Review of Dr. Amaury Herpin's habilitation thesis entitled "Evolution and plasticity of the gene regulatory networks controlling sex determination: of masters, slaves, usual suspects and usurpators"

My role of an external reviewer was here both extremely easy and extremely difficult. Easy, because it was a great pleasure to read the whole thesis. I learned a lot. I think that it is an ideal example of a habilitation thesis, i.e. that it shows how a habilitation thesis should look like. Habilitation theses are traditionally composed of published papers, the one by Dr. Herpin includes eight selected papers documenting the expertise of the author, all were published in very respectful journals. The Introduction takes nearly 80 pages. According to my experience, in many Ph.D. and habilitation theses, an introduction is not very interesting, being mostly restricted to necessary commentaries to the included papers. Here, my feeling was very different: this introduction is to me a true masterpiece. It summarizes in a dense way the research of the applicant, yet this complex task is performed in a very readable and easy to follow manner. The deep knowledge of Dr. Herpin on the evolution of sex determination were transformed to a nice text written in lively language very suitably illustrated by numerous figures. I read the whole Introduction at once, like a detective story, I was simply not able to stop reading. I appreciate that the author included on different places personal commentaries allowing to understand that we are following a personal way of research and its development, a quest of a living, enthusiastic person with own preferences and feelings about his work and wished to move understanding of the studied topics. The Introduction contains everything what should be there: nice contextualization of the various aspects of research, description of its development including finding the right collaborators, ideas why the research is important and that it has consequences for biological applications in aquaculture or medicine of sexual disorders, detailed future perspectives…

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Regardless of whether the thesis is written well or not (and it is), the discoveries are crucial. It is quite paradoxical that to me the most significant findings are just the opposite: a perfect documentation for an evolutionary stability where no one had expected it, and for a variability where no one had expected it either. The first case includes the discovery of the chaperone-mediated autophagy (CMA) in teleosts documenting that this process is much older and conserved than assumed. The opposite situation is the as minute as possible exploration of the sex differentiation pathway in the medaka demonstrating that "downstream" genes of the pathways – so called "slaves" – have conserved sequences (commonly across Eumetazoa), but not so much conserved function across vertebrates. Now I found it very logical – it is natural that a change in a behaviour of a network member, who wants to become a "master", would have an effect of the behaviour of many other members of the same network, but we needed a detailed analysis of the medaka gonadal development done by Dr. Herpin and his colleagues to view it clearly.

I admire a range of the experimental methods used in the included papers. The up-to-date techniques of developmental and molecular biology and bioinformatics were applied in the best way, I like a lot that all the research is done and the results are interpreted in the explicit phylogenetic context.

At the beginning of my review, I complaint that it was also an extremely difficult task. By it, I wanted to express that according to my feelings it is difficult to find an aspect that should be criticised, and I somewhat feel that a criticism is expected from a reviewer. Of course, as every good research, papers in the thesis are not the end of the story, but opens new research questions and possibilities… Oh no, what starts as a criticism turned again to a compliment. I better finish now with the obligatory phrase that I have no doubts that the thesis should be accepted by the committee and that Dr. Herpin is without doubt a leading researcher whose work is accepted worldwide and brings new aspects to the field.

To start a discussion, I have two questions:

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1) In the Introduction (not in the paper published in PNAS) it is stated that the presence of sex chromosomes in the herring is surprising and unexpected as such a widespread species with enormous population should have flexible, if not random sex determination. I would like to know why we should not expect sex chromosomes in such a species with extremely large effective population size (unlike to many others his contributions, I am quite sceptical to the idea of random sex determination by Prof. Perrin).
2) It appears that conservation of sex determination does not reflect degree of differentiation of sex chromosomes (rather poorly differentiated sex chromosomes appeared to be dozens of million years old in many lineages). Maybe the conservation reflects properties of sex differentiation networks. The fact that a switch in sex determination mechanisms does not require only a change in "masters" but also coordinated changes in "slaves" of sex differentiation networks would explain why sex determination systems are so stable in many lineages (many lineages of insects, many amniote lineages), and it would explain why it is difficult to switch from ESD to GSD and mainly from GSD to ESD at least in amniotes. Would you agree that sex determination/differentiation network might be responsible for the differences in the evolutionary stability of sex determination/sex chromosomes? And if yes, what would be so peculiar in these networks in some lineages of teleosts and amphibians that they have so unusual variability (medakas, Rana and Xenopus frogs…)?

With best wishes,

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