

The manuscript entitled “Correlated responses of energy metabolism, development and reproduction to evolution for increasing heat tolerant in *Drosophila subobscura*” was designed to evaluate the effects of different heat intensity selection regimens (using slow- and fast- ramping protocols) on metabolic rate, activities of four chosen enzymes of G6P branch included in metabolic pathway and life-history traits – fecundity and egg-to-adult viability. The authors hypothesized, according to literature data, that in conditions of high temperature environmental stress traits will evolve in direction of heat tolerance over metabolic depression and increase of some fitness traits, in order to allocate energy necessary for population adaptation. Metabolic rate was not decreased in selected experimental lines, but activity of two enzymes was decreased in slow-ramping in comparison to control (HEX) or fast-ramping selection in comparison to slow-ramping selection (G6PD), only in non-stressed conditions. Fertility was increased in both selected lines, compared to control lines, and viability was increased in fast-ramping selected compared to slow-ramping lines. This research contributes significantly in understanding of the population responses to temperature-changing environment, indicating complexity of adaptation responses as long-term and short-term environmental conditions can make it difficult to distinguish evolutionary and plastic responses. Generally, manuscript is well written and organized, some suggestions and corrections are listed below.

Title

Consider the title change, it is not necessary, as “correlated responses” implies an statistical correlation between traits. Maybe to exclude “correlated”. If authors accept this change, they should correct in the manuscript.

Towards this, in the manuscript is sometimes confusing terminology – correlated responses, evolutionary responses, positive responses....

Introduction

Raw 89: Reference is already cited at the beginning of the sentence.

Methodology

Heat knockdown temperature selection

Raws 139-141: As there were 4 experimental groups and each group was done in triplicate, I recommend to better explain this step in the experimental procedure. At the first glance it is confusing. In the paper from 2021 is more understandable.

Raws 143-144: Does this mean that female originated from one IF lines, and males from other IF lines? How it was done as females and males from different IFL were mixed to gain experimental lines 6 generations before the selection?

Raw 145: These 120 females are chosen from former 160 females?

Raws 155-157: These groups are also treated every generation but flies are randomly chosen? You choose same number of flies as in selected experimental groups for next generation?

Raws 160-161: I suppose that logistic reason is justified, but authors should comment in Discussion if this choice of control line could influence the results of statistical analyses. The influences of short-term exposures can be visible in next generations with no intensive selection pressures.

Early fecundity and egg-to-adult viability

Raws 213-214: Same comment as previous - Does this mean that female originated from one IF lines, and males from other IF lines?

Raw 218: “by five days-old females (age of maximal activity of oviposition in *D. subobscura*)”
Is this data from some reference? Authors should add it.

Results

The statistics for knockdown temperature analysis is not listed in the Material and methods.

RMR and body mass

Raw 266: Add Fig 1. after the number 0.79

Raw 267: Add Fig1. after the number 0.0007

Or maybe instead of these suggestions, add “The results are presented in Figure 1” after last sentence of this part of the Results.

Enzyme activity

In Figure 2 (page 33) results for each analyzed enzyme are not labeled as A, B, C and D as it is specified in this part of the Results, and in the legend of Figure 2.

Rows 276, 280, 282, 286: instead of Fig 1A, 1B, 1C and 1D should write Fig 2A, 2B, 2C and 2D

Raw 278: “whereas fast-ramping selected lines showed similar HEX basal activity than control lines” – did the authors mean “as control lines” instead of “than control lines”

Early fecundity and egg-to-adult viability

Are there any differences by day in fecundity between two selected regimes? It should be mentioned...

Raw 301: (Fig. 3A) – “A” should be deleted as in this figure there are no parts A, B...

Raw 305: (Fig. A) – it should write Fig. 4

Raw 306: delete comma and Fig.4, it is already written at the beginning of the sentence

Discussion

Raws 321-322: “we detected evolutionary correlated responses in a specific enzyme related to energy metabolism and positive correlated responses of fitness-related traits”

This sentence must be reorganized, as I suggested - it is confusing with correlation terms without statistics, sometimes authors mention positive correlation, sometimes evolutionary correlated responses...

Raws 341-342: delete Alton et al (2017) – instead: “they”

Raw 354: add “the study on” the moth...

Raws 368: Did the authors mean the same enzyme (not enzymes) – did they mean HEX enzyme?

At the end of the Discussion, the authors consider the local adaptations, temporal and spatial variability in understanding of adaptive response to ongoing and future climate change. Maybe they include in Discussion some papers with experiments on populations of different altitude or origin, and different temperature selection regimens, especially for the species *D.subobscura*. Also the paper below can be included in order to explain results of enzyme activities:

JM Flowers, E Sezgin, S Kumagai, DD Duvernell, LM Matzkin, PS Schmidt, WF Eanes, Adaptive Evolution of Metabolic Pathways in *Drosophila*, *Molecular Biology and Evolution*, Volume 24, Issue 6, June 2007, Pages 1347–1354, <https://doi.org/10.1093/molbev/msm057>