ELECTRONIC SUPPLEMENTARY MATERIAL 4

From article: Camacho C, Sanabria-Fernández A, Baños-Villalba A, Edelaar, P. 2020. Experimental evidence that matching habitat choice drives local adaptation in a wild population. *Proc. R. Soc. B.* DOI: http://dx.doi.org/10.1098/rspb.2020.0721

Effect of time of day and thermal conditions on microhabitat use by grasshoppers

Darker grasshoppers might be more likely than pale ones to use lighter, cooler microsites during the midday hours and/or under very warm conditions to reduce the risk of overheating due to solar radiation (Ahnesjö & Forsman 2006; Wennersten et al. 2012). Grasshopper colour could therefore influence habitat choice. However, even if these effects of time of day or weather on microhabitat use do exist, they will act in the direction opposite to the effect of crypsis: pale individuals would more often prefer dark asphalt (when it is cold), and dark individuals would more often prefer pale substrates (when it is hot).

To examine the potential influence of time of day and temperature conditions on microhabitat use, we ran a GLMM (binomial error structure and logit link function) using first-captured individuals and the R-package 'lmerTest' (Kuznetsova et al. 2016). We used substrate type (0 = pale pavement, 1 = dark asphalt) as the response variable and the original colour of individuals (log-transformed) as a continuous predictor. Time of day (to the nearest min), temperature conditions (on a semi-quantitative scale: 1 = cool, 2 = mild, 3 = warm, 4 = hot), and the 2-way interactions between these two variables were also included in the model as fixed effects. To achieve more comparable estimates, we z-transformed all predictors to a mean of zero and a standard deviation of one (Schielzeth 2010). Since the afternoon is the hottest time of the day, any effect of time of day would be reversed between morning and evening, requiring the modelling of additional interactions. However, we had relatively little data for the evening period. For model convergence reasons, therefore, only captures made during morning sessions (9-14 h, N = 172 captures) are considered in the exploratory GLMM. Sampling dates were grouped into 3-day periods and then included in the model as a random factor to account for the potential non-independence of observations made within the same period.

Our results showed that temperature conditions or time of day alone did not influence the probability of using one substrate type or the other, nor did any of these factors predict microhabitat use when involved in an interaction with grasshopper colour (Table SM1). However, the effect of grasshopper colour did remain significant in this smaller dataset (Table SM1). Therefore, we are confident that the effects of temperature conditions or time of day

are not alternative explanations for the observed correlations between colour and substrate use in both manipulated and non-manipulated individuals.

Table S1. Results of the GLMM analysing the effects of original colour, time of day, temperature conditions, and their 2-way interactions on the type of substrate chosen (0 = pale pavement, 1 = dark asphalt) in a the subset of unmanipulated individuals captured between 9:00 and 14:00 h.

Variable	ΔAICc	P-value
Original colour	**-32.08**	**<0.001**
Time	3.06	0.531
Temperature	3.27	0.590
Original colour × Time	2.15	0.887
Original colour × Temperature	1.79	0.536

Changes in AICc values and P-values were obtained by comparing models with versus without the variable of interest. Number of observations = 172. Number of groups for random variables: Date = 11.

References

Ahnesjö, J. & Forsman, A. 2006. Differential habitat selection by pygmy grasshopper color morphs; interactive effects of temperature and predator avoidance. Evolutionary Ecology, 20: 235–257.

Wennersten, L., Karpestam, E., & Forsman, A. 2012. Phenotype manipulation influences microhabitat choice in pygmy grasshoppers. Current Zoology, 58: 392−400.

Kuznetsova, A., Brockhoff, P. B., Christensen, R. H. B. 2016. lmerTest: Tests in linear mixed effects models. R package. https://cran.r-project.org/web/packages/lmerTest/index.html

Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients. Methods in Ecology & Evolution, 1: 103–113.