hardiness; 4. Model insects; 5. Goals of PhD project; 6. Main results of PhD project and their discussion; 7. References; 8. Scientific papers; 9. Summary and Conclusions. The first four parts introduce the reader to subject matter, the fifth section defines goal of the project, the sixth section presents main results and comparison of them in context of current knowledge, following with the list of references used in text. The eight section comprises two already publish scientific works and one submitted. The last part of the thesis summarizes and concludes the most significant achievements.

**Part 1: Introduction**

The introduction is clear and well prepared. It shortly explains the thesis topic to the reader. It describes the problems of periodical occurrence of winter in non-equatorial climate zones for small ectotherms and the importance of phenotypic alterations related to high cold resistance. The dissertation is focused on physiological responses to cold stress in two drosophilid fly species with contrasting cold resistance: *Drosophila (Sophophora) melanogaster*, species of tropical origin with relatively low tolerance to cold stress, and *Chymomyza costata*, holarctic species with highly freeze-tolerant larval stage. It also brings forth information on the conservation methods already being used for the species studied in the thesis.

**Part 2: Insects at low temperature**

This section distinguish between summer and winter phenotypes of small ectotherms, and presents changes associated to these stages of development. The summer phenotypes are characterized by lower developmental threshold (LDT), critical minimum of a body temperature (CTmin) or lower lethal temperature (LLT). Their low temperature limit is called as cold tolerance. In contrast, cold hardiness induced in winter phenotypes after entering into a dormant state, either diapause or quiescence, is mostly characterized by melting point temperature of the body fluid (m.p.), suppercooling point (SCP) and glass transition temperature (Tg). The LLT in winter phenotypes is lowered in comparison to summer phenotypes. Understanding of these basic terms is a prerequisite for the correct definition of the PhD thesis objectives.

**Part 3: Physiological mechanisms of insect cold hardiness**

This section outlines three strategies of insect cold hardiness in winter phenotypes: supercooling, freeze tolerance and cryoprotective dehydration. Author describes importance of water balance in insect body, osmotic and ionic homeostasis, metabolic changes, biological membranes, heat shock, antifreeze and other proteins, and of low molecular mass cryoprotectans as biological solutions, antioxidants and stabilizations of macromolecular complexes for their cold hardiness. These findings were reflected in the methodological approaches of the dissertation.

**Part 4: Model insects**

Two insect models with contrasting levels of cold tolerance or cold hardiness used in the study are described: *Drosophila (Sophophora) melanogaster*, a fly exhibiting relatively modest level of cold tolerance and *Chymomyza costata* emerging model for investigation into extreme freeze tolerance. Differences between these two insect species with respect to their cold acclimation and cold hardiness in particulars development stages are described. Selected species of contrast insects used in the study allowed to distinguish between different mechanisms of low temperature injury and their repair.

**Part 5: Goals of PhD project**

Two main goals of the PhD study were clearly defined: 1. Analyses of physiological processes, and detection of changes in metabolomic and transcriptomic profiles, occurring after the cold stress of varying intensity in the larvae of two model insects, *D. melanogaster* and *C. costata*, 2. Understanding the role of mitochondria in response to freezing stress in larvae of *C. costata*.

**Part 6: Main results of PhD project and their discussion**

This part is clear and well structured. It summarizes results already proceeded into the author´s scientific papers presented in Part 8. The author emphasizes the most important current knowledge in the area of study and novel findings presented in the papers. I especially appreciate the methodological approaches with regard to the holistic and sequential process of diapause induction, cold acclimatization, cold injury and repair or tolerance of cold injury. The different intensity of cold stress used in the experiments was clearly and unequivocally defined. This approach allowed to distinguish between specific effects of particular stresses accompanying the low temperature treatment and compare these findings with metabolomic and transcriptomic analyses. The wide range of methods used, the way of evaluating and interpreting the results underlines the excellent quality of scientific work of the author´s dissertation.

**Part 7: References**

This section is well organized and the list of references corresponds to current knowledge.

**Part 8: Scientific papers**

The results of the PhD thesis were proceeded into two papers, which have already gone through the standard reviewing process in scientific journals. Other paper was proceeded and submitted into a scientific journal for review process. This section allows, due to the standard paper structure, to recognize details about the material and methods used in the experiments and complete results.

**Part 9: Summary of results and conclusions**

This part summarizes main achievements, which answers the questions previously defined. The main results of PhD study in three areas of interests are summarized and concluded: 1. Recovery from cold stress in *Chymomyza costata*, 2. Recovery from cold stress in *Drosophila melanogaster*, 3. Mitochondria were identified as targets of low temperature-induced injury. A novel and original finding on the post-stress processes, including repair of injury were obtained in larvae of highly freeze-tolerant drosophilid fly, *C. costata* after the exposure to various intensities of cold stress. The global metabolomic and transcriptomic analyses revealed significant differences between control and supercooled larvae but relatively weak differences between supercooled and frozen larvae, and significant difference between from frozen and cryopreserved larvae. The obtained results identify mitochondria as potential targets of frost damage in cryopreserved larvae. In contrast to these results, the metabolomic and transcriptomic profiles were significantly different between supercooled and frozen larvae in case of relatively freeze sensitive organism, *D. melanogaster*. The obtained results indicated a failure of mitochondrial aerobic respiration in the frozen larvae but a restoration of pre-freezing pattern in the supercooled variant. Finally, the author demonstrates mitochondria functionality in relation to freezing tolerance or sensitivity.

<u>General conclusion</u>

The PhD thesis is well written and generally clear. It brings a novel and original finding on the post-stress processes in two model small ectotherms, *Drosophila (Sophophora) melanogaster* and *Chymomyza costata*. Objectives and hypotheses are clearly stated, the method used are adequate and they are well described. The methods and statistical analyses are described adequately. The main objectives have been achieved. The reviewed thesis together with enclosed publications clearly

demonstrate the author's ability of scientific work. Therefore, I recommend the presented PhD thesis for the final defense.

Questions:

1. Can you explain your statement about wrongly used term 'diapause', which is often used to describe the state of *D. melanogaster* adults overwintering in temperate regions?
2. In plant kingdom is well known so called the second stage of cold hardening at temperatures below zero. Do you know some evidence for this phenomenon in case of small ectotherms as well?
3. Can you reveal your plan, if any, to verify the defined hypothesis of the importance of the integrity of the inner mitochondrial membrane for freezing injury or tolerance in small ectotherms?

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