February 08, 2023

Dear Dr. Craig E. Franklin,

Please find the attached manuscript entitled, “Metabolic consequences of sex-reversal in lizards: a test of the like genotype and like phenotype hypotheses” submitted for your consideration and publication in the *Journal of Experimental Biology*.

This manuscript reports, for the first time, the metabolic consequences of temperature-dependent sex reversal in a vertebrate species to test whether selection favours sex-reversed individuals (Holleley et al 2015: Nature 523, 7558: 79-82). Reptiles have transitioned between these modes of sex determination many times, but evolutionary explanations for why this happens remain elusive.

To date, no studies have explored the metabolic consequences of sex-reversal in any other vertebrate, even though metabolism is essential to various fitness-related aspects. Here, we propose a new theoretical framework (Like-Phenotype/Like-Genotype framework) to provide a context for testing how metabolism and other fitness-related endpoints might impact the evolution of sex-reversal. We apply this framework using two lizard species with different patterns of temperature-induced sex-reversal and different genetic sex determining systems (male versus female heterogamety). We measure metabolism and growth rate (two traits linked to fitness) along with survival in hatchlings. We show that metabolic rates of sex-reversed individuals support the Like-Phenotype/Like Genotype framework depending on the GSD system being compared. Our findings provide some evidence for a fitness advantage from sex-reversal in both species but do not exclude energetic processes as a constraint on the distribution of sex-reversal in nature.

We declare that we have no conflict of interest with this research and that all research activities were conducted under appropriate institutional and governmental approvals.

Thank you for your consideration of the manuscript. Please contact me if there are any concerns or issues that I may address.

Kristoffer H. Wild

Division of Ecology and Evolution

Research School of Biology

The Australian National

Canberra, ACT, 2602