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Opponent review on Ph.D. thesis of Olga Bazalová:

CIRCADIAN CLOCK GENES IN INSECTS

The author presents extensive work describing molecular characterization of circadian clock genes in insects. Specifically, she studied: (1.) the impact of temperature on the behavior and the clock genes expression patterns in two dipteran species – *Drosophila melanogaster* and *Musca domestica*; (2.) how is the photoperiod signal transmitted through clock genes machinery in diapausing males of the linden bug, *Pyrrhocoris apterus*; and (3.) the role of circadian protein Cryptochrome in magnetoreception of two cockroach species – *Periplaneta americana* and *Blattella germanica*.

All the work has been done in Laboratory of Molecular Chronobiology of candidate's supervisor Dr. David Doležel (Institute of Entomology, Biology Centre CAS in České Budějovice) and the outcomes are summarized in three papers. One of them is "in preparation" state and the others are already published either in the best ranked entomological journal "Insect Biochemistry and Molecular Biology" or in the excellent interdisciplinary journal "Proceedings of the National Academy of Sciences of the USA".

The thesis itself consists of an introductory essay, aims of scope, summaries of the papers, overall discussion, conclusion and references. The copies of papers are finally attached.

The introduction is well written and also the chapter ordering is reasonable and keeps reader's attention. The cited references are up to date and cover the topic entirely. Perhaps, the description of Methoprene-tolerant protein (MET) as the receptor for insect juvenile hormone could be referenced also with a work originated from the laboratory of your neighbor (Jindra et al., 2015). Nevertheless, besides a few typos I do not have any reservation or comments to the introductory part of the thesis.

The first publication shows that the housefly, *M. domestica* displayed similar locomotor activity behavior pattern like *D. melanogaster* in response to altered temperature of surrounding environment. Moreover, also the expression patterns of selected clock genes (namely *per*, *vri*, *cwo*, *Clk* and *tim*) in different temperatures were found to be similar in both flies. On the contrary, no intron, homologous to the last *per* intron (Dmpi8) of *D. melanogaster*, was found in 3' UTR in *M. domestica* per gene. This finding suggests that these two closely relative dipteran species have different molecular mechanism, which initializes the same behavioral response. Among others, this manuscript presents nice graphical comparison of cis-regulatory motifs position in circadian genes from both fly species (Figures 10 and 11) and this analysis is not reflected in the text of present version. **I would be interested how is possible to interpret the finding that first introns of *Musca* genes are considerably larger and also contain higher number of cis-regulatory motifs than those of *Drosophila*. Could be this information a potential clue for explanation of differently regulated behavioral response?**

The second publication describes the role of circadian genes and JH receptor in male diapause in the linden bug, *Pyrrhocoris apterus*. The relative size of male accessory glands was used as a marker for reproduction or diapause states. This work brought evidences that circadian factors Clock, Cycle and Cry2 are responsible for photoperiod measurement. Further results showed that Met and its partner protein Taiman are responsible for JH reception and subsequently for the growth of male accessory glands. The peculiar finding of this study is that knockdown of the JH receptor neither lowered locomotor activity nor reduced mating behavior of males and moreover the Met RNAi males were fertile producing comparable number of offspring. This suggests JH-upstream photoperiodic regulation of reproductive behavior in *P. apterus* males. This is the new molecular evidence, which points out the differences in reproduction control of *P. apterus* males and females. **How it is with importance of food availability? Is it needed for successful mating of both *P. apterus* males and females? Which hormonal signals, other than JH, could be theoretically involved in regulation of evidently accomplished spermatogenesis of males with knockdown of JH receptor?**

In **the third paper** was established the role of CRY2 in light-dependent magnetoreception in two cockroach species and furthermore also the CRY2 importance in the geomagnetic field vector detection under intensities comparable to intensity of natural geomagnetic field. This extensive work has got a deep scientific impact since the outcomes allows comparison of molecular mechanisms present in insects with those known from vertebrates.

Is magnetic field sensing of the birds detected also via Cryptochrome(s)? Do you thing that CRY2 function mediated directional magnetoreception in cockroaches could be adapted also in other insect species (e.g. genome of sedentary plant parasite – the pea aphid, *Acyrthosiphon pisum* contains two CRY2 genes)?

In conclusion

The candidate has done an extraordinary job in the field of molecular chronobiology of insects and I recommend her thesis for defense of academic degree Ph.D.

In Prague March 3, 2017

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Ceske Budejovice, March 13, 2017

To whom it my concern;

Today, I had the great pleasure of participating in the thesis defense of Mgr. Olga Bazalova as an external reviewer. I recommended without any hesitation that she be granted the title of PhD, with "Excellence". Olga's work on circadian clock genes and their role in circadian rhythms, photoperiodisn and magnetoreception is of excellent quality and highly original. Her thesis was very well written, and her oral presentation was excellent as well. Below are the written questions I had submitted prior to the defense. These questions were addressed with great care and depth, and so were additional questions that I asked as follow-ups on her answers. I have no doubt that Olga will continue to excel as a researcher, if she decides to pursue this career.

Best regards,

Patrick Emery

1) Is circadian activity in Musca really comprised of an M and E peak? Are they both detectable in DD? In other words, how much of the LD pattern is due to masking?
2) What is know about the circadian neural circuit of Musca? Does it support the idea of a dual oscillator model for M and E activity?
3) Did you try to measure activity under natural or semi-natural conditions in Musca?
4) How do the molecular cycles you measured under different temperatures compare to those of Kidd et al. (2015) ? Do they support the idea that temperature compensation might rely on a rescaling of *tim* (or other circadian genes) expression? If not, can you find some trends or patterns that could help explaining temperature compensation, or the adjustment of behavioral phase?
5) In P. apterus. why is there so much variability of gene expression for Met, tai and Clk? Is it a technical issue, or might this have a biological meaning?
6) What does it mean that CLK RNAi blocks locomotor activity increase if the animal Is first exposed to SD, but not if it is grown under LD (fig.6) ?
7) How would you try to identify pathways that control photoperiodic response in P. apterus? In particular, do you have any hypothesis for the JH-independent pathway that regulate locomotor activity?
8) What would be the evolutionary advantage of splitting photoperiodic response into JH-dependent and JH-independent pathways?

9) It appears that clock genes are involved in photoperiod measurement in several insects, but a pleiotropic role is difficult to exclude. Can you think of any experiment that would help determining whether the circadian clock is indeed critical for photoperiod measurement? We could assume that you can do any form of genome engineering in P. apterus, for example.
10) How does a conductance bar look like, how does it work?
11) In which circumstances do you think cockroaches use the magnetic field to orient themselves? More broadly, why do non-migrating insects sense the Earth magnetic field?
12) How do you explain that CRY2 dsRNA has such a strong effect on MIR, but only weak effects on circadian behavior in B germanica? How do you interpret the increased arrhythmcity when downregulating CRY1?
13) How do you envision CRY2 working as a magnetosensor, mechanistically? How would it modulate neuronal activity? Any candidate pathways or genes you would like to test?