

Opponent's review on the Diploma thesis of Bc. et Bc. Veronika Běháľková

The aim of the present thesis was to examine the conserved synteny in two closely related butterfly species, *Plutella xylostella* and *Yponomeuta evonymella*, based on screening of 16 genes (selected from the *P. xylostella* genome) in the available BAC library from *Y. evonymella* and consequent BAC FISH of positive clones onto *Y. evonymella* chromosomes. In the broader perspective, the reason for this effort was to solve issues associated with the correct genome assemblies of *Yponomeuta* species and specifically *Y. cagnagella*. Therefore, the results from the current thesis represent an important part in a more complex workflow outlined by the student's laboratory.

The results of BAC FISH-based mapping (of 12 out of 16 initially selected genes) showed, as expected, different chromosome dynamics between neo-Z₁ chromosome and autosome 15. Therefore, the *P. xylostella* – assisted genome assembly in *Yponomeuta* species should be treated with caution and should rather include information from autosomes only. The study points on urgent need for the so-called "chromosomics" approach in an attempt to avoid major errors in reference-guided genome assemblies in closely related species.

The thesis has 34 pages of English-written text of high quality, with standard and logical structuring and only negligible amount of typos or inaccuracies (some of them are presented below, just for the author's information; but they can be discussed). Just one thing to be mentioned here: some reasons for the selected workflow are better explained in Discussion rather than in Introduction or other sections preceding Discussion (specifically, the selection of chromosomes 15 and Z₁, among other reasons, for their expected different rate of rearrangements).

49 references in the text mirror precisely the list of References and *vice versa*. All references are relevant and include primary sources including also up-to-date studies, supplemented with fitting review articles.

Material and Methods are precisely described and Figures and Tables are in publication quality. The amount of laboratory work was apparently huge and deserves appreciation. Discussion is very clear and concise and some information can be better extracted from here than from Introduction. Conclusion is straightforward and brings important outcomes for further studies. To my modest opinion, the aim of the study has been accomplished.

I suggest this Diploma thesis to be successfully defended and to be evaluated with mark "1", especially in case of successful presentation and satisfying response to my two questions below:

Question 1:

Page 16: According to the text, it seems that there was no optimization for the probe concentration or the amount of the blocking DNA; or for the conditions for slide/probe denaturation and stringent washing. Could the author clarify that, please? Also, why hapten-based FISH has not been tested? Finally: regarding the enigmatic issue of "unstoppability" of DNase I activity: why didn't you simply freeze the nick translation reaction during electrophoresis?

Question 2:

To my modest opinion (and it can be a matter for a short discussion), there is a little confusion in terminology throughout the text regarding terms: 1) synteny and 2) conserved synteny (along with not properly introduced third term "collinearity"). Could the author, please, distinguish between these three terms?

The list of minor errors/typos etc. (for author's purposes):

Page 1, paragraph 2: Absence of chronology in a bulk of cited studies starting with Brown et al. 2004

Page 5: The term "homeolog" is rather used for gene/chromosome copies generated by polyploidization.

Page 10: The meaning of *Henna* should be explained in the main text no matter its presence in the Table 1.

Pages 13 and 14: temperature profile: rather thermal profile

Page 15: 62.5 ug/ml of RNase A was added...but what was the final amount or the final concentration, is not mentioned.

page 25: legend to Table 7: two dots at the end

page 26: *Yponomeuta evonymella* once not formatted in italics

Page 27, first paragraph: Discontinuity between 2nd and 3rd sentence. Something is missing here.

Discussion: Faria and Navarro 2010 could be also cited for inversions (and their effects) in the Introduction part.

Reference list: formatting error between Refs Lukhtanov../Mank...and Yasukochi.../Zhang...

In Mělník, 18.1.2020

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