Review of the Dissertation: Barbora Konopova

The dissertation work performed by Barbora Konopova deals with a central and long-standing problem for insect biology: the identification and characterization of a juvenile hormone receptor. Based on previous studies of the MET (Methoprene-tolerant) gene in *Drosophila melanogaster*, the studies focused on a characterization of the MET protein's role in a more recent model organism, *Tribolium castaneum*, which also benefits from having a more clearly defined response to juvenile hormone. Studies of JH regulation in the fruit fly have been hampered by the absence of an endogenous phenotypic effect associated with mutations that would presumably impair JH signaling. The studies in Tribolium utilized RNAi to "knock out" MET function and observe the consequences on development. The studies led to a highly significant conclusion, as evidenced by its publication in the Proceedings of the National Academy of Science USA: The absence of MET activity leads to indications of precocious metamorphosis. Further, MET-null tissues fail to mediate the normally toxic effects of the JH analogue, methoprene. Further studies have shown that Tribolium MET regulates the expression of the early-ecdysteroid inducible gene, Broad, and confirms an evolutionarily conserved functional interaction between Broad and MET. Further, a gene target of Broad, Kruppel, has also been confirmed as a target which mediates JH response in Tribolium. Finally, a more recent study has further confirmed that the same role for MET can be ascribed to a hemimetabolous insect species, further indicating the general nature of this mechanism of regulation among insects.

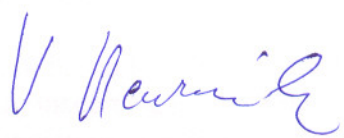
The dissertation work has been well-conceived in terms of the experimental strategy and choice of organism. Further, the work is based not only a sound understanding of the molecular genetics but also the evolution of metamorphosis in insects, so that the work resonates at both the genetic and developmental level. The findings of the work are highly significant to insect biologists as well as molecular biologists interested in molecular mechanisms of hormonal action. In fact, the work has at least partly resolved the issue of what protein is the juvenile hormone receptor in insects. Clearly, MET is one of them. Just as importantly, the work has established a framework for further mechanistic studies that will be essential for understanding hormonal regulation of metamorphosis.

In summary, the work has been well-conceived, has been well-executed technically, has led to significant findings, and established a foundation for effective and timely future discovery. I believe this is one of the finest dissertations I have encountered in my professional career, and rates alongside a dissertation performed by Michael Koelle at Stanford University under the direction of Dr. David Hogness, which reported the characterization of the Drosophila ecdysteroid receptor.

Vincent C Henrich
Professor, Biology, University of North Carolina at Greensboro
Director, Center for Biotechnology, Genomics, and Health Research

Questions:

1. Is it accurate to describe juvenile hormone as "antagonistic" to ecdysone (pg. 9)? This implies that JH "works against" ecdysone.
2. If metamorphosis evolved multiple times among the insect orders, how is the conservation of molecular receptors and their interactions explained?
3. The level of sequence diversity is relatively high. What might account for the high level of sequence diversity of Met, which carries out a highly conserved function (mediating JH effects)?
4. Mutations of the usp DNA-binding domain in flies have been associated with the abnormal derepression of Broad transcription in mutant tissues (though these mutations have no effect on ecdysteroid induction). Ectopic application of JH has also been associated with the upregulation of Broad transcription in flies. How can these effects be reconciled biologically, cellularly, and mechanistically?
5. RNAi treatment for BR-C led to "partial" metamorphosis, that is, some traits were larval and some showed signs of further morphogenesis. Was the possibility of incomplete RNAi suppression ruled out experimentally? If so, how can the progression of some traits and the suppression of others be explained? Does the pattern of traits resemble any other biologically described effects?
6. What is the possibility that there is no JH receptor, but rather, an assembly of transcriptional proteins which includes one or more JH-interacting proteins? What evidence supports and/or refutes this possibility?
7. Can Broad expression be re-initiated by ecdysteroid application in MET-null cells?
8. Broad protein-antibodies are found throughout polytene chromosomes of the Drosophila salivary gland in the late third instar. Does this suggest a role for Broad in mediating hormonal effects?
9. How specific is the effect of Broad on Kruppel-like expression?



Jean-Philippe Charles
phD, Assistant Professor

UMR CNRS 5548 DCCI
Développement et Communication Chimique chez les Insectes
Université de Bourgogne
6, Bd Gabriel
21000 Dijon
France

Dijon, November 9th, 2008

Report for the thesis manuscript of Barbora Konopová.

The manuscript is entitled "Genetic studies on juvenile hormone signalling in insect metamorphosis". It describes the results of experiments exploring the functions of three genes involved in signalling by juvenile hormones (*Met*, *Br*, *Kr-h*). The strategy used combines hormone applications, gene invalidation by dsRNAi, cloning of orthologs, phenotypic and phylogenetic analyses. Experiments were performed mostly on *Tribolium*, but also on the Neuropteran *Chrysopa*, and two Hemipterans (*Pyrrhocoris*, *Rhodnius*). The manuscript is composed of two main parts.

Background material relating to the evolution of metamorphosis and key concepts in insect endocrinology is given in the introduction. This 22 pages header addresses most important issues, and is very well written. Given the importance of the bHLH/PAS proteins in this report, the reader could have been given more information concerning this family of proteins, and for example the existence of two classes of bHLH/PAS, and how they differ in terms of dimerization properties may have been reminded. More ample description of some of the work of the Wilson lab would have been also good, in particular some details of the paper by Godlewski et al. The same applies for the *Kr-h* gene: it is described, but later, with additional results. A presentation of this gene, including a somewhat more detailed description of the work by Minakuchi et al. might have been useful here.

The second part is subdivided into published material, and unpublished results. In a first paper published in the P.N.A.S., the *Tribolium Met* gene is identified as an essential component of the JH signalling pathway during metamorphosis. The second paper, published in Development, focuses on the *BR-C* gene, and demonstrates that it is a critical effector of juvenile hormones in both *Tribolium* and *Chrysopa*. Importantly, it is also shown that the MET protein is required for the regulation of *Br-C* by JH.

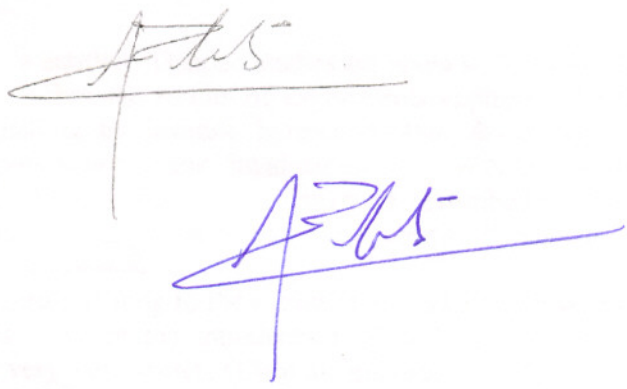
Finally, unpublished data extend these findings to non-holometabolous insects (*Pyrrhocoris*, *Rhodnius*), and demonstrate that *Kr-h* acts as an anti-metamorphic gene. Together, these results concur to establish the MET protein as a strong candidate for a receptor of JH.

The work of B. Konopova is a truly nice set of carefully designed experiments addressing important issues in the field of insect endocrinology, as underscored by the rank of the journals in which these findings have been published. Since MET is emerging as a likely JH receptor, it will be of considerable interest to explore its function in other insect

orders, and clarify its interactions with *BR-C* and *Kr-h*. In this respect, it may be regretted that the manuscript does not include a global prospective discussion, and proposed future directions. For example, given the spectacular phenotype of *Kr-h* impaired animals, it would seem reasonable to ask whether the KR-H protein is a component of the JH receptor, or if it interacts with MET, and what would be the best way to find out. Also, it would be interesting to confront these data with theoretical considerations concerning the evolution of holometaboly.

In summary, the thesis manuscript presented by Barbora Konopová is of outstanding interest and meets the highest international standards in the field. She has published two major papers, and her unpublished results will certainly soon be completed and bring more important insights into the regulation of insect metamorphosis. Without any reserves, Barbora Konopová therefore deserves the grade of pHD.

Jean-Philippe Charles



External appraisal on the thesis of Barbora Konopová entitled "Genetic studies on juvenile hormone signalling in insect metamorphosis" supervised by Dr. Marek Jindra at the Biology Centre of the Academy of Sciences of the Czech Republic, Institute of Entomology, České Budějovice

The work developed in the context of the thesis of Barbora Konopová tries to understand how insect metamorphosis is regulated by juvenile hormone at genetic and molecular scale. This is an old aim among insect endocrinologists, and although some advances have been reached in recent times, the problem is still difficult. It has been known from Wigglesworth's works in the decade of 1930 that JH exerts a sort of morphostatic action, by which it prevents that insect larvae metamorphose into adult. However, in spite of the long time elapsed, very few is known about the molecular mechanisms underlying such a morphostatic action. The challenge faced by Barbora Konopová is, thus, not a minor one.

One of the first difficulties arising when trying to unveil the molecular action of JH is that the receptor of this small lipophilic hormone is still unknown. The first step in the thesis was logically addressed in this line. The molecular and functional characterization of the gene *Met* in the flour beetle *Tribolium castaneum* made by B. Konopová has afforded much light in this sense. RNAi of *Met* in *T. castaneum* provoked a precocious metamorphosis into pupae and also conferred resistance against juvenilizing JH treatments. This suggests that Met is the JH receptor, or that it is a part of the JH receptor, or that, at least, it is involved in JH signalling. The results obtained are of paramount interest as proven by the fact that they were published in *PNAS*.

Another step accomplished by B. Konopová was the clarification of the epistatic relationships between JH, *Met* and *Broad-Complex* (*BR-C*). BR-C has been considered a factor that determines pupal development. The experiments carried out on *T. castaneum* and the lacewing *Chrysopa perla* have corroborated that (RNAi of *BR-C* inhibits pupal development). But, in addition, the mixture of larval, pupal and even adult features obtained in the RNAi phenotypes has suggested that BR-C has an additional role in temporal tissue coordination during metamorphosis. This is an additional important role of *BR-C* that had been unnoticed by previous authors. Moreover, expression studies

combined with RNAi experiments showed that JH regulation of *BR-C* is mediated by *Met*. These results obtained in *T. castaneum* and *C. perla* were published in the high visibility journal *Development*.

In my opinion, these results could on themselves grant a PhD degree. But Barbora Konopová and her supervisor Marek Jindra went beyond that. They investigated the role of *Met* in the hemimetabolous insect *Pyrrhocoris apterus*, using the same RNAi approach. Their preliminary results have shown that the interference of *Met* in young larvae induces a precocious metamorphosis, which indicates that *Met* is also involved in JH signalling in this more basal and less modified species.

Finally, and following the recent discovery that *Krüppel* gene is involved in the antimetamorphic action of JH, B. Konopová has studied this factor in the holometabolous *T. castaneum* and the hemimetabolous *Rhodnius prolixus*. In *T. castaneum*, preliminary results indicate that RNAi of *Krüppel* in young larvae induces precocious metamorphosis directly to the adult, and that *Krüppel* is a target of Met. In *R. prolixus*, RNAi of *Krüppel* provokes a subtle precocious wing metamorphosis. These results are still unpublished, but when completed and publishable, it is easily predictable that they will be readily accepted in high visibility journals.

In summary, it is clear for this external appraiser that the work carried out by Barbora Konopová is of fundamental importance to understand the genetic and molecular basis of juvenile hormone signalling in insect metamorphosis. I presume that the late Sir. Vincent Wigglesworth would smile with placid satisfaction if he could read this thesis today.

Barcelona, 10 November 2008

Prof. Dr. Xavier Bellés
Spanish Council of Scientific Research, Research Professor
Director of the Institute of Evolutionary Biology (CSIC-UPF)