



Peer Community In Evolutionary Biology

Tough as old boots: amphibians from drier habitats are more resistant to desiccation, but less flexible at exploiting wet conditions

Ben Phillips based on peer reviews by **Juan Diego Gaitan-Espitia**, Jennifer Nicole Lohr ? and 1 anonymous reviewer

T Rudin-Bitterli, JP Evans, NJ Mitchell (2019) Geographic variation in adult and embryonic desiccation tolerance in a terrestrial-breeding frog. bioRxiv, ver. 1, peer-reviewed and recommended by Peer Community in Evolutionary Biology. [10.1101/314351](https://doi.org/10.1101/314351)

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Species everywhere are facing rapid climatic change, and we are increasingly asking whether populations will adapt, shift, or perish [1]. There is a growing realisation that, despite limited within-population genetic variation, many species exhibit substantial geographic variation in climate-relevant traits. This geographic variation might play an important role in facilitating adaptation to climate change [2,3]. Much of our understanding of geographic variation in climate-relevant traits comes from model organisms [e.g. 4]. But as our concern grows, we make larger efforts to understand geographic variation in non-model organisms also. If we understand what adaptive geographic variation exists within a species, we can make management decisions around targeted gene flow [5]. And as empirical examples accumulate, we can look for generalities that can inform management of unstudied species [e.g. 6,7]. Rudin-Bitterli's paper [8] is an excellent contribution in this direction. Rudin-Bitterli and her co-authors [8] sampled six frog populations distributed across a strong rainfall gradient. They then assayed these frogs and their offspring for a battery of fitness-relevant traits. The results clearly show patterns consistent with local adaptation to water availability, but they also reveal trade-offs. In their study, frogs from the driest source populations were resilient to the hydric environment: it didn't really affect them very much whether they were raised in wet or dry environments. By contrast, frogs from wet source areas did better in wet environments, and they tended to do better in these wet environments than did animals from the dry-adapted populations. Thus, it appears that the resilience of the dry-adapted populations comes at a cost: frogs from these populations cannot ramp up performance in response to ideal (wet) conditions.

These data have been carefully and painstakingly collected, and they are important. They reveal not only important geographic variation in response to hydric stress (in a vertebrate), but they also adumbrate a more general trade-off: that the jack of all trades might be master of none. Specialist-generalist trade-offs are often argued (and regularly observed) to exist [e.g. 9,10], and here we see them arise in climate-relevant traits also. Thus, Rudin-Bitterli's paper is an important piece of the empirical puzzle, and one that points to generalities important for both theory and management.

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Reviews

Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/314351>

Version of the preprint: 1

Authors' reply, 10 July 2019

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Decision by [Ben Phillips](#), posted 05 June 2018

Minor revisions required

This is a solid piece of work on an important and fascinating topic. It is a large dataset, is well reported, and the methods and analysis are appropriate. All reviewers saw the value of the work and none found any serious flaws. Consequently, all reviewers comments are in the nature of suggestions for improvement. I would encourage the authors to consider the reviewer's comments and, where improvements can be made, revise or clarify.

In particular, I thought Lohr's suggestion for the discussion to focus less on mechanism and more on broader implications (targeted gene flow, local adaptation, conservation) was good advice. Some meditation is warranted on how the plasticity you uncovered is relevant to these broader themes.

Gaitan-Espitia's review was particularly thorough, and he raises some interesting thoughts. Being a little more precise with your use of "selection" and being clear that you have evidence for local adaptation, rather than having measured selection per se will address several of his concerns. It would also be useful to report the random effects and residual variance for the fitted models. Doing so gives us more information about the model fit, but also provides information pertaining to maternal/paternal effects, and provides hints about how heritable the traits are. I also wondered about a formal heritability analysis, but felt that the manuscript is already substantial, and there might not be sufficient family groups in many of the populations for robust estimation of heritability. Some information on timing of oviposition would be useful in the methods (how synchronised was their breeding?). Finally, it is worth discussing briefly the role of behaviour (and sexual dimorphism in behaviour and traits) and how these might affect your conclusions.

Reviewed by [Juan Diego Gaitan-Espitia](#), 10 May 2018

General comments

The main objective of the work presented in this manuscript by Rudin-Bitterli and colleagues, was to assess patterns of intra-specific variation in desiccation tolerance of a terrestrial-breeding frog, along an environmental/climatic cline (rainfall). The authors explored questions regarding phenotypic plasticity and local adaptation on desiccation tolerance using six populations of frog *Pseudophryne guentheri*, and three different ontogenetic stages (male adults, embryos and hatchlings). These populations are genetically structured with low gene flow and connectivity, offering the potential for local adaptation to climatic (and potentially edaphic) conditions. The theoretical framework of this study is based on a fundamental premise in evolutionary ecology, in which species are not considered static/uniform entities but a set of populations that vary genetically and phenotypically across spatial and temporal scales. Therefore, in order to understand the physiological, ecological and evolutionary responses of species to environmental variation, stress and climate change, scientists need to take into consideration the different environmental and genetic backgrounds of natural populations across the species distribution. For the model system used in this study (as well as for other amphibians), water availability is an important environmental factor that influences its physiology, behaviour, performance and

fitness. Hence, the authors hypothesised that low environmental water availability is likely to induce strong directional selection through its negative effects on survival, reproduction and growth, potentially leading to genetic and/or phenotypic divergence among populations. After acclimation to lab conditions, dehydration and rehydration rates were assessed on male adults from the six populations, whereas desiccation tolerance was assessed in embryos and hatchlings via analyses of time to hatching, swimming performance, hatchling wet weight and malformations. The authors found that: 1) there is significant intra-specific variation in traits associated with desiccation tolerance in *P. guentheri* at different ontogenetic stages; 2) there are some signals of local adaptation in desiccation tolerance evidenced by clinal variation in the responses of adults, embryos and hatchlings, with populations from drier sites greater tolerances in populations from xeric/drier sites compared to those populations from more mesic sites.

Overall, this work of Rudin-Bitterli and colleagues is well written and the experiments and analyses seem to be well executed and replicable. Methods were carefully described, particularly for the experimental approach and the in-vitro fertilisation. The results are short and clear, and the discussion is, in general, easy to follow. Before recommending the MS, however, I invite the authors to address the following comments. I hope these comments and suggestions will serve to improve the quality and impact of this investigation:

1) During the introduction and the discussion, the authors made a strong emphasis on the potential role of directional selection shaping the phenotypic differences on desiccation tolerance in natural populations. This is a valid argument. However, there is almost no information or discussion about geographic clines in selection and/or the role of behaviour buffering selection. These two factors are quite important for the interpretation of the results as: 1) selection can change across spatial and temporal scales shaping different fitness landscapes across the species distribution; and 2) behaviour plays a fundamental role buffering the strength and direction of selection on natural populations, particularly for traits related to desiccation tolerance and evaporative water loss in amphibians (behavioural selection of microhabitats; see for instance Mitchell & Bergmann (2016) (or in lizards Li et al., 2018). In fact, the importance of behaviour and selection of microhabitats for the burrows, are factors that require some attention in the discussion.

Mitchell, A., & Bergmann, P. J. (2016). Thermal and moisture habitat preferences do not maximize jumping performance in frogs. *Functional Ecology*, 30(5), 733-742.

Li, S. R., Hao, X., Wang, Y., Sun, B. J., Bi, J. H., Zhang, Y. P., ... & Du, W. G. (2018). Female lizards choose warm, moist nests that improve embryonic survivorship and offspring fitness. *Functional Ecology*, 32(2), 416-423.

2) If I understood well the experimental design used for the within-population crosses, then the authors have the possibility of performing some additional quantitative genetic analyses using a North Carolina II design to disentangle the genetic basis of the traits analysed (heritabilities and perhaps genetic correlations), and the parental/maternal contribution to these traits.

3) I am curious about the maternal effects on desiccation tolerance of embryos and hatchlings. There were some effects of ovum size on some of the traits. How was ovum size distributed among populations?

4) Desiccation tolerance was focused on adult males and based on this the authors made an extrapolation to the population level. It would be interesting to include some discussion about the potential responses of this trait on females (What have been found in other species?). Water availability may represent a more relevant/important physiological constraint for females than males, due to the allocation of energy and fluids into eggs.

5) It is not clear if the breeding season is synchronised across the species range or if there are some mismatches among populations. This may induce differences in the maturity of the eggs during the experiments.

6) In the discussion (line 405) the authors stated that their findings (intra-specific variation on desiccation tolerance consistent with the environmental/climatic cline) may reflect (in part) cryptic speciation. I am not convinced about this idea based on the experimental design used. Moreover, I did not find arguments in the text supporting this statement... populations genetically structured are not equivalent to cryptic species, and there is not phenotypic divergence (*sensu stricto*) among populations. There is variability and differences on plastic responses, but not in opposite directions. Moreover, it is hard to tell if this argument is correct, as the

authors developed a common garden experiment with 1 generation in which maternal and environmental effects are still present on phenotypic responses.

7) In the discussion (lines 436-437), the authors stated that "Together these results are consistent with patterns of directional selection for lower dehydration rates in areas where water is frequently scarce". Although I understand the reasoning behind that, I am not confident with this argument because: 1) The experiment was not designed to measure selection; 2) responses in the lab can be dramatically different to responses on the field; 3) selection varies across temporal and spatial scales; 4) behaviour in some cases buffers selection. Perhaps a reciprocal transplant experiment may reveal some clues about this...

Minor comments

How did the authors know that animals were acclimated after 2-5 days?

Line 186 – Similarly to similar

Reviewed by Jennifer Nicole Lohr, 10 May 2018

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Reviewed by anonymous reviewer 1, 10 May 2018

- The authors have done a good job at reviewing the literature and putting their work into the current literature framework. A weakness of this study which the authors are quite upfront about is the fact they cannot separate out genetic vs plastic effects. Yes there does seem some capacity to change your desiccation tolerance across populations, if they are indeed one species? but whether this means they have the capacity to adapt to increasingly dry environments either via plasticity or genetic adaptation remains an unknown. The authors need to be careful in their intro about the capacity to make predictions of species responses to climate change based on the current limitations of their data.

Introduction - Is the rainfall gradient ~300-1250 or ~300-788? As implied in Table 1?

Methods - How many females did the authors mate the 5 sires to? - I am presuming males were exposed to desiccation stress prior to extracting their sperm. Could this treatment have influenced the results from the sibling experiment. - What is the variance in rainfall? In the dry areas does that fall pretty much in one season?

Results - Population 5 seems to be quite invariant to the different treatments. Given this population is some distance (kms) from populations 1-3 is it possible this is another species?

Discussion - Is there any idea of the gene flow between populations? The authors mention that there may be cryptic species and strong structuring, surely they must have an idea whether there is gene flow between these populations or an approximation of how long the populations have been isolated? - Line 442- Metabolic rate has been implicated in influencing desiccation resistance, the idea being that if you have low metabolic rate you are less active, lose less water etc. I wonder if the converse would also be true, you have a lower metabolic rate which means it takes more time to take on water. Or perhaps there are constraints/trade-offs and there is a greater cost to losing water rapidly than to uptake water slowly. I imagine in this system once it rains, it really rains? I wonder if the authors could look to perhaps better studied systems to see whether this type of pattern has been documented? - Rather than populations 1,2,3,5 could they plot the annual precipitation? Or at least note under the populations high low etc