



In Prague, October 14th 2013

Peer review of the PhD thesis of RNDr. Petr Nguyen: Comparative mapping of sex-linked genes in Lepidoptera

Submitted PhD thesis represents a collection of two impacted publications accompanied with Introduction and Discussion parts. Introduction is focused on general mechanisms of evolution of sex chromosomes including plants. Special attention is paid to lepidopteran cytogenetics where supervisor's laboratory definitively has played and is still playing a leading role at the international level and achieved priority results in this field.

Core of the PhD thesis are two impacted articles. The first, "Linkage map of the peppered moth, *Biston betularia* (Lepidoptera, Geometridae): a model of industrial melanism" was published in the journal *Heredity* (IF = 4,110) and describes a construction of linkage and physical map of above mentioned species. Experiments were performed in the cooperation with University of Liverpool, Institute of Integrative Biology and Petr Nguyen strongly contributed to the physical part, especially BAC-FISH mapping of genes localized on Z chromosome. The genetic map primarily based on AFLP markers and physically localized genes revealed a relatively large synteny between orthologous autosomes of *B. betularia* and *B. mori*. On the other hand, Z chromosome probably due to the loss of recombinational partner in females showed lower conservation of gene order. Importantly, this study gave a direct explanation of lower chromosomal count of *B. mori* ($n=28$) in contrast to *B. betularia* ($n=31$) via three independent chromosomal fusions. In addition, it further supports $n=31$ as an ancestral state.

The second first-author paper "Neo-sex chromosomes and adaptive potential in tortricid pests" was published in a highly competitive journal PNAS (IF=9,737). Here, Petr Nguyen tried to confirm or dispute a predicted translocation of an autosome onto Z chromosome in the family Tortricidae as described earlier (unfortunately I couldn't find a key article: Heckel et al., 1998 in the databases). Physical mapping of genes present in *Bombyx mori* chromosomes Z and 15 on *Cydia pomonella* karyotype revealed their position

only on the one the largest chromosome Z. Autosome-Z chromosome fusion is thought to be at the start of tortricid moths radiation via translocation of battery of so called detoxification genes on Z chromosome. These genes important as a weapon against plant secondary metabolites and insecticides gained advantage from a new position through a phenomenon called postzygotic reproductive isolation.

Discussion i.e. chapter 4 "Synthesis and perspectives" is in my opinion, the most brilliant part of PhD thesis. Author clearly demonstrate that has a not only patience to "sit and observe metaphase spreads" but has also deep insight into the scenarios of sex chromosome evolution and undoubtedly is able to confront his findings with a literature. I am glad to say that submitted PhD thesis is a beautiful example of how excellent PhD thesis should be grasped, worked-out and presented. Since I had an opportunity to write a peer reviews on Petr Nguyen's bachelor and diploma thesis I also had an opportunity to see the evolutionary process of young scientist development and I am really happy with it! I would like to conclude that I fully recommend this doctoral thesis as a competent for the getting a PhD degree.

1st opponent's question: What is the supposed evolution of W chromosomes in *Cydia pomonella* and *Bombyx mori* regarding the fusion event of ancestral Z and *B. mori* chromosome 15, i.e did also the W chromosome fuse with another autosomal fragment in *C. pomonella* to get the same size as the novel Z chromosome or the W chromosome in *C. pomonella* is shorter corresponding the length of *B. mori* counterpart?

2nd opponent's question: Can you speculate, please, if mammalian (eutherian) radiation could also be similarly described by chromosomal fusion of XAR with XCR (X added and X conserved region)?

3rd opponent's question: Are there any hypotheses explaining the absence of W chromosome in the basal lepidopteran and its presence in the Ditrysian lineage? Could be this difference for example explained by extensive rearrangements of Z chromosome in the first group (leading to rapid degeneration of W) and their lower extent in the second group?

Ing. RNDr. Vladimír Krylov, PhD.



Petr Nguyen – Ph. D. thesis „Comparative mapping of sex-linked genes in Lepidoptera“

Review of External examiner

The thesis by Petr Nguyen consists of two papers in well-reputable journals (*Heredity*, *PNAS*), both publications are based on extensive experimental material and at the same time bring fresh and stimulating ideas into the field of sex chromosome evolution. The introduction and the concluding parts are very nicely written as well, and they document deep knowledge of the author in his major field. Petr is a co-author of several other important publications not included into the thesis and I have no doubts that he is an excellent candidate for the Ph.D. degree. Both included papers will definitely stimulate further research and the new data are very important and I believe that they will be used by researchers for decades. As a reviewer, I really enjoyed reading the whole thesis and it was a difficult task to find any weak spots. Particularly, I love the clever test of linkage of genes to the Z chromosome performed in the *PNAS* paper, and I have to say that inspired by this work, we successfully adopted this reliable and efficient strategy for testing homology of sex chromosomes across iguanas and we demonstrated huge conservation of their sex chromosomes.

To stimulate discussion during the defence, I raised several points:

- 1) The author uses the term “proto-sex chromosomes” (page 2). What is his definition of sex chromosomes, proto-sex chromosomes and is the latter term informative and needed?
- 2) I share with Petr admiration of papers from the lab by Doris Bachtrog about differentiation of neo-sex chromosomes in fruit flies (discussed here on pages 2-3). However, I am not sure whether they represent indeed a good general model for the evolution of sex chromosomes. The neo-sex chromosomes in drosophilas evolved several times via independent fusions of an autosome with already well-differentiated sex chromosomes and the papers nicely describe progressive adaptation of the previously autosomal part to the function of sex chromosome. My concern is that many of these adaptations (most importantly dosage compensation, degeneration of unpaired sex chromosome) were already present in the system of neo-sex chromosomes and their expansion to the newly added segments might be a very different process from the evolutionary sequence during evolution of *de novo* evolved sex chromosomes.
- 3) Pages 2, 4... I am aware that the whole field suffers from this inconsistency, but still, the author uses the term “homomorphic sex chromosomes” as a synonymy to “poorly differentiated sex chromosomes”, which might be misleading. Homomorphy concerns only morphology of the chromosomes, sex chromosomes of the same size and shape can be highly differentiated in heterochromatinization and at the sequence level, on the other hand,

heteromorphic (neo-)sex chromosomes might still highly recombine and be at these levels comparable (see please e.g. our paper Pokorná et al., *BMC Genetics* 2011 for examples).

- 4) I am not a fan of the hypothesis about ultimate loss of sex-limited sex chromosomes. This hypothesis has little support even in mammals, although it is most popular in this group (and J. Graves demonstrates it mainly in this clade and even makes catastrophic predictions about fall of masculinity in human beings). Several processes, mainly translocations of genes to Y chromosome and gene conversion in palindromes, prevent extinction of our Y chromosome. And even in mammals (*Tokudai*) without *Sry* gene and Y chromosome, the functionally important previously Y-linked genes saved themselves by translocation to autosomes.
- 5) Page 5: I doubt that “homomorphic sex chromosomes seem to be a rule rather than exception in cold-blooded vertebrates”. This belief is widespread, but among poikilothermic vertebrates, poorly differentiated sex chromosomes are more common in some lineages of fish and amphibians, not in many reptile lineages.
- 6) Considering the poor coverage of lepidopteran lineages with the putative ancestral number of chromosomes ($n = 31$) in detailed studies (genetic maps are available only for handful of species), I would be more sceptical that the studied species really represent an ancestral karyotype of Lepidoptera. All the conclusions in the *Heredity* paper are sound and recent available evidence is in accord with them, but given the old age and large diversity of the group, I would expect that the same number of chromosomes in karyotype could be homoplastic in some groups (I know two cases in reptiles where the species were incorrectly assigned as possessing ancestral karyotypes based on chromosomal numbers).
- 7) I doubt that the stability of syntenic blocks in Lepidoptera versus their instability in mammals can be attributed to differences in the female meiotic drive as stated in the Discussion of the same paper. I agree that holokinetic chromosomes could protect against female meiotic drive, nevertheless, sauropsids (including birds) have primary constrictions (centromeres) comparable to mammals and yet have very conserved chromosomes, although female meiotic drive was demonstrated in this lineage (at least in birds). Moreover, the cited paper (Pardo-Manuel de Villano and Sapienza 2001) concerns the role of female meiotic drive in the evolution of karyotypes in mammals, but it deals only with Robertsonian rearrangements, which has little effect on the degree of synteny conservation.
- 8) Although the hypothesis that the autosome-Z fusion was the key evolutionary novelty enabling evolutionary success of tortricid pests is suggestive and reasonable and I like it, it is notoriously difficult to identify the key novelties. Moreover, the statistical support for this hypothesis is weak ($n = 1$) – sorry, I have read in the conclusion that n might be 2, the same probably happened also in other lineage of Lepidoptera. We have currently reconstructed 56

putative independent origins of multiple neo-sex chromosomes (which could be different, I know) among amniotes and I have not noticed any conspicuous correlated expansions in diversification rates. Also, given that the mammalian X chromosome was involved in many of our reconstructed origins, I would hesitate to agree that mammalian X is prone to fusion with autosomes as could be inferred from the statement on page 44.

My notes are more suggestions or comments than criticism. Again, I stress that the Ph.D. thesis is excellent and I have no doubts about its success and I congratulate the author and the whole lab to this nice work.

In Prague, October 23, 2013



doc. Mgr. Lukáš Kratochvíl, Ph.D.
Dept. Ecology, Faculty of Science
Charles University in Prague



Institute of Biophysics, Czech Academy of Sciences
RNDr. Jiří Široký, CSc.
Laboratory of Plant Developmental Genetics
Královopolská 135, 612 65 Brno
tel. 541517204, fax: 541240500, e-mail: siroky@ibp.cz

Review: Comparative mapping of sex-linked genes in Lepidoptera

by RNDr. Petr Nguyen

Dr. Nguyen presents in his Thesis a topical theme of sex chromosome organization and synteny in peppered (*Biston betularia*) and codling (*Cydia pomonella*) moths with important extensions to the evolution and speciation. The Thesis is based on two articles published in *Heredity* and *PNAS* this year, although Dr. Nguyen is author or co-author of altogether eight articles in the field of insect chromosomes, dating from his Bachelor's studies (2005) until recent.

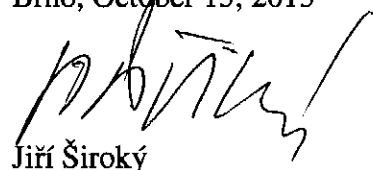
Introduction summarizes recent literature in the field and is committed strictly to the phenomena studied and not to other, non-relevant description of insect chromosomes, like that they are small and holocentric, etc.; things that readers of this particular Thesis hardly need to explain. Compendiousness of the text is, in my opinion, big favour - and not the last one - of the presented work. *Videlicet*, the author included at the end of the Thesis something unseen in similar PhD works: a chapter *Synthesis and Perspectives*, where resumes all the acquired experimental findings in the light of current scientific knowledge.

In the first of the debated articles, *Linkage map of the peppered moth, Biston betularia (Lepidoptera, Geometridae): a model of industrial melanism*, authors constructed a linkage map of the chromosomes of peppered moth using AFLPs and some genes. They have found 31 linkage groups consistent with observed 31 chromosomes. By comparing the results with chromosomes of sequenced *Bombyx mori*, they attained high degree of synteny between these fairly unrelated species with the exception of *B. betularia* Z chromosome. In the preparations of pachytene oocytes the authors observed conspicuous pairing of the W and Z chromosomes. Here, I have a question concerning the formation of the bivalent of a different W and Z chromosomes. From my knowledge of plant sex chromosomes (e.g., in *Silene latifolia*, possessing X and Y heteromorphic sex chromosomes in males) both X and Y differ to that degree, that at pachytene the chromosomes do not pair - with the only exception of a small pseudoautosomal region. Are there known molecular mechanisms taking part in the assembly of W and Z sex bivalents in insects?

The second article, *Neo-sex chromosomes and adaptive potential in tortricid pests*, traces the origin of the sex Z chromosome which had arisen by the fusion of an ancestral Z chromosome and autosome corresponding to #15 of the *Bombyx mori* reference genome. This ancestral fusion event brought together important genes responsible for deactivation of plant defence metabolites in tight linkage on the Z. This event had profound impact to adaptive radiation and speciation of phytophagous moths. I am wondering if there are other described examples of similar events in insects?

Presented work ultimately shows how careful chromosomal mapping approaches would expand our knowledge about genome dynamism and can finely trace the evolution of the species. The spectrum of methods used during this work is indeed colourful and the outcomes to the answering basic biological questions are plain enough. Taken together, in the Thesis the work is well presented and is of superior quality. I recommend Thesis for the defence.

Brno, October 15, 2013

A handwritten signature in black ink, appearing to read 'Jiří Široký', written in a cursive style.

Jiří Široký