

Review by Jennifer N. Lohr, University College London

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This is a review of the paper “Geographic variation in adult and embryonic desiccation tolerance in a terrestrial-breeding frog” from authors Rudin-Bitterli, Evans and Mitchell at the University of Western Australia.

Summary:

In this study Rudin-Bitterli et al. investigate the intra-specific variation present regarding desiccation tolerance in one species of terrestrial-breeding frog in Australia. They use six populations of frogs originating from locations with different amounts of annual rainfall and also perform crosses between the populations. I agree with the authors that intra-specific variation is an important factor for conservation efforts and in need of more extensive study.

I found it a well-written manuscript with appropriate methods and statistics. The authors measure many traits in detail, which adds a lot of power to the study. Below are a few points, which I think might help to improve the quality and clarity of the manuscript.

Major Points:

1. Purpose of crosses not stated in abstract – also in the introduction/discussion it might be good to address the value of your crosses. There was a lot of effort that went into this part of the study and so it should be stated more directly what knowledge we gain from this instead of just measuring the tolerance of adults.

2. Statistics: authors seem to make use of the proper tests, R-packages and normalization methods before hand. Maybe state at the beginning of the methods how normality was checked for all data and not just in the second section – I assume the authors also did Q-Q plots for the dehydration/rehydration data, etc.

Maybe one question about the statistics for rehydration/dehydration rates. Here the authors present ANOVA results for the 6 populations and lin reg results by rainfall measure. Could the authors maybe comment if it is justified to do both tests separately with independent P-values? As far as I understand the data set each population is also one rainfall measurement, so in fact these two tests are actually the same and this is a bit of double reporting (i.e. not independent hypotheses)? In this case it might be best to report your ANOVA and then give an R^2 value using the same model as your ANOVA. Then it is only one test and you are just reporting an effect size.

3. Tests were performed on males only. It is stated in the methods that females are harder to obtain. Could the authors add a note about the expected effects in females and what is known from other studies?

4. Discussion: there is a rather large amount of text on mechanisms in the discussion. While this is very interesting, it seems not to be either the focus of this paper nor the points to which your results can be best applied. The final summary paragraph of the discussion is actually the interesting part. Here you compare your results to other similar ones and discuss phenotypic plasticity and local adaptation – in your frogs.

I think it might improve the interest of the manuscript to expand on this last paragraph a bit and to place your evidence for local adaptation within the general framework of conservation biology as a whole, not just your species of frogs. Specifically, the last sentence of your abstract states “We emphasise the importance of considering geographic variation in phenotypic plasticity when predicting how species will respond to climate change.” And also in the introduction you talk about how “In particular, information on the environmental sensitivity of range-edge populations will be vital for understanding future changes in species distributions, since it is at range edges where colonisations and extinctions primarily occur as the climate changes”. Both interesting points. I think it would be nice to come back to this in the discussion and relate your results to those from other species to try to speak to these challenges.

Minor Points:

1. line 175-176: “The eggs of each female were divided equally into five groups and fertilised separately with sperm from one of five males.” Does this mean each combination of 1-5 males, or randomly 1-5 males – clarify.
2. line 186 – change similarly to similar
3. line 204 – change de-ionised to deionised to match with other usage in text
4. line 221-222: homogenised soil, previously collected from a *P. guentheri* breeding site – was different soil collected from the various sample sites or all from one site? Maybe state that in the methods since you are looking for between site differences the origin of the soil may also have an effect.
5. line 249: “Swimming performance was recorded 6 - 12 hours after hatching **on** a subset of hatchlings” - was recorded **for** a subset?
6. line 407: **A** recent genetic analysis

Best wishes,
Jennifer Lohr