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Instituto de Ciencias Ambientales y Evolutivas

Professor Wolf Blanckenhorn
Managing Board PCI Evol Biol

Dear Prof Blanckenhorn,

Thank you very much for the opportunity to resubmit our manuscript “Natural selection on plasticity of thermal traits in a highly seasonal environment”. We have carefully considered the constructive comments offered by the three external reviewers, and we acknowledge that their ideas promoted a noticeable improvement in the quality of our work. Accordingly, we followed all their recommendations. As major changes, in this new version we:

(1) Included the null model within our set of candidate models
(2) Included a new table with individual directional selection estimates (SE and their 95% CI)
(3) Toned down the discussion, as none of the individual selection estimates were statistically significant.

Below, you will find our point-by-point answers to all the reviewers’ comments. All changes and new additions are marked in red font in the new version of the manuscript. We hope that our changes are sufficient to make this manuscript suitable for recommendation; otherwise we are more than willing to further improve the ms.

Thank you for your attention to this manuscript. If we can provide any further information regarding the submission, then please do not hesitate to enquire.

Sincerely yours,

Juan Diego Gaitan-Espitia
Postdoctoral Fellow
Oceans and Atmosphere Division
CSIRO - Australia

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Dries Bonte

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.

R: Many thanks for your positive feedback. We hope you like this new version as well.

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.

R: Point taken. We have re-done all the analyses including the null model (see the table below). Overall, although the null model sucks up some of the variance, you can see that results do not change that much in term of the explained variance by the remaining models (the last column "Previous" shows the Akaike weight of those same models in the previous version of the ms) and that including the null model did not change at all the order of the best models. Furthermore, survival estimates also do not substantially change (results not shown) which in agreement with the results reported in the previous version still suggest that selection is rather weak in this system. Accordingly, we modified Methods (line 195) and Results (lines 225 – 230) to include this information. In addition, we included a new table with individual estimates, their SE and 95% CI. As SEs were rather large, the CI always included 0. Therefore, as a result of this, we decided to tone down even more some aspects of the discussion.

Models		K	AICc	ΔAICc	w_i	**Previous**
1	Null model (y ~1)	2	130.17	0	0.220	
2	CT_{Min_10}	3	131.40	1.23	0.119	0.153
3	MB	3	131.78	1.61	0.098	0.126
4	T_{Pref_20}	3	132.08	1.90	0.085	0.109
5	Q_{10_10}	3	132.18	2.01	0.081	0.103
6	CT_{Min_20}	3	132.25	2.08	0.078	0.100
7	CT_{Max_10}	3	132.26	2.08	0.078	0.099
8	Q_{10_20}	3	132.26	2.09	0.077	0.099

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9	$CT_{Min\_10} + CT_{Min\_20} + CT_{Min\_10} * CT_{Min\_20}$	5	133.38	3.21	0.044	0.057
10	$MB + CT_{Max\_20}$	4	133.44	3.27	0.043	0.055
11	$MB + T_{Pref\_10}$	4	133.82	3.64	0.036	0.046
12	$Q_{10\_10} + Q_{10\_20} + Q_{10\_10} * Q_{10\_20}$	5	134.17	4.00	0.030	0.038
13	$MB + T_{Pref\_10} + T_{Pref\_20} + T_{Pref\_10} * T_{Pref\_20}$	6	137.16	6.99	0.007	0.009
14	$MB + CT_{Max\_10} + CT_{Max\_20} + CT_{Max\_10} * CT_{Max\_20}$	6	137.62	7.45	0.005	0.007

Some minor points.
last sentence abstract. Something is wrong here as higher tolerance cannot be selected for and selected against.
R: Many thanks. We re-phrased completely the last part of the abstract (lines 36 – 44).

The selection on body size: why would larger frogs experience less heron predation?
R: In the text we mention that survival increases with body size. We think this might be a possibility as there is evidence in several other systems that suggest that survival increases with body mass as this trait positively covaries with several performance ones. We do not know for sure whether bigger frogs are less depredated and highlight that evaluating this possibility would be something that requires further studies (lines 299 – 303).

Nadia Aubin-Horth
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.
R: Many thanks for this positive feedback. In this new version we addressed all the minor concerns you mentioned.

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 (?).
R: Many thanks. We re-phrased completely the last part of the abstract (lines 36 – 44).

-In the discussion on line 242-241, 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.
R: Many thanks. We have now toned down several parts of the discussion (lines 242 – 247; 268 – 270; 275 – 279; 290 – 292; 293 – 294).

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!
R: Many thanks for your positive feedback. We hope you like this new version as well.

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.
R: Thanks for highlighting this. Actually we were considering THE extreme case where selection in the past has eroded ALL variation, including phenotypic, which in this case would mean that there is no directional selection on plasticity. We realized this was not the best prediction to be made and we changed it in this new version (lines 104 – 105).

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.
R: We have now included a new table with individual estimates, their SEs and 95% CI. As neither estimate was statistically different from 0, we significantly toned down our discussion. Nevertheless, we decided to keep the figure 4.

Minor comments:
P3 L132f: transpose order of two side sentences starting with "favoring organisms…" and "despite the ability…". **R: Done**

P4, L150: Start new paragraph here. **R: Done**

P4, L153/154: As mentioned above, this does NOT follow. **R: Rephrased.**

P5, L164: Again, this does not follow: Q10 might not be changing, but this doesn't mean that it is not under selection **R: We deleted part of the sentence to make it clear.**

We were not able to completely find the comments below as the line and page numbers do not coincide. Nevertheless, we believe all comments suggest that the individual estimates are not significant and therefore, that the discussion should be toned down accordingly (lines 242 – 247; 268 – 270; 275 – 279; 290 – 292; 293 – 294).

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.

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