Report on Doctoral Thesis by Mgr. Barbora Straková entitled "Evolutionary pattern and mechanism of environmental sex determination in reptiles".

This PhD dissertation encompasses four empirical studies that advance our understanding of the prevalence and evolution of environmental sex determination (ESD), focused more precisely on temperature-dependent sex determination (TSD) in reptiles. It presents outside-the-box ideas about the evolutionary trajectory that might have led to the evolution of ESD in amniotes (chapter 1), two studies that broaden the cases of TSD in squamates (chapters 2 and 3), and a study on turtle TSD that examines whether germ cell numbers might be thermosensitive and a direct gonadal formation. Overall, this work pushes the envelop of the current paradigm, and made for a very stimulating reading.

Introduction:
Overall, this section is easy to read in some parts but confusing in others, as the text jumps among topics that are only briefly mentioned without enough context. Thus, the intro would have benefitted from an expanded description of several of the concepts and issues, including a deeper discussion of the literature.

Questions - Intro

1. Birds split from their sister taxon, crocodilians, ~245Mya, whereas mammals split from reptiles ~320 Mya. Yet, in page 11 of the introduction, it is stated that sex chromosomes evolved in mammals ~150 Mya and in birds ~100 Mya. How can this be?
2. Page 13. What evidence exists that the loss of sex chromosomes in *Paroedura* did not lead to the loss of GSD?
3. Page 16. Is there strong evidence to support the hypothesis that TSD patterns MF, and FM, derived from FMF? What data would be needed to test this hypothesis?
4. Page 16. Is the reaction norm for *Pachydactylus tigrinus* similar to that of crocodilians that never reach 100% males?
5. Page 16. Biased sex ratios do not necessarily invalidate the Fisherian-Darwinian model (Fisher, 1930) of sex ratio evolution, but it is presented as if they did. What are the conditions under which biased sex ratios are expected to agree with the Fisherian model?

Chapter 1:
This is an intriguing, albeit highly speculative, paper. It proposes hypotheses based on observations from the literature that are consistent with the hypothesis of sequential hermaphroditism in non-amniotes as a potential precursor for ESD in amniotes, and of the stress response as the molecular machinery underlying the developmental pathways. It is an interesting contribution that should foster further research in this area. However, the paper would have benefited from a discussion of alternative processes that would explain the observations from the literature and possible tests that would distinguish the proposed hypothesis from alternative explanations.

Some other minor comments/questions are:

1. Page 4. The 80My and 66My underestimates the split of crocodilians and turtles.
2. Page 4 and 6. Why would the "non-homology of sex chromosomes across particular GSD lineages and the notable stability of GSD" support the idea that ESD might be ancestral to amniotes?
3. The statement that in all ESD, "*the environmental conditions for which the progeny is optimized are in action during embryonic development*" is unclear. What about when e.g. fitness of TSD taxa is affected not by temperature directly but by a correlated factor later in life?

Chapter 2:
This chapter is a nice contribution to the still understudied world of sex determination in squamates, and it adds 3 new cases of TSD to the list. It is clearly written and easy to follow.

1. Page 601. It was a missed opportunity not to discuss the explanation for the sex ratios produced by the fluctuating thermal regime.
2. Page 602: The text seems to imply that biased sex ratios reflect selection above or below the individual level (group selection or selfish gene), but they could very well reflect individual selection.

Chapter 3:
This chapter presents another study describing TSD in two gecko species and is a welcome contribution to diversity of sex determining mechanisms known in squamates. One point emphasized by the paper is the potentially important 'random' component to TSD at pivotal temperature based on the observation that in some clutches, the two eggs that are glued, develop into a male and a female. Such random effects are better expected at the temperature that produces 50:50 sex ratios as the probability of developing into one sex or the other is equal or random by definition.

1. One issue is that none of the temperatures used in the study are the pivotal temperature since no temperature produced 1:1 sex ratios.
2. In this study, this occurred in 2 and 5 clutches of *P. laticauda* and *P. nigristriata*, respectively, but it was not stated if this occurred in 2 and 5 clutches out of 19 and 22 pairs. Are these results not what would be expected under the reaction norm of a pure TSD system?
3. How different are the results for this particular study expected to be under a random vs non-random model of TSD?
4. About the effects of temperature on fitness, was there an effect of incubation temperature on hatchling size?

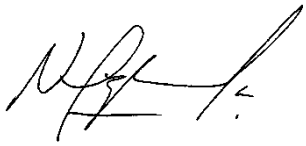
Chapter 4:
This chapter presents a study that proposes a very intriguing model about the molecular/cellular basis of TSD. The model proposes that in *Trachemys scripta* turtles, (1) warmer (female-producing, or FPT) incubation temperature induces higher number of germ cells (GC) than male-producing temperatures (MPT), that (2) this difference is established before the thermosensitive period (TSP), and that (3) higher or lower GC number directly induces ovarian formation while lower GC number induces testicular formation, but that this GC-number-induced system is particularly important at pivotal temperatures (PvT), whereas under FPT and MPT, it is somatic cells that direct gonadal differentiation. While intriguing, some observations made in the study are either not explained by the model, or explained by an alternative model, and a careful examination of the full data set leads to an interpretation that counters the conclusions reached in the paper and are instead, more consistent with previous results cited in the chapter. Namely:

1. GC numbers at the PvT overlap the range of found at MPT (Fig 2D, Supplementary Tables 1 and 2) and produced both males and females. Under the proposed hypothesis, this should only occur if GC numbers at the PvT are different between testis and ovaries. But the PvT GC counts for testis overlap entirely the range of GC counts for ovaries. How can this be?
2. Furthermore, when germ cells were ablated chemically by treatment with bisulfan at the PvT, both sexes were also produced, and the GC counts also overlap for testis and ovaries and were lower than for the control PvT testes or for testes at MPT. How were ovaries produced then?
3. How can the results of this chapter be reconciled with those from the same lab (Dinapoli and Capel 2007) and cited in the chapter, where they experimentally found that "*normal morphological development of the fetal gonad occurs in both sexes in T. scripta after germ cell*

depletion"? That study seems to be the more direct and compelling test of this hypothesis and their results are counter to it.

4. And a technical question. What was the justification to change the marker used to stain GCs in this chapter from the commonly used VASA to HuC/D?

In summary, I enjoyed reading this dissertation, and I find it to be worthy of a PhD degree.



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