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# Evaluation of the Ph.D. Thesis by Markéta Hejníková, entitled "Reproductive diapause in males of the linden bug, *Pyrrhocoris apterus*"

Robert Hanus

The PhD. thesis by Markéta Hejníková is a concise text summarizing her efforts in understanding the environmental regulation and endocrine underpinnings of (adult) reproductive diapause in the linden bug *Pyrrhocoris apterus*, with special emphasis on males. It is the so-called "short thesis", consisting of an introduction to the topic spanning over 11 pages, followed by reprints of three impacted papers, each of them being preceded by two-page summary. The reader can then go through three pages of Discussion, followed by Bibliography consisting of over 130 items cited in the author's text.

Reviewers of PhD theses sometimes wash their hands by stating that they resign on judging the quality of the papers submitted within the thesis since the reviewers in the respective journals already did so. I must slightly disagree here, because a GOOD PhD thesis is made of a GOOD accompanying text and of GOOD papers that are not off topic and that are bringing important new knowledge. Therefore, I will try to evaluate Markéta's thesis as a whole, including how the selected papers fit to the entire story and contribute to the field.
And this evaluation is overall truly positive, because this thesis indeed does consist of GOOD papers and a GOOD accompanying text. The thesis is first of all very FOCUSED and does not dilute the important information and findings by unnecessary bi-passes, deviations or speculations, etc. At the same time, the thesis goes in DEPTH of the biological question studied, i.e. the male reproductive diapause. I see four main reasons for this success. First, regulation of reproductive diapause in *P. apterus* became Markéta's principal topic since the very beginning of her studies, because she dedicated herself to this biological problem already in her Master thesis. Second, her work builds on a solid base of previous knowledge about *P. apterus* reproductive biology and endocrinology obtained by multiple generations of Czech scientist interested in this model. Third, she was lucky to have good advisors driving her through the studies and assisting her in the experimental design and paper writing, etc. And finally, and most importantly, she did a good job.

A short overview of the thesis. The introduction part takes the best from the available literature on insect diapause and provides a broad viewpoint of the problem. Beside the expected detailed dissection of proximate (neuro)endocrine aspects of insect diapause, I was also pleased to read a text searching for ultimate factors and adaptive significance of this interesting life history trait of most temperate insect species. Such a strategy of introducing the thesis is an excellent way to invite for further reading also the readers who are not experts in the field. Last chapter of the introduction then introduces the model organism, *P. apterus*.

The first included paper was published in 2016 in *J. Insect Physiol.* with Markéta as the first author with major contribution. In this paper, Markéta builds on her results presented already during her Master thesis. The authors prove the participation of the JH receptor protein *Met* and its binding partner *Tai* in JH reception. They do so via RNAi experiments and also show the downstream effect of the silencing of these genes on males, i.e. reduction of accessory gland proteins amount, increase of hexamerin protein content in the haemolymph, and ultimately extension of the male's lifespan upon *Met* silencing. They compare these experiments with those made on males after *corpora allata* ablation and also conclude short-day living conditions have stronger biological effect than CA removal or receptor silencing.

This paper then paved the way for the second first-author paper of Markéta, this time published in *Insect Biochem. Mol. Biol.*, an article which in my view represents the main scientific output of this PhD. thesis and maximum experimental effort invested by the author herself. In this paper, an extensive set of environmental and endocrine factors is tested with respect to their influence on the reproductive diapause of both *P. apterus* sexes, such as temperature induced diapause termination, application of a JH mimic, RNAi of *Met* and *tai*, CA removal, photoperiod manipulation, etc. It is a comprehensive study testing in detail all meaningful combinations of the listed factors, and considering, beside the phenotypic outputs in terms of the readiness to reproduce, also other interesting downstream effects, such as JH (JHSB3) levels, including its level in male accessory glands, expression of JHAMT (an enzyme involved in last steps of JH biosynthesis),

etc. I am not going to list all the individual findings about ecophysiological and endocrine regulation of diapause resulting from this study, because I expect we will hear them from the author herself during the public defense. I will only highlight here one of the figures from this paper (Figure 4), which nicely summarizes all the experiments made into a diagram that proposes how this regulation likely proceeds. It shows on the one hand how the diapause control is complex and sexually dimorphic, and how many different factors the authors considered before coming to these conclusions. In fact, in my view, this schematic figure is in some way the summary of this thesis in general.

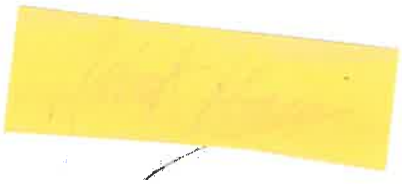
In the third included paper, also published in *Insect Biochem. Mol. Biol.*, Markéta joined, this time as a regular member of the author team, the effort of her labmates to understand the functional significance of the neuropeptide EFLa, an arthropod ortholog of the vertebrate thyrotropin-releasing hormone. To do so, they fully characterized the gene and protein in *P. apterus*, and performed localization and functional experiments with, among others, EFLa null mutants. The functional study focused on the putative role of this neuropeptide in development, oviposition, lifespan, circadian rhythmicity, and, last but not least, the diapause. How much the authors succeeded in this effort will be, once again, presented Markéta herself.

The Discussion part of the thesis is well written as well. It succeeds in stepping aside from technical details and adopting a comparative viewpoint across other model insect species. It also formulates relevant questions for the future research.

A balanced review of a Ph.D. thesis should also contain some critical point(s). Here is one: To this purpose, I will use a quotation of myself from the review of Markéta's Master thesis back in 2013: "A lesson to take is that a good scientific work needs not only to be written, but also needs a careful proofreading". Likewise, this Ph.D. Thesis would benefit from one more round of reading to make sure that every sentence finishes with a full stop, that the references are in correct and unified format, etc.

**Final recommendation: I am more than happy to recommend this interesting Thesis for public defense and I am looking forward to participating in person.**

In Prague, September 19, 2022



**My questions:**

1. related to the second and main paper (IBMB 142: 103721, 2022): This paper uses a series of *in vivo* manipulations of both *P. apterus* sexes to address the relationship between diapause, its endocrine regulation and the onset of reproduction once diapause terminated. The first bioassay uses males and females exposed for different periods (1, 3, 4, 6, 9, 13) to low temperatures (0°C) as a mean to terminate their diapause. Afterwards, their ability to reproduce (mate) has been scored in intact individuals as well as individuals whose JH sensitivity was manipulated by means of RNAi against the JH receptor *Met*.
Figures 1 and 2 show the data as cumulative curves of the onset of mating. However, I do not see any stats comparing the differences among these curves, equivalent to survival curves. Instead of statistics, I read the statements such as "weaker diapause", "slightly prolonged diapause", "accelerated mating", "slight delay", etc. Intuitive question: why were not these trends tested statistically and how would one do so?

2. On the same note: It appears from Figure 1 B-E that a simple injection of anything (dsMet but also Ringer's solution) dramatically accelerates the readiness of males to mate. Can you comment on this?

3. On many places across the thesis I spotted the immature *P. apterus* stages to be called "larvae" instead of the expected "nymphs". Is there any reasoning behind the selection of this term?



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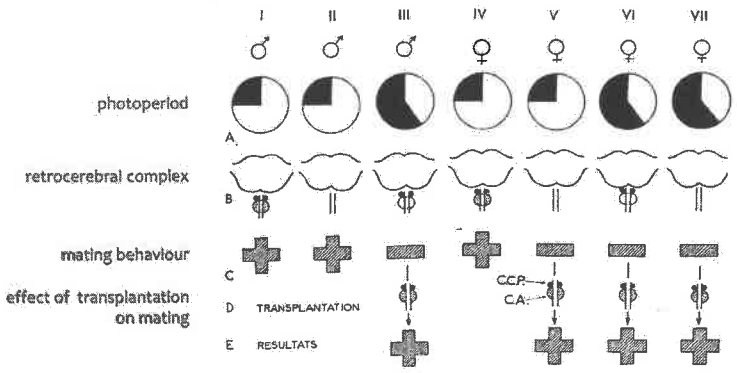
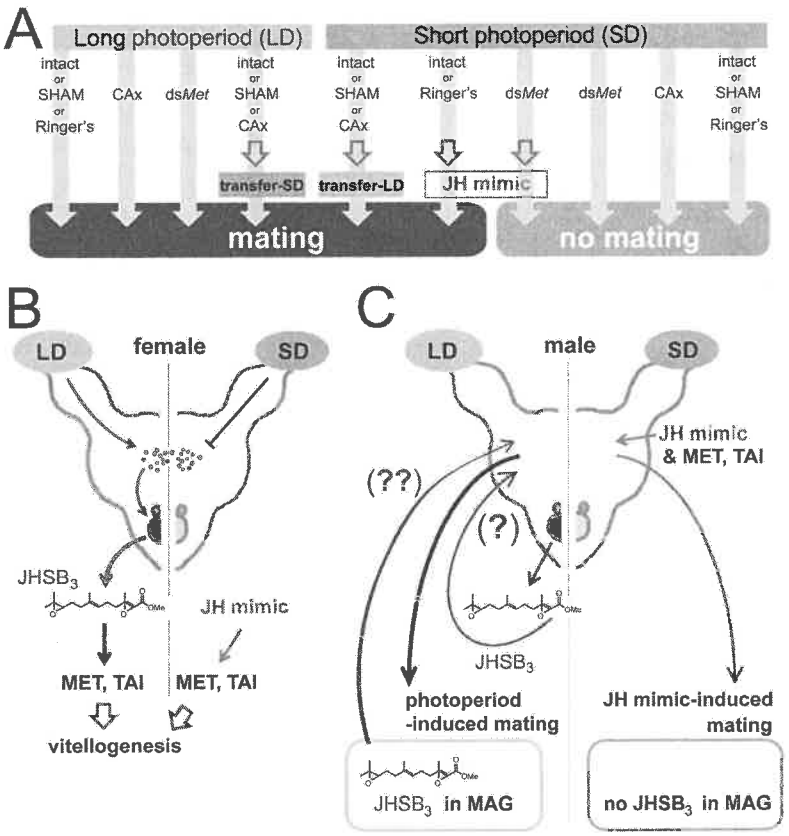
4. Very general question to develop: I am pasting below two figures. The first one dates back deep to the past century and summarizes the knowledge on endocrine and photoperiodic regulation of *P. apterus* reproduction, while the second one is from your IBMB 2022 paper. Can you pinpoint what have we learnt over the past half a century (including your contribution), and what are eventual contradictions between the obsolete view and the modern perspective?




5. I keep a few more questions for the public defense itself.



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