September 28th, 2022

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Editors
PCI Evolutionary Biology

Thank you for the opportunity to submit a new revised version of our manuscript entitled "***Evolutionary responses of energy metabolism, development, and reproduction to artificial selection for increasing heat tolerance in Drosophila subobscura.***". Below you will find a detailed list corrections that we have taken to improve the manuscript V3 following the suggestions from the reviewer.

We hope the manuscript will now clarify all of their comments. Each comment is in Calabri bold followed by our reply in italic text.

Sincerely,
Andrés Mesas and Luis Castañeda

Editors comments
We thank the authors for the detailed revision of the first version of the paper.
The reviewer has some few more comments that should be addressed, but we are happy to write a recommendation once the authors incorporate the final suggestions from the anonymous reviewer.
by Inês Fragata and Pedro Simões, 26 Sep 2022 15:55

Manuscript: https://www.biorxiv.org/content/10.1101/2022.02.03.479001v2
version: https://doi.org/10.1101/2022.02.03.479001

Review by anonymous reviewer, 10 Sep 2022 08:27
The authors have done a good job of editing, revising and placing their work in a much broader context. Thank you. I have read the revised manuscript here and have made some suggestions that are not exhaustive. Please check them and revise them accordingly. The line numbers mentioned here are for the v2 document:

L29- Drosophila subobscura needs to be *'Drosophila subobscura*'
We modified this point

L114- delete 'associated'
Removed.

L149- in a vial 'with'
Added it

L158- ends='ended'
We modified this point

L167-select='selected'
We modified this point

L170-171- Logistic to 'logistical'
We modified this point

L174- individuals='individual'
We modified this point

L214-215: The OD to 340nm was measured; re-word. The meaning is not very clear.
This section was rewritten "Immediately, changes in optical density (OD) at a wavelength of 340 nm was measured with multiple reads per well"

L217-218: triplicated=triplicate?
We modified this point

L227- change: counted daily instead of daily counted
We modified this point

L229- change: better to call accumulated fecundity as total fecundity? I will leave the decision to you? One reads better than the other and makes more biological sense.
RESPONSE:
We understand its point, but we still think that the term "accumulated fecundity" highlights that fecundity was measured every 24 hours and that it was added to the fertility of the next day, allowing us to evaluate the interaction of the egg oviposition through the days of experiment. On the other hand, the term "total fecundity" could be associated with fertility at the end of the oviposition activity on the eighth day or partial totals to each day.

L340- change- is a conspicuous environmental to important abiotic variable that....
We modified this point

L345- change to- However the evolution of heat tolerance was not associated...
We modified this point

L351-353- delete ". While several" and change to "cold habitats (REFS....) and other studies have found....."
We modified this point

L354- delete on the other hand, and change to- "In line with our findings reported here,"
We modified this point

L358-359 - which would difficult the detection of change to: would be difficult to detect metabolic changes under.....
We modified this point

L360- change to- A possible explanation could be that, our control and.....
We modified this point

L361- change: which could hide to "which could override correlated responses"
We modified this point

L367- change:organismic=organismal?
We modified this point

L369- change:selection acts on enzymes= selection can act....?
We modified this point

L377- agree with= is concordant
We modified this point

L377- delete the second 'that' and add 'D. simulans populations evolving...' after found... so it should read "a study that found D. simulans populations evolving.....";
This section was rewritten "…with a study that found D. simulans populations evolving in thermally fluctuating environments, with a reduction in the gene expression…"

L378- delete "of D. simulans"
We removed this point

L379- participation = involved in?
We modified this point

L382- withstand = survive the?
We modified this point

L388- delete both the 'a's in the sentence: 'a' fast 'a' slow
We removed this point

L389- delete ' , but' so the sentence should read "metabolism at the cost...."
We removed this point

L392-393- A bit confused here: "the higher fecundity might be linked to their physiology as heat can drive higher offspring production, but it does not mean that greater numbers = higher quality? So, greater fecundity might not be better quality per se? Like you hint in lines

396-397 that fast-ramping individuals did not differ in egg to adult survival from the controls?" Can you clarify this or I could have mis-understood something!

RESPONSE:

We propose that the pace-to-life syndrome could explain the evolution of higher heat tolerance, associated with a reduction in enzyme activity and increased reproductive responses, which were found in our experiment. In short and applied to our work, this syndrome proposes that individuals with lower metabolism should show an increase in reproductive traits (see in detail in the text, lines 386-390). This response was found in the slow-ramping selected lines, which show that the evolution of thermal tolerance was associated with a reduction in the activity of the HEX enzyme and an increase in fertility and viability from egg to adult. In contrast, in the fast-ramping selected lines an increase in fertility was found that cannot be attributed to any change in the activity of the enzymes evaluated in this work and, in addition, a higher fertility was not associated with an increase in egg viability to adult. This does not mean that viability will not be increased by a reduction in egg quality, but that this trait was not statistically differentiated from the unselected control lines (possibly by the greater dispersion of the data). Possibly, the responses associated with the evolution of a high tolerance to heat by fast heatings are different from those observed in slow-ramping selected lines, and that were not evaluated in this work.

L393- delete we can observe; change to: observing;

We modified this point

L394- change: this difference was not significantly different to 'was not statistically significant'

We modified this point

L402- has=had

We modified this point

L404-406- ', we have..... (Castaneda et al. 2019)' "you say several works; but only one study is cited. Also, upper limit of thermal limit for other traits like fertility does not evolve in other studies (for e.g., van Heerwarden et al. 2021). Do you mean upper thermal limits for tolerance? or survival? (CT max)?" This final point has to be precise!

This section was rewritten "Despite some evidence showing that upper thermal limit (CTmax) had limited evolutionary potential (Kellermann et al. 2012; Kelly et al. 2012), our research group has found that CTmax of D. subobscura populations have enough genetic variation (Castañeda et al. 2019), to adapt to local conditions (Castañeda et al. 2015) and respond to artificial selection (Mesas et al. 2021; but see Santos et al. 2022 for no evolutionary response to warm temperatures)."