

Opponent's review of bachelor thesis of Magdaléna Vališová: Cytogenetic analysis of sex chromosomes in representatives of the tribe Heliconiini

Bachelor thesis of Magdaléna Vališová focuses on cytogenetic analysis of sex chromosomes in two species of Heliconiini: *Dryas iulia* and *Eueides isabella*. The main purpose of this work is to study the size and structure of their W chromosomes. Moreover, the results disprove conclusion about W chromosome in *D. iulia* made in controversial publication by Lewis et al. (2021), which describes it as noticeably smaller than the other chromosomes based on genomic data.

This thesis emphasizes the importance of cytogenetic verification of *in silico* generated data, and brings valuable contribution to the field. The thesis is well written, logically structured and contains minimum amount of typographical errors.

However, I noticed discrepancies in the use of some citations; I believe that e.g. Yoshido et al. 2005 or Dalíková et al. 2017 do not fit well to support the statements in the text and more suitable papers should be cited instead. Further, I have some comments listed below and nine questions to be answered during the defense:

1. Introduction:

Questions:

- 1) section 1.1: „For instance, only XYZ bivalents were identified in *Bombyx mori* by Traut (1976).“ – Typical sex chromosome constitution in *B. mori* is WZ/ZZ (female/male). Can you explain the XYZ bivalents?
- 2) section 1.2: „W chromosome has been found in 98 % of the species in the order Lepidoptera“ - Can you support this statement? Although 98% of Lepidoptera belong to Ditrysia, we do not know how many % of Ditrysia actually have the W chromosome.
- 3) section 1.3: „There are two hypotheses on the origin of the lepidopteran W chromosome (Lukhtanov, 2000). The first suggests that the W chromosome originated from fusion of the ancestral Z chromosome with an autosome (Figure 1). This would mean that the W chromosome is in fact a neo-W of autosomal origin (Traut et al., 2008). The second hypothesis says that W chromosome evolved from a supernumerary chromosome (Figure 2), the so-called B chromosome, in Tischeriidae and Ditrysia (Dalíková et al., 2017).“ – In Dalíková et al. 2017 we actually suggested that in Tischeriidae the W chromosome originated from Z-autosome fusion. Moreover, recent research actually favors the theory of non-canonical origins of the W chromosomes in Lepidoptera. Can you elaborate on that?
- 4) section 1.5: „It is because the Y and W chromosomes are partly or fully eliminated from the process of recombination during meiosis.“ – Why is the W chromosome in Lepidoptera eliminated from meiosis and to which extent?

2. Aims

- 5) Why and how did you select *E. isabella* for this purpose? It is actually never mentioned in the thesis.

3. Methods:

6) section 3.5: Did you verify nick translation results on a gel? If not, why?

7) section 3.7: „For reprobing, the nail polish and cover slip were removed from the slide with forceps, and the slides were washed in 2x SSC for 5 minutes at room temperature.“ – How many times were the slides washed with 2x SSC? Since you are using the same Cy3 fluorochrome for the reprobing experiment, how can you be sure that the hybridization results are not mixed with the previous genomic probe?

4. Results:

Comments:

- I believe the second paragraph would better fit into the Introduction or Methods, since it describes FISH and GISH principles.
- section 4.2: „The W chromosome in *E. isabella* displayed strange behavior concerning the morphology and chromosome pairing.“ – as mentioned later in the discussion, there are more species with such W chromosome. In my opinion it is actually quite common phenomenon.

Questions:

8) Do you have any data/information on males? It would be good to use them as a control group.

9) section 4.1: How would you explain the weaker signal on one of the ends of the W chromosome in *D. iulia*? Is it possible that this chromosomal end could somehow relate to the Lewis et al. 2021 conclusions?

5. Discussion:

Comments:

- section 5.2: This section contains a lot of redundant information which is not directly linked to your results. Moreover, the existence of achiasmatic meiosis in Lepidoptera is never mentioned.

To sum up, the bachelor thesis by Magdaléna Vališová is very well written, the obtained results are of publication quality and the final conclusions bring interesting and important insight into the related field. I appreciate the courage which inspired this project and the precision of presented work, and I believe it fully meets the expected criteria.

Overall, I enjoyed reading this thesis and it is my pleasure to recommend it for defense.

Sincerely

RNDr. Martina Hejníčková, Ph.D.

in České Budějovice, 11 May 2023