Peer Community In
Evolutionary Biology

Is thermal plasticity itself shaped by natural selection? An assessment with desert frogs

Wolf Blanckenhorn *based on peer reviews by* ***Nadia Aubin-Horth***, ***Wolf Blanckenhorn*** *and* ***Dries Bonte***

Leonardo Bacigalupe, Juan Diego Gaitan-Espitia, Aura M Barria, Avia Gonzalez-Mendez, Manuel Ruiz-Aravena, Mark Trinder, Barry Sinervo (2018) Natural selection on plasticity of thermal traits in a highly seasonal environment. bioRxiv, ver. 1, peer-reviewed and recommended by Peer Community in Evolutionary Biology. 10.1101/191825

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It is well known that climatic factors – most notably temperature, season length, insolation and humidity – shape the thermal niche of organisms on earth through the action of natural selection. But how is this achieved precisely? Much of thermal tolerance is actually mediated by phenotypic plasticity (as opposed to genetic adaptation). A prominent expectation is that environments with greater (daily and/or annual) thermal variability select for greater plasticity, i.e. better acclimation capacity. Thus, plasticity might be selected per se. A Chilean group around Leonardo Bacigalupe assessed natural selection in the wild in one marginal (and extreme) population of the four-eyed frog Pleurodema thaul (Anura: Leptodactylidae) in an isolated oasis in the Atacama Desert, permitting estimation of mortality without much potential of confounding it with migration [1]. Several thermal traits were considered: CTmax – the critical maximal temperature; CTmin – the critical minimum temperature; Tpref – preferred temperature; Q10 – thermal sensitivity of metabolism; and body mass. Animals were captured in the wild and subsequently assessed for thermal traits in the laboratory at two acclimation temperatures (10° & 20°C), defining the plasticity in all traits as the difference between the traits at the two acclimation temperatures. Thereafter the animals were released again in their natural habitat and their survival was monitored over the subsequent 1.5 years, covering two breeding seasons, to estimate viability selection in the wild. The authors found and conclude that, aside from larger body size increasing survival (an unsurprising result), plasticity does not seem to be systematically selected directly, while some of the individual traits show weak signs of selection. Despite limited sample size (ca. 80 frogs) investigated in only one marginal but very seasonal population, this study is interesting because selection on plasticity in physiological thermal traits, as opposed to selection on the thermal traits themselves, is rarely investigated. The study thus also addressed the old but important question of whether plasticity (i.e. CTmax-CTmin) is a trait by itself or an epiphenomenon defined by the actual traits (CTmax and CTmin) [2-5]. Given negative results, the main question could not be ultimately solved here, so more similar studies should be performed.

References:

[1] Bacigalupe LD, Gaitan-Espitia, JD, Barria AM, Gonzalez-Mendez A, Ruiz-Aravena M, Trinder M & Sinervo B. 2018. Natural selection on plasticity of thermal traits in a highly seasonal environment. bioRxiv 191825, ver. 5 peer-reviewed by Peer Community In Evolutionary Biology. doi: [10.1101/191825](https://doi.org/10.1101/191825)

[2] Scheiner SM. 1993. Genetics and evolution of phenotypic plasticity. Annual Review in Ecology and Systematics 24: 35–68. doi: [10.1146/annurev.es.24.110193.000343](https://doi.org/10.1146/annurev.es.24.110193.000343)

[3] Scheiner SM. 1993. Plasticity as a selectable trait: Reply to Via. The American Naturalist. 142: 371–373. doi: [10.1086/285544](https://www.journals.uchicago.edu/doi/10.1086/285544)

[4] Via S. 1993. Adaptive phenotypic plasticity - Target or by-product of selection in a variable environment? The American Naturalist. 142: 352–365. doi: [10.1086/285542](https://www.journals.uchicago.edu/doi/abs/10.1086/285542)

[5] Via S. 1993. Regulatory genes and reaction norms. The American Naturalist. 142: 374–378. doi: [10.1086/285542](https://www.journals.uchicago.edu/doi/abs/10.1086/285542)

Reviews

Evaluation round #2

DOI or URL of the preprint: **10.1101/191825**
Version of the preprint: 2

Authors' reply, 27 February 2018

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Decision by Wolf Blanckenhorn, posted 27 February 2018

Decision on Bacigalupe et al based on 3 external re-reviews: REVISION NECESSARY

The second version of this manuscript is much improved. In particular, the context has been changed completely, and the selection analysis now conforms more to standard analyses in the field. Nevertheless, I still believe that the AIC analysis does not add much to the study, but in fact rather obscures the fact that no selection on physiological plasticity has been found. This is an important (negative) result, which of course can always be argued away due to sample size limitations (cf. P11, L379ff), but I would not go as far here. I think these negative results should be recommended after another revision!

Reviewed by Dries Bonte, 05 February 2018

This is the revision of a manuscript on the natural selection of thermal plasticity in a single frog desert populations. I am happy to see that my previous comments were taken into account, and also that the focus has shifted to the basics, i.e. selection on reaction norms rather than the focus on a climate change framework. I like the introduction as it gives a neat overview of the state-of-the art, and the discussion clarifies the authors interpretation to the best.

The analyses greatly improved and in the end, the authors do show the lack of selection differentials on plasticity, but actually neither on the thermal traits as well (to my opinion). Although the manuscript is well written, I keep having problems with the interpretation of the model selection. As evidenced from figure 4, any sign of directional selection is weak to say the least. Of course, variance explained by the models is largely determined by the amount and type of models included in the model selection. the null model has not been included, and I would suggest to do this, as this will be informative on whether survival can be explained by any trait at all, or whether a no-trait model does eventually do best. This is not a trivial issue as the authors now suggest the thermal traits to be under selection. I am not convinced and would like to see some quantitative evidence on this. This does not make the manuscript less interesting because failure to detect selection based on demography in the wild is an important finding. If the null model would have by far the highest Aikaike weight, it should result in a clearer message and avoidance of any over-selling, or speculations that might eventually be taken as a fact.

Some minor points.

last sentence abstract. Something is wrong here as higher tolerance cannot be selected for and selected against.

The selection on body size: why would larger frogs experience less heron predation?

Reviewed by Nadia Aubin-Horth, 05 February 2018

This new version of a manuscript by Bacigalupe and colleagues aims to quantify genetic variation for plasticity in thermal traits, as well as if and how selection acts on this inter individual variation in nature in a population found at the limit of geographical distribution of that species. As I underscored in my first review, I think this is a very interesting question. Seeing the actual individual reaction norm for these traits is an important dataset for the field of eco-physiology.

My main concern in the original manuscript was the focus on climate change and how it would change the selection regime on thermal traits and plasticity in thermal traits. The authors have now taken this concern into account and have rewritten their manuscript without focusing on this aspect.

My second concern was regarding the fact that the authors measure selection and want to predict evolution under a new selection regime in a climate change scenario. I was worried that not having any information on the heritability of the traits studied would hinder making predictions about the response to selection. However, now that the authors are not focusing on climate change, my comment is less relevant.

Finally, I thought upon reading the manuscript that some results were not well explained in the methods (mainly testing selection on the absolute value of the thermal traits and not only their plasticity). The authors have modified their analysis in the new version of the manuscript to quantify plasticity in a different way and now analyze and present their data in a more consistent way.

Therefore, as the authors have responded to my comments in a satisfactory manner, I do not have additional major comments to make on the new version of the manuscript. I only have a few minor comments:

-In the abstract on line 40-42, this sentence would need to be rewritten so we really separate the prediction of higher tolerance being selected or selected against (?).

-In the discussion on line 2402-241, this sentence: "Furthermore, survival decreased as values of most of the traits increased in both warm and cold acclimated conditions » is strange, since as a reader, looking at figure 4 I could not see significant differences in survival between individuals with different trait values. The sentence could be modified to tamper/nuance this affirmation.

Reviewed by Wolf Blanckenhorn, 05 February 2018

Review by Wolf Blanckenhorn

The second version of this manuscript is much improved. In particular, the context has been changed completely, and the selection analysis now conforms more to standard analyses in the field. Nevertheless, I still believe that the AIC analysis does not add much to the study, but in fact rather obscures the fact that no selection on physiological plasticity has been found. This is an important (negative) result, which of course can always be argued away due to sample size limitations (cf. P11, L379ff), but I would not go as far here. I think these negative results should be published!

Conceptually, my main concerns are as follows:

Fig. 1b: This prediction does NOT follow. Only because there was much selection in the past, this does NOT mean that there is no longer current selection. Selection is exerted on the phenotype, so it may go on forever. There might not be a RESPONSE to selection (because eg. genetic variation is low and/or depleted. But this is not the same thing. Doesn't matter what you predict, actually.

Fig. 4 is bad. Looking at that figure, I only possibly see negative selection in panel top left (Ctmin10) and positive selection in panel top center (body mass). All other lines are flat, but you still discuss some effects in the text that I think are not there.
I suggest to replace Fig. 4 by a table listing the selection coefficient estimates (gradients) with SE or 95%CI, as is standard. Then everybody can see the direction of selection and whether it is strong and significant or not. Discussion of non-significant coefficients should be kept to a minimum.

Minor comments:
P3 L132f: transpose order of two side sentences starting with "favoring organisms..." and "despite the ability...".
P4, L150: Start new paragraph here.
P4, L153/154: As mentioned above, this does NOT follow.
P5, L164: Again, this does not follow: Q10 might not be changing, but this doesn't mean that it is not under selection.
P8, L258f: I do not understand this sentence.
P10, L338f: I don't think so! Possibly only CTmin10.
P10, L344f: Again, I don't think so!
P11, L380f: Yes, and no! If you can report linear selection here, you can and should report non-linear (i.e. stabilizing) selection as well. They may indeed all not be significant and hence weak. Indeed, if an optimum has been reached, stabilizing rather than directional selection is expected.
P11, L394-398: Again, I don't see this in Fig. 4. Supply a specific test and significance.
P12, L401f: Were daily high extremes indeed frequent? Ctmax lines flat in Fig. 4.
P12, L407ff: Indeed an interesting result implying increased thermal breadth, if it is real!
P12, L409-414: Again, I don't see that! That is, I don't follow which data support this conclusion.
P13, L430f: Again, I don't see this in Fig. 4.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/191825
Version of the preprint: 1

Authors' reply, 28 December 2017

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Decision by Wolf Blanckenhorn, posted 28 December 2017

Revise

We obtained 3 external reviews for the paper ”Natural selection on plasticity of thermal traits in a highly seasonal environment” by Leonardo Bacigalupe et al. All reviewers saw merit in this study and think that it can eventually be recommended by PCI EvolBio after extensive revisions, which however are necessary and strongly recommended before. The reviews were consistent in the sense that the reviewers identified two aspects that need extensive revision. First, the context of climate change needs to be revised, as only a single population of this frog was studied in its natural habitat, thus experiencing no climate change but natural seasonal fluctuations. Second, the statistical analysis needs extensive revision according to the comments of the reviewers.

Reviewed by Nadia Aubin-Horth, 28 November 2017

Review for PCI Evol Biol

October 2017

Bacigalupe et al.

Natural selection on plasticity of thermal traits in a highly seasonal environment Main comments

This manuscript by Bacigalupe and colleagues aims to study natural variation in thermal plasticity in a poikilotherm and if this plasticity is itself the target of selection in a natural setting. They present their question in a context of global warming, proposing that although the strength of selection and type (directional, stabilizing) would affect the response to a changing environment, we have almost no information on selection acting on plasticity of thermal traits itself. To test this, they sample wild frogs and bring them to the laboratory to acclimate them in a controlled environment to two temperatures (each individual is tested in both temperatures sequentially): 10 and 20C. Various thermal traits (critical temperature as a measure of performance, behaviour, metabolism) are then tested at each acclimation temperature to quantify the plasticity in these traits. They then release the frogs back in their natural habitat and estimate survival in the following months, in order to test for the presence of natural selection on plasticity of each of these thermal traits. The authors make 3 predictions about the relationship between the plasticity of each of the 4 measured traits and fitness (measured as adult survival) that they test with this dataset. They find that survival is principally affected by mass and is also partly explained by various traits, each with their particular relationship, suggesting that several trait combinations may bring about equal fitness.

I think the ms has great potential and presents solid data, however I have concerns that must be addressed in a new version before I can recommend this manuscript.

The authors place their work within the framework of predicting the response of populations to global warming. However, they do not test survival in a warm-world scenario (in the lab for example), but rather in current temperatures in the wild. Therefore, they test if plasticity is under selection in the actual thermal regime. Selection could therefore have already acted on plasticity. The authors themselves seem to suggest it did on line 73-74 “However, we still do not know whether that physiologicl plasticity, WHICH RESULTS FROM INHABITING A HIGHLY VARIABLE ENVIRONMENT, is being targeted by natural selection” Their selection measurement is thus not really related to their introduction that aims to quantify the direction and strength of selection on plasticity in a global warming context, since it is not done in conditions that mimic these higher expected temperatures. Why was this type of survival measurement chosen, given the context for the study? If there is selection now, what does it tell us about future response to global warming, if the selection regime changes anyway?

Second, they present selection gradients but never mention the second half of the prerequisite to a response to selection: heritability of the traits. The interindividual variation in plasticity of each trait must be underlined by genetic variation, otherwise there can be selection on the current generation but there will be no evolutionary response in the next generation. This is not the direct topic of the manuscript, but must be addressed to highlight how these selection measurements are relevant to make predictions about response to climate change.

Because of these two points, I think the manuscript introduction must be rewritten so that the core of the work is presented in an appropriate context. I agree that we need to know more about selection on plasticity itself, and that acclimation to temperature is an ecologically-relevant trait in a frog species. In my opinion, this important and novel dataset could be presented without relying on climate change as the background context for doing this study, but a large amount of work on the text must then be done.

My other main point is that the discussion is on the absolute values of the traits that were associated with survival (line 214-2017). However, these results are not clear at all from the results section (where can we see this result?), and since this question was not presented in the introduction, the reader is left wondering what is the link with plasticity (the main question at the beginning of the ms). If these results become more central in a revised version, I would suggest using a 3-D graph showing survival and an associated physiological trait with the two acclimation temperature on the third axis, so we can see the different fitness peaks in different "trait combinations" Minor comments:

*Predictions presented in the introduction (line 77) should be represented graphically

*Some terms such as Q10 etc should be defined for non physiology specialists if a broad readership is targeted. Similarly, terms like norm of reaction should be defined

*Please include how many frogs were tested first at 10 C and then at 20C and vice-versa. Was it about equal?

*Why not include mass as a covariate of some sort (non-specialist question here) instead of using it in the MCR model

*The calculation made on line 200-2002 need to be explained in more details.

*Figures: figure 2 need finer lines, maybe color, so the reader can follow individual norms of reaction. Figure 3: need to re-order the panels so they match the 3 predictions (and the new figure showing the predictions graphically)

Reviewed by Wolf Blanckenhorn, 28 November 2017

Re-review of manuscript "Natural selection on plasticity of thermal traits in a highly seasonal environment" by Bacigalupe et al. for BioRXiv.

In this study, the authors assess viability selection in the wild as a function of plasticity in several thermal traits (xi: CTmax – critical maximal temperature, CTmin – critical minimum temperature, Tpref – preferred temperature; Q10 – thermal sensitivity of metabolism) in one marginal (or extreme) population of the four-eyed frog Pleurodema thaul. Animals were first captured in the wild and subsequently assessed for thermal traits in the laboratory at two acclimation temperatures (10 & 20C), defining the plasticity in all traits xi as the difference between the two acclimation temperatures. Thereafter the animals were released again in their natural habitat and their survival was monitored over the subsequent 1.5 years, covering two breeding seasons, to estimate viability selection. The authors conclude that, in addition to larger body size, selection seems to favour frogs that can tolerate high temperatures during the cold breeding season and frogs that increase their activity during the warm periods of the year.

This is definitely an interesting study because selection on plasticity in thermal traits, as opposed to selection on the thermal traits themselves, is rarely reported in animal species. The manuscript is overall clear although several small (language and style) edits are still needed to streamline the presentation. However, I had difficulties following the conclusions of the authors, as the data analysis in my opinion is convoluted and suboptimal, in the extreme even flawed. I suggest reanalysis in a more standard way as outlined below, which should lead to clearer results and make this manuscript more understandable.

Major comments: 1) The authors investigate one apparently marginal, or in some way special ("northermost") population of this species (L93). That's fine, and the desert habitat seems to be indeed practical for monitoring survival in a thermally extreme environment because it is nicely contained. However, only ONE population is assessed here, so any conclusions about the adaptive fit of this species to this special habitat remain unreplicated and hence of limited generality. What remains is a study of thermal plasticity and selection in a single population.

2) Most crucially, I believe the analysis is suboptimal if not flawed, hampering clear interpretation and discussion of results. a) Analysis of ratios and differences is always problematic because the same number can result from a change in the numerator/first term or a change in the denominator/second term. Hence interpretation of results is complicated. As a result, I could not follow the main conclusions in the first paragraph of the Discussion. b) Furthermore, the authors only analysed the difference (i.e. plasticity, essentially the slope, as depicted in Fig. 2) in the traits xi but NOT the actual measured traits themselves (at the two acclimation temperatures). The raw data are depicted in Fig. 1, but then the selection analysis apparently uses only the slopes depicted in Fig. 2. This again hampers full interpretation of the result. A proper analysis would analyse the actual traits xi(10) and xi(20) PLUS the interaction term between the two (xi(10)*xi(20)), the latter being something like the difference analysed here. (Body size of course needs also be included as a covariate.) This way all terms are being analysed and interpreted. c) I do not see the added value of the AIC analysis in Table 1. In particular, it does not seem to help the authors in interpreting their results, as it is unclear whether any effects are significant or not. The logic of AIC is that the model(s) with the lowest AICc should be preferred (if delta AIC is within ca. 2). Here this means, if Table 1 is correct, that most of the terms do not add significantly to the model. d) Related to (c) above, it is unclear if the linear selection gradients depicted in Fig. 3 are significant or not. I would suggest using standard uni- and multivariate selection analyses for both the traits xi and plasticity (although xi(10)*xi(20) would show up as correlational selection in such an analysis), adding linear and quadratic terms (as for term 27 in Table 1) plus the above correlational term. Terms can be removed if not significant. This would make the analysis much more palatable and clear to the average reader.

Minor comments: L76 and L130: It is unclear to me why Q10 was assessed as the difference in oxygen consumption between 20C and 30C, whereas the plasticity treated here was assessed as the difference in acclimation between 10C and 20C. The latter would also have allowed calculation of Q10.

L228ff: The argument that frogs died due to thermal effects rather than predation is plausible, but in the end cannot be proven. Why not add actual field temperature parameters in the model to obtain indirect evidence that indeed temperature is the culprit?! Further, why not separate analyses for breeding and non-breeding season to correctly identify which frogs died when?

L248-267: I could not follow the arguments discussed here.

Best regards,

Wolf Blanckenhorn

Reviewed by Dries Bonte, 28 November 2017

The work of Bacigalupe and colleagues addresses an important topic in evolutionary biology, i.e. the evolvability of thermal reaction norms. The authors study different component of thermal biology (ranging from acclimation strategies to plasticity in thermal preferences and thermal sensitivity of metabolism in two populations of a frog. The species had been demonstrated to fulfil the conditions by which thermal reaction norms can be expected to be subject to selection. The paper is in general well written and all experiments and analyses seem to be well executed, and repeatable. The authors use model inference approaches of estimated survival rates in function of different plasticity measures to detect signals of stabilising or directional selection. By means of model averaging, the authors do not detect strong and univocal signatures of selection, and instead survival to be primary size dependent. Of the remaining models, 25% of the AIC weights suggest directional selection in one of the measures traits. The authors use this evidence to demonstrate that thermal acclimation is subject to selection. This interpretation of course highly depends on the set of contrasted models, and the interpretation is subject to debate as especially the estimates on the directional component (Fig 3) show large variation, what frequentist statisticians would interpret as non-significant. I do not think that this absence of 'significant' results does jeopardise the importance of the study, but it may overall need to be toned down. In the abstract you write that the results suggest a complex fitness landscape. One may equally argue it is very simple as none of the directions are really convincing- and none of the complex models showed strong support. I am also wondering to which degree the different thermal components are correlated at the individual level. Such phenotypic correlations may be important for further interpretation. Now, only correlates with body size are reported This is my main point of concern for this study. I here-under list additional issues that the authors may want to consider:

Abstract: line 27-31: this sentence is hard to follow. Consider splitting in two The last sentence (line 37-39) is based on the suggestion/insight that the impact is strong and convincing. I would prefer to stick to a rather open conclusion about the lack of any clear signal instead of 'forcing' a strong conclusion on the adaptive value of any of the strategies.

Introduction line 58. The references 8-11 are relevant for your study, but sure some more seminal references could be used here? line 72: what do you mean with the environmental opportunities? Is anything known on whether the thermal traits themselves are under selection (compared to plasticity). This seems like a first logical step in the research. If yes, please add a couple of sentences on this as this may be important to further understand the relevance of your findings on acclimation.

Methods line 163. Explain what you mean with the survival rates extracted as individual covariates? line 170: the selection of alternative models is highly responsible for this interpretation. You need to explain clearly why you always had body size as covariate from the beginning.

Results nicely reported; no comments

Discussion In general, you find somehow opposing selection pressures following acclimation in the warm and cold season (on different traits). I would be useful to see some discussion on the (lack of) individual phenotypic correlations as this will be relevant to understand how the environment is actually selecting. Line 271-280: this is interesting, but why not included in the main results?