Title: Long-term dynamics of adaptation to a warming environment is dependent on historical background

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Abstract

Climate change is leading to biodiversity decline at an unprecedented pace. Evolutionary responses may be crucial for organisms' ability to cope with prolonged effects of climate change. It is thus fundamental to understand the dynamics of adaptation to warming environments in order to predict and mitigate the impacts of climate change on biodiversity. In particular, addressing how reproductive success evolves in deteriorating environments is extremely relevant, as this trait is more likely constrained at lower temperatures than upper physiological thermal limits. Experimental evolution udner a warming environment can illucidate the potential of populations to respond to rapid environmental changes. The few studies following such framework lack analysis of long-term response. We here focus on the long-term adaptive response of Drosophila subobscura populations evolving under a warming environemnt, analysing the reproductive success after 39 and 52 generations of thermal evolution. We found that long-term adaptation to higher temperatures can occur but the pace of such response is slow and likely dependent on low rates of environmental change. Furthermore, we observed that the magnitude of the response is relatively mild. In addition, the evolutionary dynamics differs between populations of distinct geographical origin, with the higher latitude populations only showing an adaptive response to the warming environment in a more advanced generation. This study reinforces the need for long-term evolution experiments to fully reveal the potential for thermal response. It also highlight that the scrutiny of several populations in this context is needed for a measure of variation within a species. Accounting for these sources of variation - both temporal and spatial - will allow for more robust assessments of climate change responses.

Keywords: Thermal adaptation, Experimental evolution, Long-term evolution, Climate change, Fertility, Drosophila

Introduction

Climate change is promoting biodiversity decline at an unprecedented pace and is likely to continue to do so in the future (IPCC 2023). Evolutionary responses may play an important role in enhancing organisms' ability to cope with prolonged effects of climate change (Urban et al. 2016). It is crucial to understand the dynamics of adaptation to warming environments to predict impacts of climate change. In particular, addressing how reproductive success evolves in deteriorating environments is extremely relevant, as it has been shown that this trait is more likely constrained at lower temperatures than physiological impairment(Walsh et al. 2019a,b; Parratt et al. 2021; van Heerwaarden and Sgrò 2021). In fact, fertility patterns have been shown to track species' geographical distribution in nature, highlighting its role as a predictor of biodiversity changes due to climate change (Parratt et al. 2021; van Heerwaarden and Sgrò 2021).

The possibility of an adaptive response to a deteriorating environment is shaped by two main factors: the standing genetic variation within populations and the rate of environmental change (Bell 2017). When genetic variation for fitness-related traits is low and/or evolutionary constraints between traits occur, the speed of the adaptive response will be particularly limited. In that scenario, adaptive evolution may be only evident (if present at all) after prolonged imposition of the new environmental challenges and/or when the rate of environment change declines or ceases (Hoffmann and Sgró 2011; Lindsey et al. 2013; Bell 2017).

Experimental evolution is a powerful tool to deepen our understanding about the tempo and mode of thermal evolution, as it allows to follow the real-time evolution of populations under relevant thermal settings (Kawecki et al. 2012; Rogell et al. 2014; Kellermann and van Heerwaarden 2019; Santos et al. 2021b; van Heerwaarden and Sgrò 2021). Furthermore, it allows to directly test the role of specific environmental factors, e.g. such as temperature, while confounding environmental effects are minimized relative to field studies. Some studies in ectotherms (mostly

Drosophila) have focused on evolutionary responses of reproductive traits to experimental warming but most have covered a limited temporal range – around 20 generations or less (Schou et al. 2014; Kinzner et al. 2019; van Heerwaarden and Sgrò 2021).

In general, these studies have found limited evolutionary response to warming in reproductive traits. In an exceptionally longer-term experimental study which spanned 41 generations, Rogell et al. (2014) found an increased reproductive performance in response to a fluctuating and increasingly warmer environment, with populations of seed beetle Callosobruchus maculatus evolving under higher temperatures presenting increased offspring production relative to their controls. Importantly, this effect on fecundity was only observed after the incremental setting (of 0.3ºC per generation) was halted upon 18 generations of selection (Rogell et al. 2014) and not immediately after the increment (Hallsson and Björklund 2012). These experiments suggest that the tempo of adaptive change to new thermal conditions is likely low – possibly due to limited genetic variation for reproductive traits - and that a rapid rate of environmental change poses serious limits to that response. In addition, reductions in genetic variation might result from prolonged evolution under stressful conditions due to the continuous effects of genetic drift when effective population size is rather low and/or there are population crashes causing bottlenecks. Nevertheless, it is possible - though not very likely - that a reshaping of the genetic architecture following such bottlenecks contributes to the release of genetic variation and increase of evolutionary potential (Bryant and Meffert 1993). Considering these different sources of genetic variation, long-term responses to incremental temperatures may be highly variable between populations and species. One caveat of the thermal experimental evolution studies reported above is that they neglect effects of variation at the inter-population level - namely by studying different geographical populations of a species - and how it affects the adaptive response.

Importantly, these real-time evolution studies of adaptation have also the potential to test for costs of adaptation to the warming conditions, that is whether adaptation to a specific thermal environment promotes a reduced performance in the ancestral (thermal) environment, for instance,

due to the expression of genes with antagonistic pleiotropy (e.g. (Kawecki and Ebert 2004). However, detection of costs of adaptation in general has been evasive possibly because these are seldom strong (Hereford 2009).

Drosophila subobscura is a species that shows evolutionary responses to climatic factors with repeatable clines of body size (Huey et al. 2000; Gilchrist et al. 2004) and chromosomal inversions (Prevosti et al. 1988) that track global warming (Balanyá et al. 2006; Rezende et al. 2010). As expected, this species has also been found to respond plastically to thermal challenges (Fragata et al. 2016; Simões et al. 2020; Santos et al. 2021a). Our team has been studying how populations of this species evolve under an increasingly warmer environment (Santos et al. 2021c). We have been focusing on populations from northern (high latitude) and southern (low latitude) European locations to address the relevance of different historical backgrounds during thermal evolution (Santos et al. 2021c). The thermal setting in the warming regime was incremental (0.2ºC per generation) until generation 24 and then the thermal environment was kept constant across generations until the last assay, with a daily fluctuating regime spanning both high and low temperatures (with daily peaks of respectively 11ºC and 5ºC above and below 18ºC, the control conditions). We previously found that the evolutionary response of populations to warming conditions was slow and specific to the history of the populations. In fact, thermal adaptation was only observed in the populations of low latitude after 39 generations, but not after 22 generations in a warming environment (Santos et al. 2023b). We also found minor evidence supporting costs for this thermal adaptation (Santos et al. 2023b). These findings highlight the importance of a better understanding of the evolutionary dynamics of thermal responses in different populations. It is an open question whether longer-term evolution would allow for an adaptive response also in the higher latitude populations.

We here focus on the long-term adaptive response of our warming-evolved populations by analysing their reproductive success after more than 50 generations of thermal evolution. With this analysis we also aim to test the repeatability of patterns relative to those observed at generation 39 (Santos et al. 2023b). Coupling data from two long-term assays (generations 39 and 52) we expect to

find a more robust adaptive response. Specifically, we expect that the reduction in the rate of environmental change - with the warming regime being kept constant for more than 25 generations by the time the last experiment was conducted - will have likely enabled a general adaptive response in both high and low latitude populations. Specifically, we expect to observe a previously undetected selective response in the high latitude populations, provided sufficient genetic variation is present. It is possible that evolutionary constraints such as trade-offs with other traits and costs of adaptation emerge during this longer-term response, contributing to a mild magnitude of evolutionary change.

Material and methods

Population maintenance and thermal selection regimes

The experimental populations used in this study were obtained from two collections of natural populations of Drosophila subobscura, done in August/September 2013 in Adraga, Portugal (38º48' N) and Groningen, The Netherlands (53º13' N). 213 founder females were obtained in Adraga and 170 in Groningen (Simões et al. 2017). These collections gave rise to the PT and NL laboratorial populations. These populations were maintained in 30 cm3 glass vials (in general 24 vials for both the adult and developmental stages) with discrete generations of 28 days, 12L:12D photoperiod with constant 18ºC temperature, controlled densities in eggs (70 eggs per vial) and adults (40 adults per vial), and reproduction close to peak fecundity (seven to ten days-old flies). By generation four, these populations were three-fold replicated, leading to PT1-3 and NL1-3 populations. After 70 generations, a warming regime was created from each population (Warming, WNL1-3 and WPT1-3 populations). This regime had a daily fluctuation cycle that ranged from 15ºC and 21ºC and increased its daily mean temperature (0.18ºC) and amplitude (0.54ºC, with a lower minimal and higher maximal daily temperature) every generation. All experimental populations were subjected to the maintenance protocol described above, only differing in the thermal regime imposed. Evolution in the warming regime led to a decoupling in generation time – from 28 days to 24 days by the time of the experiment - given the faster developmental time of populations evolving under this thermal regime. PT and NL

138 populations, representing the ancestral state of the WPT and WNL populations, were used as control
139 populations in the experiment.

140 By generation 22 the peak temperature in the warming regime reached 30.2ºC. This
141 originated a significant crash in adult census sizes due to high juvenile mortality for all warming
142 populations in generations 22 and 24 (average sizes of 200 and 250 respectively). As a result of this,
143 we reversed the warming cycle to the cycle of generation 20 (Santos et al. 2023b). This cycle, with a
144 mean temperature of 21.4ºC and a fluctuation between 13.5ºC to 29.4ºC, was then maintained till
145 the end of the study and was the warming environment applied in phenotypic assays (see details
146 below). Apart from the population crashes referred above that led to the halt in the thermal cycle
147 (with census sizes below 250 individuals), very low population sensus sizes were also observed by
148 generation 44 that motivated a two-generation transfer of the warming populations to control
149 conditions to allow for population recovery. Apart from these previously referred generations,
150 populations sizes were generally high throughout the study (between 600-1000 individuals) – see
151 Table S1 with sample sizes across generations.

152

153 *Experimental assays*

154

155 This study includes data from the reproductive success of warming populations analysed at
156 generations 39 and 52 of thermal adaptation. Data from the assay performed at generation 39 was
157 already published elsewhere (Santos et al. 2023b). In both assays, an orthogonal design was applied
158 with Warming and Control populations being tested in each (Warming and Control) environment.

159 The assay of generation 39 involved twenty pairs of flies per replicate population and
160 environment. In total, 480 pairs of flies – placed in individual glass vials - were studied in this assay
161 (20 pairs × 3 replicate populations × 2 selection regimes × 2 historically differentiated populations ×
162 2 assay environments) – see also (Santos et al. 2023b). At generation 52, an additional assay involved

sixteen pairs of females and males per replicate population and environment, in a total of 384 pairs of flies (16 pairs × 3 replicate populations × 2 selection regimes × 2 historically differentiated populations × 2 assay environments). In both assays, we estimated reproductive success as the total number of offspring derived from eggs laid by each mating pair at day 9 of adult age (a 24-hour laying period), that emerged during a 10-day period starting from the first day of emergence. The ninth day of adult life was chosen as it is within the interval of the age of individuals contributing to the next generation during our population maintenance system (~ 6 to 10 days of age). Maternal environmental effects were minimized by maintaining all assayed populations for one full generation in the control environment prior to the assay.

Statistical methods

Individual data from each mating pair was used as raw data in the analyses. These included Linear mixed effects models, defining a "sum to zero" contrast option for each factor. Generalized linear mixed-effects models (GLMM) were applied on the whole dataset and tested with different distributions - poisson, quasipoisson, and negative binomial. The best overall model, based on the lowest values of Akaike information criterion (AIC), was the one assuming a quasipoisson distribution accounting for zero inflation. This distribution was used in all analyses. Significance levels were obtained by applying Type III Wald chisquare tests.

We applied two overall models to the data of assays from generation 39 and 52, that varied in the random factor defined (interactions with random factors are not presented but were included):

$$(1)\quad Y = \mu + History + Selection + Env + Generation + AP\{History\} + History \times Selection + History \times Env + History \times Generation + Selection \times Env + Selection \times Generation + Env \times Generation + History \times Selection \times Env + History \times Selection \times Generation + History \times Env \times Generation + Selection \times Env \times Generation + History \times Selection \times Env \times Generation + \varepsilon$$

(2) $Y = \mu + History + Selection + Env + Generation + Block + History \times Selection + History \times Env + History \times Generation + Selection \times Env + Selection \times Generation + Env \times Generation + History \times Selection \times Env + History \times Selection \times Generation + History \times Env \times Generation + Selection \times Env \times Generation + History \times Selection \times Env \times Generation + \varepsilon$

In model (1), AP{History} is a random factor, that represents the Ancestral population nested in the fixed factor History (PT low latitude vs NL high latitude) (e.g. NL1 is the ancestral population of NL1 and WNL1, nested in NL origin, etc.). Block is a random effect in model (2), being the set of same-numbered replicate populations assayed in the same pseudo-randomized experimental rack. Y is the trait under study, reproductive success. Selection is a fixed factor corresponding to the two selection regimes (Warming and Control), Env is the fixed factor Environment, with two categories (Warming and Control Environments), and Generation is the fixed factor corresponding to the two generations assayed (39 and 52). All other terms in the model represent the interactions between the fixed factors. Model (2) – block as random factor - was chosen based on lower AIC values. Additional models were applied to each latitudinal population separately and to each generation separately.

All analyses and figures were done in R v4.3.3, with glmmTMB, car and ggplot2 packages (Wickham 2016; Brooks et al. 2017; Fox and Weisberg 2019).

Results

To assess long-term adaptation in our warming populations we performed an overall analysis including both assays, done at generations 39 and 52. Significant effects were observed for the terms Environment and Selection (Table S2). Differences in selection corresponded to a general better performance of the warming populations relative to the controls (with the exception of the patterns for the low latitude populations in the control environment, see below). Differences observed between environments corresponded to a general decline when populations where assayed in the warming environment (see Figure 1). Importantly, a significant Selection × Environment interaction was observed (Table S2), indicating that differences between selection regimes changed across environments, with the warming populations showing a better performance than controls in that environment but less so in the control environment. This was particularly evident in the case of the low latitude populations where the sign of differences between populations reverses between environments (see Figure 1). Importantly, although we found general differences between generations (significant factor Generation), we did not find any significant interactions of other fixed factors with generation, indicating that the differences between populations of different selective regimes across environments that were mentioned above were maintained across generations (e.g. non significant Selection × Environment × Generation interaction see Table S2).

Analyses were performed to separately assess the performance of populations with different historical origin. In both cases, as expected there was a significant Environment effect (see Table 1). For the low latitude populations (but not for the high latitude ones) we also observed a significant effect of generation as well as a significant interaction between selection and environment (see Table 1 and Figure 1). As referred above, this interaction resulted from a better performance of the warming populations of southern origin relative to their controls in the warming environment, while the reverse pattern occurred in the control environment, particularly at generation 39 (see Figure 1). As for the high latitude populations, we also found significant differences between generations (significant factor Generation) and a marginally significant effect of selection and of the interactions between selection and generation and between environment and generation factors (Selection x

232 Generation and Environment x Generation, see Table 1). The interaction between selection and
233 environment was not significant for these populations.

234 Given the marginal interaction between selection and generation in the high latitude
235 populations we further analysed data from each generation separately to search for possible
236 discrepant patterns between generations. Besides the expected effect of environment, we did not
237 find any effect of selection or interaction between selection and the environment by generation 39
238 in the high latitude populations (see Table S3). On the other hand, by generation 52 a significant effect
239 of selection and of the interaction between selection and environment was observed (see Table S3).
240 This interaction corresponds to a higher difference in performance between warming and control
241 populations in the warming environment, although performance was consistently higher in the
242 warming populations in both environments (see Figure 1). Applying a similar analysis to the low
243 latitude populations, selection was not significant in either generation, while the interaction between
244 selection and environment was statistically significant at generation 39 but not at generation 52 (see
245 Table S3).

246 Discussion

247 Here we provide evidence for long-term adaptation to the warming conditions in Drosophila
248 subobscura populations evolving in a warming environment. In fact, we found that those populations
249 showed an increased reproductive success than the controls when tested in their own (warming)
250 environment while differences were reduced in the ancestral (control) environment. Importantly, the
251 dynamics of the response was different between populations with distinct geographical origins, being
252 faster in the southern – lowe latitude - ones (see below). Our findings point to the existence of
253 sufficient standing genetic variation to allow for an adaptive response in our populations despite the
254 occurrence of sporadic bottlenecks associated with thermal stress during development in particular
255 generations. It might also be the case that some of these episodes of high stress have opened the
256 opportunity for interactions between drift and selection (Cohan 1986; Cohan and Hoffmann 1989)
257 and/or reshuffling of the genetic architecture with increased additive genetic variation following

258 bottlenecks (Bryant and Meffert 1993) that may allow for further evolutionary potential and
259 subsequent responses. In any case, the slow response suggests that levels of genetic variability are
260 not high in our populations. Apart from the expected low heritability in traits closely related to fitness
261 (Mousseau and Roff 1987), reduced genetic variation may also result from lower effective population
262 sizes in some generations as a consequence of repeated episodes of high selection during
263 development. In addition, high environmental effects on the phenotype also result in low
264 heritabilities.

265 It is important to note that the adaptive responses we observe were only obtained after the
266 thermal increase per generation in the warming cycle was halted by generation 24 (Santos et al.
267 2021c, 2023a,b). This finding is in agreement with those of another evolution experiment addressing
268 adaptation to incremental warming in seed beetles (Hallsson and Björklund 2012; Rogell et al. 2014).
269 These results reflect the importance of the rate of environmental change in allowing for evolutionary
270 rescue to occur (Bell 2017). This has been clearly demonstrated in experimental studies in bacteria,
271 with more adaptive responses observed under slower environmental change (Liukkonen et al. 2021)
272 and particular genotypes not being evolutionarily accessible under rapid environmental change
273 (Lindsey et al. 2013). Experimental studies in ectotherms addressing short term evolution under
274 continuously incremental temperatures have shown a lack of adaptive response (Schou et al. 2014;
275 Kinzner et al. 2019; van Heerwaarden and Sgrò 2021). We here show that adaptive responses can
276 arise after prolonged evolution in a thermally fluctuating environment but probably not when
277 populations face a fast pace of environmental perturbation. Consequently, this raises concerns on
278 the ability of populations to respond to sudden thermal shifts and in particular to sustained high rates
279 of environmental change.

280 Our findings reveal differences in the evolutionary dynamics of populations of different
281 geographical origin. In fact, the high latitude populations showed a more tardy response to thermal
282 selection, being only evident after more than 50 generations of thermal evolution, contrary to the
283 response already obtained for the low latitude populations by generation 39 (Santos et al. 2023b).

Apart from the tempo of the response, it is also important to note that the patterns observed in each geographical population were also somewhat different, with stronger evidence suggesting that the relative performance of thermal selection regimes varied between assay environments in the low latitude populations but less so in the high latitude ones. Specifically, part of this variation between geographical populations is associated with performance in the control (ancestral) environment where the low latitude populations show some indication of performing below the controls particularly at generation 39, which was not the case for the high latitude populations. This pattern is suggestive of costs of adaptation in the low latitude populations (see (Santos et al. 2023b). Nevertheless, by generation 52 such differences in the control environment are no longer conspicuous, suggesting that such costs of adaptation were not particularly relevant. This is not a surprising finding considering other literature (see (Hereford 2009; Bono et al. 2017) for a review). It might be the case that evolution under fluctuating (heterogeneous) environments, as we use here, is less likely to allow for measurable costs of adaptation than homogeneous environments since in the latter case organisms are not exposed to different environmental conditions and consequently selection will not act to adjust/increase performance in a wider range of environments (Bono et al. 2017). Furthermore, our finding of an adaptive response with no clear costs involved suggests a possible advantage in future climatic scenarios where warmer seasons are projected to be warmer (IPCC 2023), while populations will need to keep their ability to cope with lower temperatures during colder seasons. However, this is most likely not enough to ensure population persistence, given the pace of the evolutionary response of the populations under study.

In summary, we found that long-term adaptation to higher temperatures can occur in D. subobscura but the pace of such response is slow and likely dependent on low rates of environmental change. Thus, this finding supports the evidence that ectotherms may have limited capability to respond evolutionarily to temperature shifts (Kellermann and van Heerwaarden 2019) and may struggle to show an adaptive response to a fast-paced global warming. In addition, we observed that such adaptive response is not particularly strong. The long-term evolutionary dynamics differs between populations of distinct geographical origin, with the higher latitude populations only

showing an adaptive response to the warming environment in a more advanced generation (cf. Santos et al. 2023b). This study reinforces the need for long-term evolution experiments and the scrutiny of several populations in the analysis to account for variation within a species. Both these components will allow for more robust assessments of species responses to sustained climate change.

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Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article. The senior author (PS) is recommender for PCI Evol Biol.

Data, scripts, code, and supplementary information availability

Raw data and scripts are available online at https://doi.org/10.6084/m9.figshare.25431880.v1. Simões, Pedro (2024). Complete dataset and scripts for manuscript entitled: "Long-term dynamics of adaptation to a warming environment is dependent on historical background"; Authors: Marta A. Antunes, Afonso Grandela, Margarida Matos & Pedro Simões (2024). figshare. Dataset. https://doi.org/10.6084/m9.figshare.25431880.v1

Supplementary information is available online: https://www.biorxiv.org/content/10.1101/2024.02.15.580572v3.supplementary-material

References

Balanyá, J., J. M. Oller, R. B. Huey, G. W. Gilchrist, and L. Serra. 2006. Global genetic change tracks global climate warming in Drosophila subobscura. Science (1979) 313:1773–1775.

Bell, G. 2017. Evolutionary Rescue. Annu Rev Ecol Evol Syst 48:605–627.

Bono, L. M., L. B. Smith, D. W. Pfennig, and C. L. Burch. 2017. The emergence of performance trade-offs during local adaptation: insights from experimental evolution. Mol Ecol 26:1720–1733. Blackwell Publishing Ltd.

Brooks, M. E., K. Kristensen, K. J. Van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Mächler, and B. M. Bolker. 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. R Journal 2:378–400.

Bryant, E. H., and L. M. Meffert. 1993. The effect of serial founder-flush cycles on quantitative genetic variation in the housefly. Heredity (Edinb) 70:122–129.

Cohan, F. M. 1986. Genetic divergence under uniform selection. II different responses to selection for knockdown resistance t o ethanol among. Genetics 114:145–163.

Cohan, F. M., and A. A. Hoffmann. 1989. Uniform selection as a diversifying force in evolution: evidence from Drosophila. Am Nat 134:613–637.

Fox, J., and S. Weisberg. 2019. An {R} Companion to Applied Regression. Third Edit. SAGE, Thousand Oaks CA.

Fragata, I., M. Lopes-Cunha, M. Bárbaro, B. Kellen, M. Lima, G. S. Faria, S. G. Seabra, M. Santos, P. Simões, and M. Matos. 2016. Keeping your options open: Maintenance of thermal plasticity during adaptation to a stable environment. Evolution (N Y) 70:195–206.

Gilchrist, G. W., R. B. Huey, J. Balanyà, M. Pascual, and L. Serra. 2004. A time series of evolution in action: A latitudinal cline in wing size in South American Drosophila subobscura. Evolution (N Y) 58:768–780. Society for the Study of Evolution.

Hallsson, L. R., and M. Björklund. 2012. Selection in a fluctuating environment leads to decreased genetic variation and facilitates the evolution of phenotypic plasticity. J Evol Biol 25:1275–1290.

Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. American Naturalist 173:579–588.

Hoffmann, A. A., and C. M. Sgró. 2011. Climate change and evolutionary adaptation. Nature 470:479–485.

Huey, R., G. Gilchrist, M. Carlson, D. Berrigan, and L. Serra. 2000. Rapid evolution of a geographic cline in size in an introduced fly. Science (1979) 287:308–309.

IPCC. 2023. Summary for Policymakers. Pp. 3–34 in Climate Change 2022 – Impacts, Adaptation and Vulnerability. Cambridge University Press.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecol Lett 7:1225–1241.

Kawecki, T. J., R. E. Lenski, D. Ebert, B. Hollis, I. Olivieri, and M. C. Whitlock. 2012. Experimental evolution. Trends Ecol Evol 27:547–560.

Kellermann, V., and B. van Heerwaarden. 2019. Terrestrial insects and climate change : adaptive responses in key traits. Physiol Entomol 44:99–115.

Kinzner, M. C., A. Gamisch, A. A. Hoffmann, B. Seifert, M. Haider, W. Arthofer, B. C. Schlick-Steiner, and F. M. Steiner. 2019. Major range loss predicted from lack of heat adaptability in an alpine Drosophila species. Science of the Total Environment 695: 133753. Elsevier B.V.

Lindsey, H. A., J. Gallie, S. Taylor, and B. Kerr. 2013. Evolutionary rescue from extinction is contingent on a lower rate of environmental change. Nature 494:463–467.

Liukkonen, M., I. Kronholm, and T. Ketola. 2021. Evolutionary rescue at different rates of environmental change is affected by trade-offs between short-term performance and long-term survival. J Evol Biol 1–8.

Mousseau, T. A., and D. A. Roff. 1987. Natural selection and the heritability of fitness components. Heredity (Edinb) 59:181–197.

Parratt, S. R., B. S. Walsh, S. Metelmann, N. White, A. Manser, A. J. Bretman, A. A. Hoffmann, R. R. Snook, and T. A. R. Price. 2021. Temperatures that sterilize males better match global species distributions than lethal temperatures. Nat Clim Chang 11:481–484.

Prevosti, A., G. Ribo, L. Serra, M. Aguade, J. Balaña, M. Monclus, and F. Mestres. 1988. Colonization of America by Drosophila subobscura: Experiment in natural populations that supports the adaptive role of chromosomal-inversion polymorphism. Proc Natl Acad Sci U S A 85:5597–600.

Rezende, E. E. L., J. Balanyà, F. Rodríguez-Trelles, C. Rego, I. Fragata, M. Matos, L. Serra, and M. Santos. 2010. Climate change and chromosomal inversions in Drosophila subobscura. Clim Res 43:103–114.

Rogell, B., W. Widegren, L. R. Hallsson, D. Berger, M. Björklund, and A. A. Maklakov. 2014. Sex-dependent evolution of life-history traits following adaptation to climate warming. Funct Ecol 28:469–478.

Santos, M. A., M. A. Antunes, A. Grandela, A. Carromeu-Santos, A. S. Quina, M. Santos, M. Matos, and P. Simões. 2023a. Past history shapes evolution of reproductive success in a global warming scenario. J Therm Biol 112:103478. Elsevier BV.

Santos, M. A., M. A. Antunes, A. Grandela, A. S. Quina, M. Santos, M. Matos, and P. Simões. 2023b. Slow and population specific evolutionary response to a warming environment. Sci Rep 13. Nature Research.

Santos, M. A., A. Carromeu-Santos, A. S. Quina, M. Santos, M. Matos, and P. Simões. 2021a. High developmental temperature leads to low reproduction despite adult temperature. J Therm Biol 95:102794.

Santos, M. A., A. Carromeu-Santos, A. S. Quina, M. Santos, M. Matos, and P. Simões. 2021b. No evidence for short-term evolutionary response to a warming environment in Drosophila. Evolution (N Y) 75:2816–2829. Society for the Study of Evolution.

Santos, M. A., A. Carromeu-Santos, A. S. Quina, M. Santos, M. Matos, and P. Simões. 2021c. No evidence for short-term evolutionary response to a warming environment in Drosophila. Evolution (N Y), doi: https://doi.org/10.1111/evo.14366.

Schou, M. F., T. N. Kristensen, V. Kellermann, C. Schlötterer, and V. Loeschcke. 2014. A Drosophila laboratory evolution experiment points to low evolutionary potential under increased temperatures likely to be experienced in the future. J Evol Biol 27:1859–1868.

Simões, P., I. Fragata, S. Seabra, G. S. Faria, M. Santos, M. R. Rose, M. Santos, and M. Matos. 2017. Predictable phenotypic, but not karyotypic, evolution of historically differentiated populations. Sci Rep 7: 913.

Simões, P., M. A. Santos, A. Carromeu-Santos, A. S. Quina, M. Santos, and M. Matos. 2020. Beneficial developmental acclimation in reproductive performance under cold but not heat stress. J Therm Biol 90:102580.

Urban, M. C., G. Bocedi, A. P. Hendry, J. B. Mihoub, G. Pe'er, A. Singer, J. R. Bridle, L. G. Crozier, L. De Meester, W. Godsoe, A. Gonzalez, J. J. Hellmann, R. D. Holt, A. Huth, K. Johst, C. B. Krug, P. W. Leadley, S. C. F. Palmer, J. H. Pantel, A. Schmitz, P. A. Zollner, and J. M. J. Travis. 2016. Improving the forecast for biodiversity under climate change. Science (1979) 353:6304. American Association for the Advancement of Science.

van Heerwaarden, B., and C. M. Sgrò. 2021. Male fertility thermal limits predict vulnerability to climate warming. Nat Commun 12:2214. Springer US.

Walsh, B. S., S. R. Parratt, A. A. Hoffmann, D. Atkinson, R. R. Snook, A. Bretman, and T. A. R. Price. 2019a. The Impact of Climate Change on Fertility. Trends Ecol Evol 34:249–259. Elsevier Ltd.

Walsh, B. S., Steven R. Parratt, David Atkinson, Rhonda R. Snook, Amanda Bretman, and Tom A.R. Price. 2019b. Integrated Approaches to Studying Male and Female Thermal Fertility Limits. Elsevier Ltd.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Tables

Table 1 - Patterns of local adaptation for generations 39 and 52 in high latitude (a) and low latitude (b) populations

a) High latitude populations

Model parameters	χ2
Selection	3.468 m.s.
Environment	79.449 ***
Generation	5.214 *
Selection:Environment	2.289 n.s.
Selection:Generation	2.943 m.s.
Environment:Generation	3.362 m.s.
Selection:Environment:Generation	1.414 n.s.

b) Low latitude populations

Model parameters	χ2
Selection	4.218 *
Environment	172.83 ***
Generation	19.070 ***
Selection:Environment	10.650 **
Selection:Generation	0.230 n.s.
Environment:Generation	0.003 n.s.
Selection:Environment:Generation	0.822 n.s.

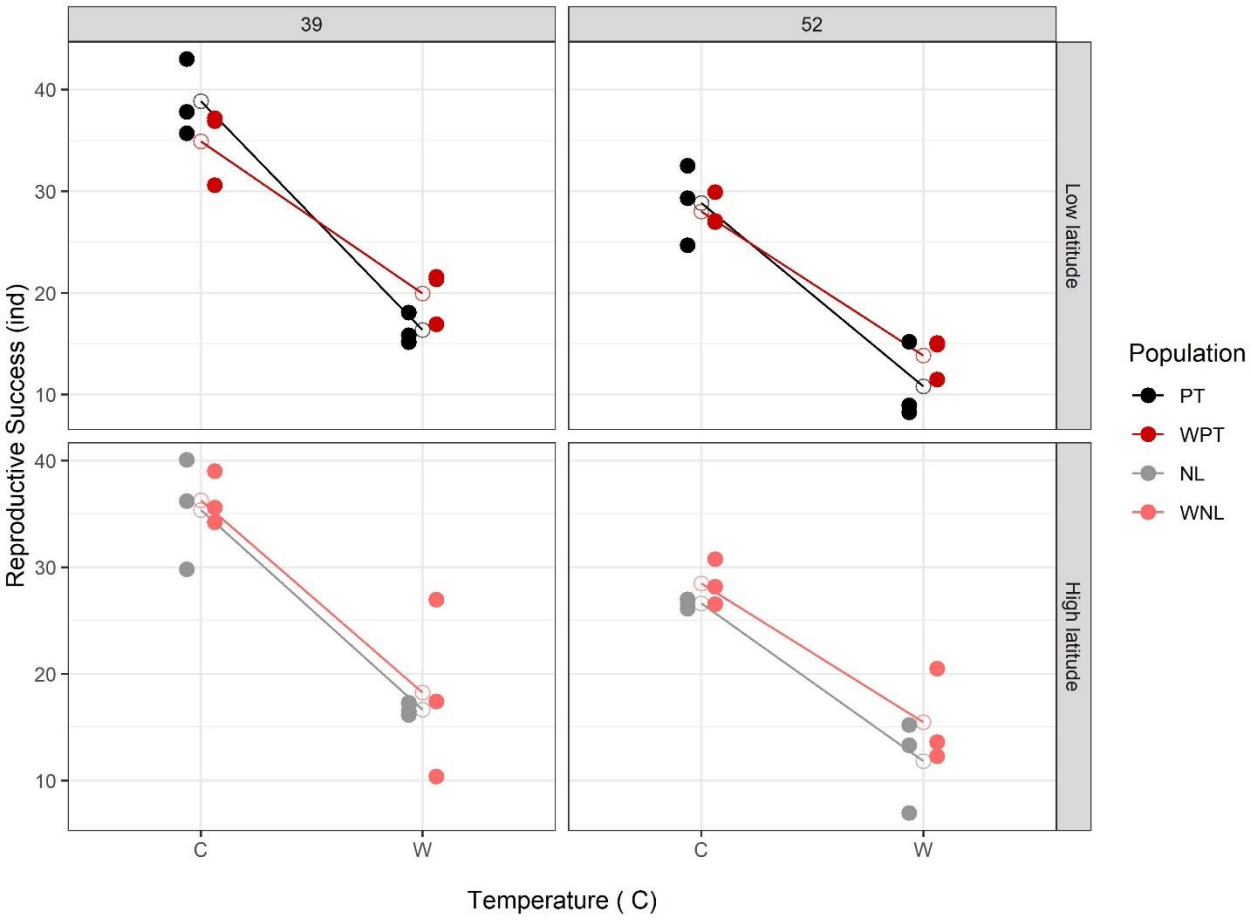
Note: significance levels: p> 0.05 n.s.; 0.05>p>0.01*; 0.01>p>0.001**; p<0.001 ***; d.f.=1 for all analyses

Figure Legends

Figure 1 - Patterns of local adaptation for low latitude and high latitude populations at generations 39 and 52.

Note: Low latitude populations are represented in the first line and high latitude in the second line; Generation 39 is represented in the first column and generation 52 in the second column.

Figure 1


39
52
Low latitude
High latitude
Reproductive Success (ind)
40
30
20
10
C
W
Temperature (C)
Population
PT
WPT
NL
WNL