



## Review of the master thesis

Title: **Reconstruction of the evolution of multiple sex chromosomes in *Leptidea* wood white butterflies**

Author: Kristýna Pospíšilová

The master thesis of Kristýna Pospíšilová is a very good piece of work about an interesting topic and it was a pleasure to read. The thesis focuses on the evolution of multiple sex chromosomes in three species of wood white butterflies. I found this model group fascinating, as in three closely related and even cryptic species the sex chromosomes originated in different ways. The main achievement of the thesis is that it uncovered and described these origins in great detail.

The topic of the thesis is well thought through and the plan is clearly explained. It is obvious that the topic is part of a larger project but the scope and outcome of the thesis is absolutely worthy of standing on its own and is enough to be a very strong diploma thesis. Kristýna Pospíšilová followed up the results of her colleagues and built on top of these data. With the knowledge of Z linked genes she performed many rounds of hybridizations to map the physical position of these genes. This synteny was then compared with the position of orthologs in *Bombyx mori* to uncover the evolutionary pathways leading to the fascinating system of multiple sex chromosomes in the studied group.

The Introduction provides a very good overview of the whole project, introduces the model organisms and describes why their sex chromosomes are so intriguing. It informs the reader about current knowledge in respect to the topic of the thesis. The Aims are well formulated and clear. Materials and Methods are well described and enable the reader to easily follow how the author achieved her results. I think the methodological design is very clever; combining modern methods of molecular cytogenetics, data from array-CGH and gene mapping. The Results are clearly written, and I especially appreciate the impressive high quality of the images. The Discussion puts the data into the context of the evolution of sex

chromosomes in other groups of Lepidoptera. It also brings a possible explanation about the origin and detailed evolution of sex chromosomes in the studied species. I agree that presented scenario seems to be the most plausible. The literature sources are well used, and I believe complete.

What I am missing however, in either the Introduction or the Discussion, is information about the phylogenetic position of the studied group within Lepidoptera. Especially as the author compared the positions of genes in wood white butterflies with the position of orthologs in *B. mori* and in the Discussion she made a wider comparison with her results, so I believe that the thesis would benefit from either an additional figure or at least a more detailed description of the phylogenetic relationships.

In conclusion, I found the thesis of Kristýna Pospíšilová very interesting. In particular I value her commendable efforts untangling such an intricate evolution of this fascinating system of sex chromosomes. I really appreciate that the thesis in entirety was coherent, all parts were well linked together, and the results presented in this thesis are novel. Also, I highly value the author's insight into the topic and her determination to solve the whole mystery and provide a complete scenario of the origin and evolution of this system of sex chromosomes supported by evidence. I would also like to highlight that Kristýna Pospíšilová has a real writing talent. Her text was stylistically clean, logical, and engagingly written. Even complicated parts were clearly explained, and her English is excellent with almost no typos or stylistic mistakes. I am convinced that the results of the thesis will make a broadly appreciated publication.

For all the reasons mentioned above I fully recommend this thesis and strongly suggest the highest mark.

Detailed comments and questions:

1. We can read in the introduction that Lepidoptera (in general) have a conserved number of chromosomes. At the same time the author is stating the range of chromosome number found in Lepidoptera is between  $n = 5$  and  $n = 226$ . That is an incredibly high range. Is variability in chromosome number in Lepidoptera limited to certain taxa or is it spread through the whole phylogeny? Is it known what is the evolutionary origin of these extreme cases?
2. Regarding the evolution of sex chromosomes. In the Introduction we can read that sex chromosomes may have evolved from the B chromosome which carry the female sex

determining gene. I understand how sex chromosomes may have originated in B chromosomes and there are known cases when this happened (including the proposed origin in the work of the author's colleagues). However, it is still hard for me to imagine that at the beginning of this evolution the B chromosome would carry the sex determining gene as this gene is crucial for the liveability of the individual and population and the presence of the B chromosome is not essential. Could the author please further explain this hypothesis?

3. Regarding the FISH with the female, how did you identify W1, W2, W3 and W4?
4. In the thesis all the positions of the genes are compared to positions of their orthologs in *B. mori* which has a well annotated genome. However, in several places the text implies that the karyotype of *B. mori* is ancestral and the changes happened in the lineage leading to *Leptidea* or inside *Leptidea*. Is the *B. mori* karyotype really ancestral within Lepidoptera? What if the rearrangement didn't happen in *Leptidea* but in the lineage leading to *B. mori*? I found that the Bombycoidea are a crown taxon in Lepidoptera, therefore I am not sure whether the proposed direction of the documented rearrangement is always reflecting the phylogenetic position of the group. What is the author's opinion of this?

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