



Prague, 17/2/2023

Review of the Ph.D thesis entitled “W chromosomes in Lepidoptera: evolution, diversity and molecular features” by Mgr. Martina Hejníčková

I read the Ph.D thesis of Martina Hejníčková with great interest. The thesis is focused on the cytogenetic analysis of lepidopteran species, with emphasis on the evolution of sex chromosomes. The candidate aspired to test alternative hypotheses on the evolution of W chromosomes in lepidopteran insects, by applying a combination of methodological approaches, which are among the “golden” standards in the field: conventional and molecular cytogenetics to identify the presence of W chromosomes, flow cytometry to estimate the lack of W chromosome from genome size differences between sexes and bioinformatic analysis of Illumina sequencing to identify the repeatome of W chromosomes. The research project resulted in three publications with the candidate as leading author, providing novel findings on unstudied species and allowing us to have a clearer view of the (probably) independent emergence of W chromosomes among lineages and the variability of the W chromosome genomic content.

Overall, I enjoyed reading the thesis and the three manuscripts. They are well-written, nicely structured, provide a wide comparative view, novel insights and follow the expected current (high) standards of research. In this context, I undoubtedly recommend to award the Ph.D title to Martina Hejníčková.

Minor comment:

Introduction, paragraph 1.6: “However, relatively high frequencies of sex chromatin deviations were recorded in this group: out of 23 examined species, 7 of them showed abnormalities despite being phylogenetically distant (Traut & Mosbacher 1968; Ennis 1976).”. I think this sentence is a little bit confusing as the meaning is not clear. I think the author would like to express that 7 out of the 23 species show sex chromatin deviations, and this is unexpected because all these 7 species are phylogenetically closely related?

Questions:

1. In many cases, it is mentioned that the sex chromatin body seems to vary between species. Did you detect variation between individuals of the same species or between different developmental stages?
2. In species with ZZ/ZW, is the W chromosome always heterochromatic in Lepidoptera? Is it possible that it might exist differences in either (1) heterochromatin constitution, or (2) genomic content in some lineages, and therefore, it does not form the sex chromatin body?
3. Since you have both metaphases (from many species) and the LINE probes, did you try to test the variability of repetitive elements on W chromosomes between the two major lineages with potential independent emergence of W? This could also allow you to check if W emerged more than once even inside Ditrysia.
4. Although several lepidopteran insects have been examined/sequenced, and Z chromosome gene content seems to be homologous, do you consider that part of the



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variability on Ws might be because of not identified sex chromosome turnovers (i.e. some species might not have homologous Z)?

5. If all possible know-how and sufficient budget will be provided, which should be your experimental design and future direction of research to resolve the evolution of W chromosome in Lepidoptera?

Sincerely yours

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