



Universidad de Granada
Facultad de Ciencias
Departamento de Genética
E-18071 Granada, Spain
Juan Pedro M. Camacho

Fax 34 958 244073
E-Mail <jpmcamac@ugr.es>
Telephone 34 958 248925

PhD thesis review

Title: Mechanisms of molecular differentiation of sex chromosomes in Lepidoptera and their evolution

Author: Mgr. Martina Dalíková

Reviewer: Juan Pedro M. Camacho, Granada (Spain) 19 June 2017

The PhD thesis by Martina Dalíková deals with the study of several aspects of chromosome evolution in Lepidoptera, and is structured into three papers already published in reputed journals (Science, Heredity and Chromosome Research), a manuscript sent to Journal of Heredity, and an additional chapter including unpublished results. Martina Dalíková is first author in two papers, and her contribution is clearly specified in all of them, and it is substantial. The thesis is well written and easy to follow. This nice collection of papers demonstrates that Martina Dalíková has managed a lot of molecular-cytogenetic techniques and that she is able to put scientific results together with their interpretation into very well-written papers.

The main goal of this thesis is clearly stated in it: "The main aim of my thesis was to bring new information about mechanisms of molecular differentiation of sex chromosomes in Lepidoptera and to contribute to the understanding of their evolution." Even though the immense majority of topics are properly discussed, I would like to raise some questions most likely evidencing my expertise bias.

1) page 13: Does the "carbonaria chromosome" contain an identified DNA sequence allowing its differentiation from the "typical chromosome" by BAC-FISH analysis?

2) In chapter 3.3, entitled "New insight into evolution of W chromosome in Lepidoptera", it would have been advisable to place Figures and Tables intermingled with text (as in the section on unpublished data), as this would have made this chapter more easy to read. Anyway, I found it highly appropriate the use of molecular phylogenetic information to infer the direction of chromosome changes. Anyway, I would like to comment that Camacho et al. 2000 (DOI: 10.1098/rstb.2000.0556) remarked the high similarity between B chromosomes and sex chromosomes, in many molecular aspects. Perhaps this paper would have contributed to enrich the discussion on B chromosome adoption for sex chromosome role.

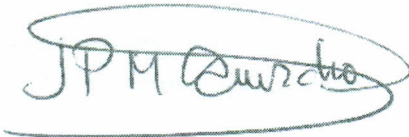
3) Regarding chapter 3.4 entitled "W-enriched satellite sequence in the Indian meal moth, *Plodia interpunctella* (Lepidoptera, Pyralidae)", I would like to ask about the presence of

PiSAT1 in male genomes (page 86), as this suggests its presence on chromosomes other than W. Do you have evidence for its presence on any other chromosome? I would like to comment here that satDNA can be present in many genomic locations but at arrays shorter than the minimum detected by FISH. This is the case of the non clustered satDNA families recently reported in the migratory locust (Ruiz-Ruano et al. 2016: doi:10.1038/srep28333).

4) Adoption of B chromosomes: a) Would it be compatible the origin of W chromosome through B chromosome adoption and the full pairing shown by Z and W during meiotic prophase? b) Are there cases of B chromosomes in Z0/ZZ lepidopteran species? c) page 11: "the W chromosome exhibits a rapid and random evolution, while the Z chromosome exhibits conserved synteny of Z-linked genes".... in case of B chromosome adoption, the observed rapid evolution could be mistaken for long period of independent evolution prior to adoption. d) The presence of palindromic structures containing multiple copies of a few genes in the human Y chromosome (first line in page 3) is reminiscent of recent finding of protein-coding genes on B chromosomes, where they are also found in multiple copies. These palindromic structures are also present in Y chromosomes of Pan and Gorilla. Could this be suggestive of Y chromosome origin through B chromosome adoption in an ancestor of these species?

5) page 105: "Thus, it seems that MSCI is mainly a specialized version of meiotic silencing of unsynapsed chromosomes (Turner et al. 2004)"....The hypoacetylation of X and B univalents in the grasshopper *Eyprepocnemis plorans* adds good evidence to this claim (see Cabrero et al. 2007).

As a whole, I consider that this thesis represents a highly valuable contribution to scientific knowledge and meets the highest standards, for which reason I highly recommend it to be defended.



Signed: Juan Pedro M. Camacho
Professor of Genetics
Department of Genetics
University of Granada
Granada (Spain)

19 June 2017

Oponentský posudek na doktorskou práci



Biofizikální ústav Akademie věd České republiky
RNDr. Roman Hobza, Ph.D.
Oddělení vývojové genetiky rostlin
Královopolská 135, 61200, Brno
e-mail: hobza@ibp.cz

Název práce: Mechanisms of molecular differentiation of sex chromosomes in Lepidoptera and their evolution

Autor: Mgr. Martina Dalíková

Předložená doktorská práce Martiny Dalíkové je zaměřena na studium struktury a evoluce pohlavních chromozomů a to zejména u motýlů. Práci vypracovala v etablované a mezinárodně uznávané laboratoři pod vedením prof. Marce. Jádro práce je tvořeno čtyřmi publikacemi, kdy u dvou je doktorandka první autorkou. Dle vyjádření ostatních autorů v úvodu práce je doktorandka specialistkou na cytogenetické experimenty (hlavně u prací, kde je spoluautorkou) a do značné míry komplexní badatelkou, která své experimenty navrhuje, realizuje a zveřejňuje v podstatě sama, což se týká dvou prvoautorských prací.

Úvod k disertační práci je čtivě psaný a i svým relativně úsporným rozsahem (bráno pozitivně) dostačující. Následující výsledky shrnuté ve zmíněných publikacích je obtížné nějak zásadně hodnotit, protože už prošly komplexním recenzním řízením, a jejich kvalita je do určité míry podložena kvalitou časopisů, ve kterých byly zveřejněny. Ve svých komentářích se zaměřím spíše na dotazy směřující k ujasnění některých pasáží.

U práce *New insight into the evolution...* autorka používá k určení syntenie (počet kopií genů) mezi pohlavními chromozomy u vybraných druhů motýlů qPCR. Jak obtížné by bylo provést křížení a mapování jednotlivých genů u zmíněných druhů? Jak obecně je uplatnitelná klasická genetika u motýlů (generační doba, množství potomků, možnost trvale udržitelných laboratorních chovů...)? Dále se autorka zmiňuje (v diskuzi), že pozoruje zcela degenerované W chromozomy (zcela heterochromatické) u studovaných druhů. Je to určitě tak, že míra degenerace odpovídá míře heterochromatizace? Jsou pro to nějaké důkazy, kdy by nějaká studie ukázala míru exprese genů v různých chromatinových (globálních) stavech? Autorka též diskutuje míru akumulace repetitivních sekvencí a označuje ji za rychlý a

náhodný proces během evoluce pohlavních chromozomů. Je to důsledek zmíněné heterochromatizace nebo jeho příčina?

Autorka se částečně zabývá i imunolokalizací modifikovaných histonů u meiotické inaktivace pohlavních chromozomů. V práci jsem nenašel (možná jsem přehlédl) informace o úloze metylace DNA v tomto procesu. Jak to tedy je? Jaký je vztah metylace DNA a modifikace histonů? Kdy se během evoluce objevují na scéně epigenetické procesy? Jak spolu tyto procesy komunikují, a který z nich má podle vás zásadnější roli v regulaci exprese?

V práci W-enriched satellite sequence... autorka klonuje fragmenty W chromozomu a získává relativně omezený počet klonů. Přestože je nakonec úspěšná a identifikuje zajímavý satelit, není v dnešní době jednodušší postupovat pomocí NGS metod? Sekvenování i s malým pokrytím by přineslo obrovské množství dat a i hybridizace s genomickou sondou by v podstatě šla provést in silico pomocí mapování sekvencí genomové DNA na sekvence odvozené z W chromozomu. Tím by autorka získala rychlý a komplexní přehled o struktuře celého W chromozomu.

Jaké jsou v současné době zásadní otázky a horká témata v oboru evoluce pohlavních chromozomů? V čem konkrétně mohou motýli být nápomocní (co v jiných živočišných nebo rostlinných systémech nenajdeme) při objasňování procesu degenerace pohlavních chromozomů?

Závěrem bych chtěl konstatovat, že cíle vědecké přípravy byly v plném rozsahu splněny. Případné kritické poznámky a otázky uvedené v mém posudku nemají snižovat úroveň předkládané práce. Protože se jedná o oponentský posudek, je mou snahou spíše upozornit na případné nejasnosti než na klady této práce. V odpovědích na otázky by pak měl doktorand ukázat způsobilost vést vědeckou diskuzi o řešené problematice. Závěrem konstatuji, že Martina Dalíková prokázala schopnost samostatně a tvořivě řešit aktuální problémy současné biologie a doporučuji přijetí její disertační práce k obhajobě.

V Brně dne 29. června 2017

RNDr. Roman Hobza, Ph.D.





FACULTY OF SCIENCE Charles University

In Prague, July 13th 2017

Appraisal of Martina Dalíková's PhD thesis „Mechanisms of molecular differentiation of sex chromosomes in Lepidoptera and their evolution

The PhD thesis represents a collection of three impacted publications and one manuscript submitted to the Journal of Heredity. In general, it could be divided into two major parts, the first one concerning the genetic and evolutionary studies of industrial melanism on the textbook model British peppered moths (*Biston betularia*) and the second one focused on the sequence composition and evolution of Lepidopteran W chromosome. Manuscript and one paper in Chromosome Research are the first-author publications.

The PhD thesis is accompanied with a well written Introduction describing the general aspects of the sex chromosome evolution in both, XY and WZ systems, meiotic sex chromosome inactivation and one chapter aimed on Lepidopteran sex chromosomes. Technical and methodical aspects of cytogenetic studies, necessary for the elucidation of the sex chromosome evolution are subjects of the last introduction section.

Interestingly, Martina Dalíková included also unpublished results about the analysis of histone modifications such as methylation and acetylation in Mediterranean flour moth (*Ephestia kuehniella*) and the silkworm (*Bombyx mori*) and compared their level among W and Z chromosomes and autosomes. Unsurprisingly, strong accumulation of dimethylated form of H3K9 in W chromosome but not in Z and autosomes confirmed its heterochromatization. On the other hand, positive gene activity marker, H4 hyperacetylation observed also in W chromosome, indicates the existence of bivalent histone modifications, well described in mammalian embryonic stem cells.

Regarding publications included in the PhD thesis, the first paper "Industrial melanism in British peppered moths has a singular and recent mutational origin" published in a highly prestigious journal, Science describes a genetic mapping of carbonaria morph to a 200-kb region orthologous with a silkworm region in the chromosome 17. Martina Dalíková and collaborators constructed AFLP and mRNA based (via BAC clones) linkage and physical map of *Biston betularia* chromosome 17 and employed this genetic map for the identification of an



FACULTY OF SCIENCE Charles University

above mentioned 200-kb region. Interestingly, SNP analysis of six marker loci within the carbonaria region revealed its singular and recent origin.

Second publication "Linkage map of the peppered moth, *Biston betularia* (Lepidoptera, Geometridae): a model of industrial melanism", published in the journal *Heredity* describes the construction of *Biston betularia* genetic map based mainly on AFLP markers and physically mapped BAC clones. Importantly, this study provided an explanation of lower chromosome count in *B. mori* (n=28) if compared with *B. betularia* (n=31) through three independent chromosomal fusions.

Next publication "New insight into evolution of W chromosome in Lepidoptera" is in the manuscript form and was sent to the *Journal of Heredity*. Paint-FISH technique altogether with CGH approach revealed strong sequence conservation of Z sex chromosome among all tested Lepidopteran species. On the other hand, results support multiple origins of heterochromatinized W chromosome and offer their various scenarios encompassing the B chromosome or fusion of Z chromosome with autosome (neo-Z). In my opinion this manuscript shows the best of author's experimental and mental work. Moreover, it undoubtedly brought a new insight into the W chromosome evolution, since previous studies focused on this topic were limited by the lack of genetic and cytogenetic data from basal Lepidopteran taxa.

Last but not least first-author paper "W-enriched satellite sequence in the Indian meal moth, *Plodia interpunctella* (Lepidoptera, Pyralidae) published in the journal *Chromosome Research* describes the isolation and sequence characterization of new satellite DNA (PiSAT1) derived from the W chromosome. The identification of its polyadenylated transcripts opens a relatively new field concerning the function of regulatory RNAs in Lepidopteran species.

In general, the PhD thesis is well written in English language with a minimum of typing errors. All papers are published in a high quality impacted journals and the first one even in the journal *Science*. Taking into account that the median of impact factor for the cytogenetic research is relatively low, the publication of such results in a so prestigious journal is a great success for all co-authors. In conclusion, it is my pleasure to recommend the submitted PhD thesis for the defence and as a relevant for the getting a PhD degree.



FACULTY OF SCIENCE Charles University

Q1: What is your opinion on the evolutionary convergence regarding the definition of homologous structure (eye development for example). What exactly the homology is, how is it defined?

Q2: Could you, please, compare the melanization process between insects and vertebrates including the differentiation and migration of pigment bearing cells?

Q3: Do you have some hypothesis or personal opinion on the inconsistency of the dosage compensation among various Lepidopteran species?

doc. RNDr. Ing. Vladimír Krylov, PhD.

A handwritten signature in blue ink, appearing to be "Krylov", written over a faint blue horizontal line.