



Přírodovědecká  
fakulta  
Faculty  
of Science

Jihočeská univerzita  
v Českých Budějovicích  
University of South Bohemia  
in České Budějovice



BIOLOGICKÉ  
CENTRUM  
AV ČR, v. v. i.

## **Review of the Ph.D. thesis “Evolution of sex determination systems in toxicoferan reptiles” by Mgr. Barbora Augstenová**

The Ph.D. thesis of Barbora Augstenová deals with sex chromosomes and sex determination in toxicoferous reptiles. It focuses mainly on snakes, but other groups such as chameleons and anguimorphan lizards are also involved. The thesis is part of a comprehensive study by the research group of Lukáš Kratochvíl and Michail Rovatsos, which aims to reveal the evolution of sex determination in reptiles. This involves the use of both classical and molecular cytogenetics methods, such as comparative genomic hybridization and mapping of rDNA and microsatellites, including the telomere, which are known to frequently accumulate on sex chromosomes in some vertebrate taxa. This work will not only lead to a reconstruction of the evolution of sex chromosomes in reptiles, but will also help us understand the general mechanisms responsible for sex chromosome evolution and turnover. The thesis consists of a well-written introduction and six articles published in good journals, in four of which the applicant is the first author.

I have the following comments and questions:

Comments:

1) On page 24, the author says: "The presence of heterochromatic W in *Acrochordus javanicus* along with the lack of accumulation of the tested repetitive sequences questions the role of microsatellite accumulation in the heterochromatinization of the W chromosomes of caenophidian snakes as was previously suggested." I do not think this is necessarily true, because it just means that the microsatellites tested in the study are not accumulated on the sex chromosomes, but there could be other STRs that are accumulated in this respective genome.

2) In Rovatsos et al. 2018 (Sex. dev.), you presented data on a triploid male columbrid snake with sex chromosomes ZZW. The animal had multiple issues and was sterile with one testis-like gonad. Your interpretation, if I understand it correctly, was that sex determination in this species is based on the ratio of Z chromosomes and autosomes, but with a contribution from the dominant W. I do not think the dominant W is necessarily involved here, as this situation largely resembles the sex determination in *Drosophila melanogaster*. In *Drosophila*, sex is determined by the X:A ratio, while the Y chromosome is only necessary for male fertility, and the triploid XXY individual (with an X:A ratio of 0.67) becomes intersex.

3) I appreciate the knowledge game implemented in papers with CGH data which are part of the thesis. To understand the interpretation of CGH pictures, one needs to know what color a male or female probe represents. Usually such information is given somewhere in the text, which is dull. Here, however, one must deserve the information by knowing that avidin binds to biotin and that the fluochrome FITC is green.

Questions:

1) You write in your text that the animals studied were from pet trade (Augstenová et al. 2018, Genes). I wonder what happened to these animals when you no longer needed them?

2) In Augstenová et al. 2018, you compared the number of telomeric repeats between males and females using qPCR. How do you interpret the fact that in *Natrix natrix* the telomeric repeats are more abundant in males?

3) In many species, the telomeric repeats are accumulated in various chromosomes. Do you plan to investigate the genomic context of these repeats to find out the possible mechanism of this spread, e.g. the presence of an adjacent mobile element?

4) In the last paper on anguimorphans lizards (Augstenová et al. 2021), you write that in *Abronia lythrochila*, C-banding revealed the putative W chromosome, which, however, did not show the female-enriched signal in CGH. Why the GATA and other microsatellites used in previous studies were not used here? They could potentially reveal the sex chromosome not

only in this species but also in related species. Also, it would be nice if you used the same set of markers in all your studies to make the data comparable.

In conclusion, Ph.D. thesis of Barbora Augstenová provides a lot of data that will contribute significantly to the understanding of the evolution of the sex chromosomes not only in reptiles, but in general. It is noteworthy that the papers in the thesis are only a selection from nine papers to which she contributed and that her work already attracted attention of scientific community, as it has been cited 107 times. It shows that the author is well prepared for a successful scientific career. Therefore, with no hesitation I recommend this theses to be successfully defended.

In České Budějovice 14.9.2021

RNDr. Magda Zrzavá, Ph.D.