

Neuchâtel, the 14th of February 2023

PhD Thesis Evaluation Irena Provazníková

The PhD thesis "Drivers of karyotype evolution in Lepidoptera" presented Irena Provazníková contains four research chapters of which two are published in peer-reviewed journals at the time of writing this report. For two of these chapters, the candidate is listed as equal first author and based on the author contributions listed on page 4 of her thesis, she provided substantial inputs to these chapters. The latter include the design of the study, performing experiments, the data analysis and writing.

The thesis combines more classic cytogenetic approaches with current state-of-the-art genomic methods providing novel methodological frameworks to study karyotype evolution. Overall, the presented research provides exciting and original insights on the mechanisms that underlie karyotype evolution in Lepidoptera, one of the most diverse taxonomic groups across the tree of life. Importantly, the candidate worked on different species that are taxonomically far away from current lepidopteran model species. The findings of this thesis are thus of broader importance for the field of chromosomal evolution, especially in groups with holocentric chromosomes. The thesis chapters are overall nicely written and well-crafted and will surely all be published soon.

In her *General Introduction*, the candidate sets the stage for her dissertation by first nicely outlining the history of studying chromosomal rearrangements (CRs) in an evolutionary context, albeit starting a bit too broad. The introduction then continues with a discussion of the potential role of CRs during speciation, with a focus on CRs that cause reproductive incompatibilities when they evolve in allopatry. However, are there also scenarios possible where speciation is feasible under different geographic modes of speciation? This continues with a discussion on how CRs could promote local adaptation, focusing especially on inversions and chromosomal fusions, but how would speciation be possible under this scenario remains an open question. The candidate then introduces sex chromosomes in general followed by a nice overview of the situation in Lepidoptera. Here, I wonder if the candidate could elaborate why some groups are karyologically more diverse than others and if there might be lower and upper boundaries for the number of chromosomes within a given species, especially in the light of the results of this thesis? On another note – unlike suggested by the candidate, there are macroevolutionary inferences using phylogenetic approaches that suggest that taxonomic groups with a higher diversity in chromosome numbers also have higher rates of speciation. The *General Introduction* provides an original and broad synthesis of our current knowledge on CRs, their evolution and evolutionary implications, although the link between chromosomal speciation and sex chromosomes could have been stronger.

The *General Introduction* is followed by four research chapter where, in *Chapter 1*, the candidate provides an interesting comparative analysis that assessed the suitability of various, putative universal, cytogenetic markers across Lepidoptera. A key finding of this study is that histone related markers can be broadly used for cytogenetic studies, while some of the other markers seem to only work in deeply divergent ancestral lineages. I found the latter to be an exciting and novel result. Overall, the chapter is original and provides unparalleled insights on part of the genomic architecture across Lepidoptera, which is then also neatly discussed.

Specific comments on this chapter are: First, given that species specific probes were generated for most of the species that were investigated, is there any sequence data available that would allow



to predict if a cytogenetic marker could work in a specific taxonomic group? Such *in silico* approaches could be especially interesting given the increasing availability of chromosome-resolved reference genomes. For some of the studied species, such genomes are available and I wonder if the candidate has mapped the primer sites to the respective genomes.

Secondly, in the introduction, it is suggested that CRs such as inversions, fusions or fissions play an important role during speciation in Lepidoptera. Cited is a though a paper that deals with sex-chromosome evolution in *Leptidea*. To promote speciation, the evolution of reproductive isolation is required. So, the question at hand is, how would CRs promote speciation in the first place and under which conditions might they result in complete isolation?

Combining short and long fragment genomic data with cytogenetic approaches, the candidate investigated the role of repetitive DNA for genome architecture in Chapter 2, focusing especially on two species with extensive ribosomal DNAs (rDNA) clusters. A key finding of this chapter is that the spread of rDNA clusters could be mediated by satellite-like sequences in the genome. The results of this chapter are especially interesting as they shed light on the genomic architecture of telomeric regions in Lepidoptera and may hold the key for the factors that mediate the (in-)stability of chromosomes in some groups of Lepidoptera but not others. Overall, the chapter sets the stage for future explorations of the repeat landscape that are now becoming possible through long read sequencing approaches. The chapter is well written albeit the discussion could emphasize more on the macroevolutionary implications of the results.

Further specific comments are: As outlined in the discussion, the study does not allow to distinguish whether rDNA spreads emerged via ectopic recombination or the integration of extrachromosomal circular rDNA. What further experiments or data would be needed to disentangle this?

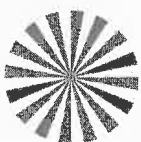
As a general question, why were explicitly *A. urticae* and *I. io* from the Darwin Tree of Life Project used? Were these the only ones available at the time of analysis and even if so, what species or taxonomic groups would have ideally been added to the study? Also, different sequencing platforms were used for the long fragment data (Oxford Nanopore and PacBio). Given the higher error rate associated with Oxford Nanopore but the shorter fragments that can be sequenced by PacBio, could these technical differences have affected part of the interpretations and/or the detectability of repetitive regions?

In Chapters 3 & 4 the candidate studied sex chromosome evolution in two different groups of moths. Both chapters highlight the astonishing diversity of sex chromosomes in Lepidoptera and provide new insights on how they could evolve. This is especially important as many phylogenetic based macroevolutionary inferences do not differentiate between autosomes and sex chromosomes. Neo-sex chromosomes could thus provide yet another, however, often overlooked twist to chromosomal speciation. In her third chapter, the candidate used genomic in situ hybridization to identify sex chromosomes and qPCR to identify sex linked genes. Doing so, she found a likely ancestral fusion of sex chromosomes and autosomes in the superfamily Gelechioidea as well as further rearrangements.

A first specific comment is that the introduction highlights that sex chromosomes allow for the evolution of postzygotic isolation and therefore promote speciation. However, if neo-sex chromosomes constitute such strong barriers, how would they emerge in the first place? Secondly, the abstract suggests that the autosome-sex chromosome fusion observed in this study could be selected for, but in this case why would it not fuse in other species with similar ecology where it could also be adaptive? The potential for selection is moreover based on the assumption that the chromosome-scale synteny is conserved between the studied species and *Biston betularia* to which they were compared to, but is such an assumption warranted?

Lastly, a key assumption of chapter 3 is that the autosome-sex chromosome fusion is ancestral to Gelechioidea, but if fusions are adaptive could the observed pattern also have occurred through recurrent parallel evolution?

In her last chapter, the candidate then investigated sex chromosome evolution in *Yponomeuta* moths using several lines of evidence ranging from transcriptomic data over BAC-FISH to qPCR. The results suggest the presence of multiple sex chromosomes including a neo-W chromosome within all but one of the studied species. The candidate suggests in her chapter that sexual antagonism could be a driver for sex chromosome turnover, given that the W chromosome got fused with an autosome



containing ovary specific gene clusters, providing a novel evolutionary mechanistic framework. While this framework might explain the repeated fusion and the emergence of neo-sex chromosomes the questions remains though if it would also apply to groups like *Polyommatus*, where species can also undergo massive chromosomal fission and syntenic blocks are likely to be separated.

In her *Synthesis*, the candidate then discusses the broader implications of her four research chapters in two subsections on repetitive sequences and sex chromosomes, respectively. The discussion is overall very convincing, although the link between the different research chapters could have been stronger. Especially bridging between the potential role of repetitive elements and the propensity for the evolution of novel (sex) chromosomes could have been further discussed. Given that the thesis uses various systems from across Lepidoptera, including some of the very basal clades, the macroevolutionary implications of the findings could similarly have been further examined. For instance, how similar are sex chromosome turnovers in more recently evolved clades and what is the role of syntenic blocks?

Overall, Irena Provazníková provided a convincing dissertation with many novel ideas and approaches. I therefore recommend this thesis for defence.

With kind regards



Prof. Dr. Kay Lucek

