A review on the doctoral thesis of Nicola R Fornaini: „Evolutionary dynamics of chromosomal rearrangements within the family Pipidae“

The doctoral thesis of Nicola R. Fornaini focuses on karyotype and genome evolution in two model systems (i) frogs of the family Pipidae and (ii) fishes of the genus *Carassius.* In both model systems, polyploidization has occurred independently several times and the selected species represent nice model systems to study the consequences of polyploidization on genome evolution. The present thesis uses a wide array of classical as well as modern cytogenetic approaches to describe the genome and karyotype changes between the species. I also appreciate that the cytogenetic approaches were intended to be complemented by genomic approaches to get a deeper insight into the genome evolution in polyploids, but the genomic data as presented in the thesis are rather preliminary and for the most part not yet analysed.

The PhD thesis consists of 5 papers published in genetic and zoological journals, two of which Nicola R. Fornaini is the first author. Three of them focus on Pipidae frogs and two on Carassius fish. Given this it was a little bit confusing to me that the title of the thesis as well as the whole introduction focus only on the frog system, with no mention of the fish system. Two papers included in the doctoral thesis are thus not introduced in any way, although it would be nice given that two systems were analysed by similar methods and were used to address similar questions.

In general, the papers are interesting, well written and provide interesting insights into the karyotype evolution after polyploidization or the taxonomy of Xenopus frogs and Carassius fishes from a cytogenetic perspective. However, since the papers represent a collaborative work of multiple co-authors and, according to the authors' contributions were not written by Nicola R. Fornaini, with one exception, I will focus my review mainly on the introductory part of the thesis, which was written solely by the student.

The introductory part consists of 4 main chapters: Introduction, Materials and Methods, Discussion and Conclusion. Although some parts of this text are well written, some other parts would need more work to make a comprehensive and well-rounded introductory text to the papers. For example, the Introduction (1.1 – 1.2) is according to me too much focused on model systems and their role in developmental biology studies and human medicine, which is not a main topic of this thesis. On the other hand, some important issues of the thesis, such as subgenome evolution in polyploids, are not very well addressed in the Introduction. I also missed a chapter describing the aims of the thesis after the Introduction. Without this, it was quite difficult to understand the following chapters. For example, I found the chapter Materials and Methods confusing without specifying aims and other circumstances of the research. On the one hand, it stated that all cytogenetic methods were described in the accompanying papers. On the other hand, it described the methods of DNA and RNA isolation and analysis of sequence data. I later understood that this was because there were some unpublished results in the thesis, but without explaining this, the structure of the text was confusing.

The Discussion part summarizes the results of the three papers on the frog system. I found this part of the text nice and comprehensive. Only the last part, describing the unpublished results of genomic analysis of subgenomes in Silurana and Xenopus, was rather superficial and the results were not adequately presented (no specific data, figures or statistics are shown). I understand that the results are preliminary, and most analyses are still in the progress, but in this case, I think, it would be better not to include this part in the thesis.

The figures also need a little more care: Some figures (e.g. Figure 5) are entirely taken from the accompanying paper, including the text of the legend. Although the source paper is cited at the end of the legend, it is not common practice to take over the entire figure, including the legend. For other figures (e.g. Figures 6, 9), the legend is inadequate, and the figure is difficult to understand. Some figures (e.g. Figures 3, 4) have very poor resolution.

In conclusion, Nicola R. Fornaini presented nice cytogenetic work, covered by five published papers, which is definitely sufficient to obtain the PhD degree. However, the thesis itself suffers from several flaws and would need some more care to make it well balanced and comprehensive.

Questions:

On page 12 the author says: „However, in higher vertebrates, as amphibians, reptiles, birds, and mammals, animals that are characterized by having a well-developed brain, a backbone and a more complex structure compared to lower vertebrates like fish, they don't seem to have an high tolerance to polyploidy very well." Does this mean that „well-developed brain and more complex structure" hinder polyploidization? How would you explain that some taxa tolerate polyploidization more than others?

On page 13 the author says: „Polyploidy is common in plants, fish, and frogs, indicating clear fitness advantages." I would be a little bit more careful with that statement. Neopolyploidy is common, but if you look at the number of paleopolyploidization events, they are much less common, which suggests that polyploids are not always competitively superior over diploids. Can you discuss a little bit more under which conditions the polyploidy can be advantageous and under which not?

Given the content of the papers, I missed some text in the introduction about subgenome evolution in allopolyploids. For example, why is one genome usually dominant over the other, and which one? Can you elaborate on this topic a little bit?

Paper 1: Homologous chromosomes within and between Carassius species have been determined just based on centromeric index, which can be, however, very similar for some chromosomes. Isn't it possible that the high variability in centromeric index for some chromosomes (Figure 2) is caused by the fact that the chromosomes have not been homologized properly? How could you improve the method of chromosome homologization?

Paper 4: In GISH experiments, the whole-genome probe did not paint the B chromosome. This is strange as most B chromosomes are composed of sequences duplicated from other regular chromosomes. The authors interpret this result as a methodological artefact caused by higher heterochromatinization of the B chromosome. Can you think of another reason why B is not stained?

In Prague, 3.1.2025 RNDr. Radka Reifová, Ph.D