Beneficial developmental acclimation in reproductive performance under cold but not heat stress

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Abstract

Thermal plasticity can help organisms coping with climate change. In this study, we analyse how laboratory populations of the ectotherm species *Drosophila subobscura*, originally from two distinct latitudes and evolving for several generations in a stable thermal environment (18ºC), respond plastically to new thermal challenges. We measured adult performance (fecundity traits as a fitness proxy) of the experimental populations when exposed to five thermal regimes, three with the same temperature during development and adulthood (15-15°C, 18-18°C, 25-25°C), and two where flies developed at 18°C and were exposed during adulthood to either 15°C or 25°C. Here, we test whether (1) flies undergo stress at the two more extreme temperatures; (2) development at a given temperature enhances adult performance at such temperature (*i.e.* acclimation) and (3) populations with different biogeographical history show plasticity differences. Our findings show (1) an optimal performance at 18ºC only if flies were subjected to the same temperature as juveniles and adults; (2) the occurrence of developmental acclimation at lower temperatures; (3) detrimental effects of higher developmental temperature on adult performance; and (4) a minor impact of historical background on thermal response. Our study indicates that thermal plasticity during development may have a limited role in helping adults cope with warmer - though not colder - temperatures, with a potential negative impact on population persistence under climate change. It also emphasizes the importance of analysing the impact of temperature on all stages of the life cycle to better characterize thermal limits.

Keywords (6): Thermal plasticity; Heat stress; Cold stress; Developmental acclimation; Fecundity; Drosophila

1. Introduction

Temperature rise and increased thermal extremes associated with current climate changes are likely to pose important new challenges to organisms (Buckley and Huey, 2016; Merilä and Hoffmann, 2016). This might be particularly troublesome for ectotherms, as recent evidence suggests high constraints in upper thermal tolerance limits (Hoffmann et al., 2013; Hoffmann and Sgrò, 2018; Kellermann et al., 2012; Porcelli et al., 2015; Sgrò et al., 2016). To avoid extinction, organisms can move to new habitats or adapt to the new conditions (Porcelli et al., 2015). Both adaptive thermal plasticity and genetic thermal adaptation may be solutions if populations remain in their habitats of origin (Chevin and Hoffmann, 2017).

Plasticity might allow a quicker response than evolutionary adaptation to changing conditions (e.g. Sgrò et al., 2016; Gibert et al., 2019). However, plasticity can also be maladaptive (Murren et al., 2015; Snell-Rood et al., 2018; Gibert et al., 2019). Examples of plastic thermal response are widespread both in nature and laboratory studies, with levels of genetic variation for plasticity varying between populations, traits, and environments (Sgrò et al., 2016; Sorensen et al., 2016). Populations adapting to different thermal environments are expected to present distinct thermal reaction norms – the function relating phenotypic change with temperature (Angilletta, 2009). Evidence for this has been equivocal, with some studies indicating non-parallel reactions norms ~~, and~~ thus suggesting genetic variation for plasticity (e.g. (Angilletta et al., 2019; Austin and Moehring, 2019; Clemson et al., 2016; Fallis et al., 2014; Klepsatel et al., 2019; Mathur and Schmidt, 2017; Rajpurohit and Schmidt, 2016; Sarup and Loeschcke, 2010), and others showing no plasticity differences among populations (e.g. Cooper et al., 2012; Klepsatel et al., 2013; Clemson et al., 2016). One important question that has received little attention is the extent to which plastic thermal responses can be lost when

populations evolve in stable thermal environments for several generations. It is a general expectation that, under such uniform conditions, organisms can lose their homeostatic ability to cope with more extreme temperatures, particularly if it is costly (Hallsson & Björklund, 2012; Murren et al., 2015; Sorensen et al., 2016). Experimental evolution studies indicate that loss of thermal plasticity in constant environments is lower than expected (Hallsson & Björklund, 2012; Ketola et al., 2013; Manenti et al., 2015; Fragata et al., 2016), suggesting that the costs of this plastic response are not high. However, few studies compared plasticity patterns between differentiated populations (but see Fragata et al., 2016).

Variation in thermal plasticity may also occur across life stages, particularly in holometabolous insects (Sgrò et al., 2016; Porcelli et al., 2017; Austin & Moehring, 2019). Plastic changes during the developmental stage may have persistent effects in the thermal performance of adults (Beaman et al., 2016; Kellermann et al., 2017). These changes can be beneficial, if they prepare organisms for stressful conditions experienced later in the adult stage (Beaman et al., 2016; Porcelli et al., 2017), or negative, if they lead to reduced adult fitness. For instance, exposure to thermal stress during development can lead to increased thermal tolerance at those temperatures in adults – beneficial developmental acclimation (Castañeda et al., 2015; Huey et al., 1999; Kellermann et al., 2017; Schou et al., 2017; Sgrò et al., 2016), e.g. due to the upregulation of heat-shock proteins (Hoffmann et al., 2003; Telonis-Scott et al., 2014). Yet, recent evidence in *Drosophila* indicates that the magnitude of developmental acclimation response near the upper thermal limits is typically low (e.g. Castañeda et al., 2015; van Heerwaarden et al., 2016; Kellermann & Sgrò, 2018), although with contrasting patterns across species (Schou et al., 2017). The impact of high juvenile temperature on adult performance might even be negative, if there is a mismatch between development and adult temperatures or

under particularly stressful developmental conditions (Beaman et al., 2016; Schou et al., 2017). For example, heat stress during development can lead to reduced body size, with potential negative implications for life-history traits (Kingsolver and Huey, 2008). Also, oogenesis and spermatogenesis pathways can be compromised by increased temperatures, leading to reduced adult reproductive performance. A stronger focus on the effects of juvenile thermal stress on adult performance is needed for a better understanding of population persistence under climate change (Porcelli et al., 2017; Walsh et al., 2019).

Drosophila subobscura is a Palearctic species that invaded South and North America around 40 years ago. It is an excellent model to address thermal adaptation, with emphasis on the clinal variation of chromosomal inversion frequencies and their response to changing thermal conditions (Balanyá et al., 2006; Rezende et al., 2010; Rodríguez-Trelles et al., 2013). Clinal variation in thermal tolerance has been observed in the South American cline of this species (Castañeda et al., 2015). Differences in reproductive performance following heat stress during development have been found by Porcelli et al. (2017) using two *D. subobscura* populations of the European cline. They reported that heat stress only in juveniles had more detrimental effects on adult performance than stress only in adults, particularly in the northern populations. However, it is an open question whether the reproductive performance of northern populations would be better at lower developmental temperatures. Other experiments in northern European *D. subobscura* populations showed beneficial developmental acclimation in thermal tolerance to lower, but not to higher extreme temperatures (MacLean et al., 2019; Schou et al., 2017). Interestingly, in a thermal plasticity study of historically differentiated *D. subobscura* populations - sampled from three different latitudes along the European cline (Portugal, France, and Netherlands) - we found that the northern *D. subobscura* populations showed an initial better thermal performance across different (both low and high) adult

temperatures than their southern counterparts. These differences disappeared during evolution in a constant, laboratory environment (Fragata et al., 2016). However, the effect of different developmental temperatures on the plastic adult performance of these populations was not addressed.

Here, we analyze two newly founded populations derived from ~~the most~~ contrasting latitudes (Portugal and Netherlands) and maintained in the lab under similar conditions as in the previous study (18ºC) for more than 60 generations. By generation 30, we observed a clear adaptive response to this new environment, with convergence between them for most adult traits, except body size (Simões et al., 2017). We have now exposed these populations to new thermal environments - both colder (15ºC) and warmer (25ºC) temperatures - during the developmental and adult life stages. The choice of temperatures was based on the expectations of a climate change scenario, predicting more extreme temperatures in the future. Specifically, we ask: 1) do these lab populations show a clear thermal plastic response? 2) Do reaction norms indicate stress at the two more extreme temperatures? 3) And does it occur when individuals experience such temperatures only in the adult stage? 4) Does exposure to new temperatures during development affect reproductive performance at those temperatures? 5) Are there differences between the two geographical populations in their response to new thermal conditions?

In general, we expect a higher performance at 18ºC as populations have been evolving for tens of generations at this temperature (the control conditions), with the more extreme 15ºC and 25ºC potentially showing a lower performance. In addition, development at the new temperatures may enhance adult performance at such temperatures when compared to adults developed at the control conditions, showing a beneficial acclimation response (*sensu* Huey et al., 1999). Finally, despite the overall

147 phenotypic convergence expressed in their present evolving environments, it is possible
148 that differences between populations will appear in these novel environments due to their
149 contrasting genetic backgrounds.

150

151 ## 2. Material and Methods

152 *2.1 Origin and maintenance of Laboratory Populations*

153 Two sets of laboratory populations were analysed in this study, resulting from
154 collections performed in late August/early September 2013 in two contrasting European
155 latitudes: Adraga (Portugal; 38º47'N, 9º28'W; hereafter referred to as PT) and Groningen
156 (The Netherlands; 53°14'N, 6°33'E; hereafter NL). Average monthly temperatures
157 between 1982 and 2012 near Adraga ranged between 10.2ºC (in January) and 19.6ºC (in
158 August), and in Groningen between 1.3ºC and 16.1ºC (data retrieved from
159 https://en.climate-data.org/). The number of founding females was 213 for PT and 170
160 for NL – see details in Simões et al., 2017. All laboratory populations (three-fold
161 replicated after founding of each latitudinal population, PT_{1-3} and NL_{1-3}) evolved under
162 the following conditions: discrete generations with synchronous 28-day cycle; 12L:12D
163 photoperiod and constant temperature of 18ºC; controlled densities in both adults (around
164 50 adults per vial) and eggs (around 70 eggs per vial), in a total of 24 vials per generation;
165 reproduction for the following generation at around peak fecundity (seven to ten days old
166 imagoes). At each generation, emergent imagoes from the several developmental vials of
167 each population were thoroughly mixed under CO_2 anaesthesia, for a final adult census
168 size between 500 and 1200 individuals (see also Fragata et al., 2014, 2016; Simões et al.,
169 2017). The experiments were done when the PT and NL populations had evolved for 67
170 generations in the laboratory environment.

171

172 *2.2 Thermal Plasticity assay*

173 To characterize how different temperatures in the juvenile and adult stages affect adult
174 performance, we studied the fecundity and wing size of individuals from the six
175 populations – PT_{1-3} and NL_{1-3} – subjected to five thermal treatments (Figure 1). In three
176 of these treatments we exposed individuals to similar developmental and adult
177 temperatures, 15ºC, 18ºC or 25ºC (treatments designated 15-15, 18-18, and 25-25,
178 respectively). We also analysed the effect of different adult temperatures on reproductive
179 performance after development at control temperature by additionally assaying
180 individuals at 15ºC or 25ºC after development at 18ºC (treatments 18-15 and 18-25). For
181 each replicate population and developmental temperature combination, we collected 12
182 vials with about 70 eggs per vial. By the third day of imago emergence (synchronous for
183 all samples that developed at 18ºC), 16 mating pairs (virgin males and females) were
184 formed for each population and treatment, with a total of 480 pairs (16 pairs*6
185 populations*5 temperature treatments). Flies were transferred daily to fresh medium and
186 the eggs laid by each female were counted for 10 days. Since during this period we expect
187 different values as a function of age, several fecundity-related traits were then analysed:
188 age of first reproduction (number of days until laying the first egg, related with rate of
189 sexual maturity), early fecundity (total number of eggs laid between days 1 and 7, also
190 affected by age of maturity, as well as initial rate of egg laying), peak fecundity (total
191 number of eggs laid between days 8 and 10, close to the age when eggs are collected for
192 the next generation). Finally, we used the entire data set, characterizing the total fecundity
193 (days 1 to 10). Flies were then stored in a mixture of alcohol and glycerol (3:1) for later
194 wing size scoring. Wing size of the females that developed and assayed at the same
195 temperature (15-15, 18-18, and 25-25) was measured through geometric morphometric
196 analysis (Dryden and Mardia, 1998). The procedure consisted in recording thirteen

morphological landmarks of the wing using the Fly Wing 15Lmk plug-in of the IMAGEJ 1.33u software (http://rsb.info.nih.gov/ij/). The wing size of each fly was estimated as its centroid size, that is the square root of the sum of the total 26 squared Euclidian distances of the 13 landmarks to the centroid (see details in Santos et al., 2005). These data allow to analyse both developmental thermal plasticity for wing size and the possible impact of wing size on adult performance (see below).

2.3 Statistical Methods

To analyze thermal plasticity data linear mixed models were fitted by REML (restricted maximum likelihood). Estimation of p-values for differences between latitudinal populations (PT or NL) and temperatures were obtained through analyses of variance (Type III Wald F tests with Kenward-Roger degrees of freedom. The following model was applied:

$$Y = \mu + Pop + Rep\{Pop\} + Temp + Pop \times Temp + \varepsilon,$$

Where *Y* is the trait studied (age of first reproduction, early and peak fecundities), *Pop* is the fixed factor "latitudinal population" (with two categories: PT and NL), *Rep{Pop}* is the random factor replicate population nested in each latitudinal population (using as raw data the mean value for each replicate population and treatment, e.g. PT_1 for the 18-18 treatment), and *Temp* is the fixed factor corresponding to the different temperature treatments. The effect of different adult temperatures was assessed by using the 18-15, 18-18 and 18-25 treatments. To test for the effects of both developmental and adult temperature in adult performance we used data from the 15-15, 18-18 and 25-25 treatments. Wing size was also analysed as a dependent variable in those treatments, using the model above. To test for the effect of different adult temperatures after development

at 18ºC, we analysed data from the 18-15, 18-18 and 18-25 treatments. The model described above was also applied to test for developmental acclimation at colder or warmer temperatures: one model using treatments 15-15 vs 18-15 for lower developmental temperature and another model with treatments 25-25 vs 18-25 for higher temperature. Developmental acclimation occurs when adult performance is higher in the 15-15 (or 25-25) treatment than in the 18-15 (or 18-25) treatment.

To measure the effect of wing size on the fecundity of adults maintained at the same temperature as juvenile and adults (15-15, 18-18, and 25-25), analyses of covariance (ANCOVA) were applied for each fecundity trait. The analysis was based on the model above, including the centroid size (log transformed) as covariate and its interactions with other factors. Interactions with the covariate were dropped from the model as these were non-significant for all traits. Models with and without defining wing size as covariate were then compared with the best model being elected based on the lower values for AIC. Normality and homoscedasticity assumptions for analysis of variance were checked. Small deviations from normality were accepted, and homoscedasticity was verified by the Brown-Forsythe test, which has great robustness and statistical power even when significant deviations from normal distributions occur (Olejnik and Algina, 1987).

All statistical analyses were done in R v3.5.3, using the *lme4* (Bates et al., 2015), *car* (Fox and Weisberg, 2019) and *lawstat* (Hui et al., 2008) packages.

3. Results

3.1 Thermal plasticity of fecundity traits

A clear plastic thermal response was observed for all fecundity traits, with significant differences (factor Temp), when considering all five treatments or the three

where temperature was the same in adults and juveniles (Table A.1 and A.2; Figure 2). In general, adult performance at the lower (15-15) and higher temperatures (25-25) was reduced relative to control conditions (18-18) – see Figure 2 and Figure B.1.

We will now focus separately on the effects of adult and developmental temperature in adult performance. Focusing on the adult performance of flies developed at 18ºC, we found significant effects of varying adult temperature (18-15, 18-18 and 18-25 treatments) for all traits (Table 1; factor Temp). Performance at 25ºC was in general the highest across traits, and the worst at 15ºC (Figure 2 and B.2). All pairwise comparisons between adult temperatures were significant across traits ($P<0.05$), except for the 18-25 and the control (18-18) conditions in peak fecundity ($F_{1,4} = 0.078$, $P>0.05$; see Figure 2).

We addressed the effect of development temperature by testing whether development at a different temperature enhanced adult fecundity at such temperature (i.e. 15-15 vs 18-15 treatments and 25-25 vs 18-25 treatments). Flies that developed at 15ºC had significantly better performance at that temperature (15-15) than those that developed at 18ºC (18-15) for all traits (see Figure 2 and B.3, factor Temp – Table 2). For early fecundity there was a significantly different plastic response of PT and NL populations (*Pop x Temp* - Table 2). This significant interaction resulted from a higher fecundity for PT populations when development occurred at 15ºC ($F_{1,4} = 12.981$, $P=0.023$) while no differences were found when development occurred at 18ºC (Figure 2 and B.3; $F_{1,4} = 0.443$, $P>0.542$). Given this significant interaction, the effect of developmental temperature on early fecundity was tested separately for NL and PT. Differences between temperatures were significant for PT ($F_{1,2.05} = 321.78$, $P<0.003$) but not for NL ($F_{1,2.26} = 13.85$, $P>0.05$). As for adult performance at 25ºC, flies that developed at 18ºC performed much better than flies developed at 25ºC (see Figure 2 and B.3, Temp – Table 2). In this

case NL and PT flies responded similarly to the exposure to the two thermal treatments (25-25 and 18-25) for all fecundity traits (*Pop x Temp* - Table 2).

3.2 Thermal plasticity of wing size and its effect on fecundity traits

Wing size was significantly affected by developmental temperature, with lower wing size at higher temperatures (Table A.3; Figure B.4). No significant differences were found between latitudinal populations in the thermal response of wing size (*Pop*Temp* - Table A.3).

Analyses including wing size as covariate were performed for the treatments 15-15, 18-18 and 25-25, to account for its effect on fecundity traits. As the interaction terms with the covariate were not significant for any trait, these interactions were dropped from the analysis (see Table A.4). This new model provided very similar results to the model excluding wing size, also with a significant effect of temperature (see Table A.4). AIC values indicated that the model including wing size was the best for all traits, although the effect of the covariate was not significant (see Table A.4).

Finally, a model using wing size as covariate was applied to the early fecundity data at 15ºC to analyse whether variation in wing size could account for the significant differences between NL and PT populations at this temperature. There was no significant interaction between wing size and population ($F_{1,2}$ = 0.502, P>0.552) so this term was dropped from the model. Comparing models with and without covariate a lower AIC value was obtained for the latter model (36.66 vs 37.16).

4. Discussion

This study analysed the thermal plastic response of two populations of *Drosophila subobscura* derived from contrasting European latitudes that have been evolving in a stable thermal environment for several generations. Overall, we found that these populations show a clear plastic thermal response, with both reproductive performance and wing size varying due to exposure to distinct thermal environments. Other studies have also shown that populations evolving at constant temperatures respond plastically to different thermal environments (e.g. Hallsson & Björklund, 2012; Ketola et al., 2013; Fragata et al., 2016). As somewhat expected, when individuals were submitted to the same temperature as juveniles and adults, there was a better adult performance at the control, 18ºC conditions, the temperature at which populations have been evolving for tens of generations.

4.1 Negative impact of high developmental temperatures on adult performance

We showed that exposure to high temperatures during the developmental and adult stage – in this case, 7ºC above the control conditions – can be highly detrimental for reproductive performance in *D. subobscura*. However, high temperature only in the adult stage led to improved performance during the first week of life of both populations, when compared to the controls exposed to 18ºC during developmental and adult stages. Constantly high developmental temperatures may have a negative impact on both oogenesis and spermatogenesis (David et al., 2005; Walsh et al., 2019), thus diminishing reproductive output. Our results are comparable to those found by Porcelli et al. (2017) in a study of the effect of heat stress in the reproductive performance of *D. subobscura*.

There, one northern and one southern European population showed decreased fertility when exposed to a high temperature at both development and adult stage (23.5ºC). Also, no negative effect on fertility was observed when experiencing 23.5ºC only during the adult stage. The fact that individuals developed at higher temperatures have in general smaller body size might contribute to a lower fecundity at those temperatures if a positive association between body size and fecundity occurs (Kingsolver and Huey, 2008). Here, we observed the expected decrease of wing size at higher developmental temperatures, but this did not explain the variation in fecundity. Although wing size is in general taken as a good proxy of body size in Drosophila (Huey et al., 2000; James et al., 1995), some caution is needed when analysing flies reared at different temperatures due to the different scaling relationships between body and wing size in response to temperature (Mirth and Shingleton, 2012).

Evidence for negative effects of heat stress during development on adult performance has also been described in other insect species (e.g. Zhang et al., 2015; Klockmann et al., 2017; Walsh et al., 2019). Furthermore, direct evidence indicates that heat tolerance in *Drosophila* is lower at the developmental stage than at the adult stage (Lockwood et al., 2018). This constraint is likely enhanced by the fact that many insects have a relatively sessile developmental stage with restricted opportunity to avoid exposure to heat stress, namely through behavioural thermoregulation (Dillon et al., 2009; Huey and Pascual, 2009; Rajpurohit and Schmidt, 2016). This is troublesome for these organisms, particularly considering the current climate warming and the associated occurrence of heat waves (Kingsolver et al., 2013), which can lead to sudden population decline.

4.2 Beneficial developmental acclimation at low temperatures

In contrast with what was observed for higher temperatures, flies developed at the lowest temperature showed higher adult performance at that temperature than flies developed at the 18ºC control conditions and later exposed to colder conditions, as expected by beneficial developmental acclimation (see Beaman et al., 2016; Huey et al., 1999; Sgrò et al., 2016; Sorensen et al., 2016). This pattern was observed in both populations across traits, but with a higher magnitude in the southern ones for early fecundity (see below). In *D. subobscura*, the highest ovariole number is obtained when development occurs at lower temperatures (12-14ºC) followed by a steady decrease as temperature increases (Moreteau et al., 1997). This might help explain the higher fecundity of our flies developed at 15ºC relative to those developed at 18ºC. Further research is needed to test whether development at colder temperatures leads to a general better reproductive performance across a range of adult temperatures, a pattern predicted by the "colder is better" hypothesis (Huey et al., 1999). The patterns of acclimation response for the reproductive performance we report here match those found for thermal tolerance in this species: beneficial acclimation at lower temperatures (CT_{min}) but not at higher ones (CT_{max}) – see MacLean et al., 2019; Schou et al., 2017).

Studies addressing developmental acclimation to lower temperatures in fecundity in *Drosophila melanogaster* have provided contradictory results, with evidence for such acclimation in some (Nunney and Cheung, 1997) but not all studies (Angilletta et al., 2019; Huey et al., 1995; Klepsatel et al., 2019). In particular, our findings in *D. subobscura* contrast with a recent study on the effects of developmental plasticity in *D. melanogaster* which reported that individuals developed at an intermediate temperature showed a better reproductive performance across three different adult temperatures

(Klepsatel et al., 2019) – the "optimal acclimation temperature" hypothesis (see Huey et al., 1999; Klepsatel et al., 2019). It is possible that the mechanisms associated with the acclimation response in reproductive performance differ across *Drosophila* species (or even populations), for instance as a result of adaptation to different thermal environments (see Schou et al., 2017, for evidence of this in heat tolerance).

4.3 The importance of thermal reproductive limits

Recent literature on thermal adaptation has acknowledged the need for more thoroughly addressing the temperature effects on reproductive traits, instead of focusing almost exclusively on the study of physiological thermal tolerance limits (Sorensen et al., 2016; Porcelli et al., 2017; Walsh et al., 2019). Porcelli et al. (2017) found reduced fertility in *D. subobscura* females developed at 23.5ºC, a much lower temperature than the upper thermal limits obtained in physiological assays (higher than 35ºC – e.g. Castañeda et al., 2015). In our experiment, we also observed a clear reduction of reproductive performance after development at a moderately high temperature (25ºC), with low fecundity and no egg hatching for all populations. Heat stress during development likely caused male sterility as found by Porcelli et al. (2017) because spermatogenesis is more thermally sensitive than oogenesis (David et al., 2005). On the other hand, when 25ºC were experienced in the adult stage only reproductive performance was enhanced. These findings emphasize the importance of analysing several traits in all stages of the life cycle to better characterize the thermal limits of populations (e.g. see Austin & Moehring, 2019).

Some authors have called for caution when studying developmental plasticity effects on the adult response, due to the possible confounding effects of selection at the juvenile stages as a result of differential mortality across thermal environments (Santos et al., 2019). In this study, we did not observe clear differences between juvenile viability across the developmental temperatures assayed (based on visual inspection of vials at the different temperatures). Thus, while it is possible that some selection during the developmental stage is occurring in our experimental setup, it is unlikely to be a major factor affecting our results.

4.4 Does history play a role in the plastic response?

Overall, we found that populations derived from different geographical locations showed a generally similar plastic response to the new thermal environments. In a previous study with other populations from the same locations, we found that initially differentiated populations converged in thermal reaction norms (temperatures of 13ºC, 18ºC and 23ºC) during 28 generations of evolution in a stable lab environment (Fragata et al., 2016). This suggests that the similar plastic response observed in the current experiment might result from evolutionary convergence in the reaction norms. Contrary to our findings in *D. subobscura*, Porcelli et al. (2017) found geographical differences in reproductive responses to heat stress in two European populations, with the southern population (Valencia, Spain) showing improved performance relative to the northern one (Uppsala, Sweden). The precise origin of the populations might explain these differences as the more marginal *D. subobscura* Scandinavian populations (as the one from Uppsala) have likely less genetic variation than other northern populations such as those from Groningen (see also Simões et al., 2012). Other factors may also influence the results,

411 such as (1) the distinct procedure for founding and maintaining the laboratory
412 populations, and (2) the different number of generations in the laboratory. These factors
413 might also explain differences between our study and others that reported geographical
414 variation in thermal reaction norms for fecundity in *Drosophila* (Klepsatel et al., 2013;
415 Clemson et al., 2016).

416 In spite of the above, we found some evidence for a historical signature of thermal
417 plasticity for early fecundity with a higher cold acclimation ability for our southern (PT)
418 populations. Wing size between PT and NL individuals was very similar at 15ºC, so it is
419 very unlikely that this trait explains the fecundity differences. Our results indicate, thus,
420 that our populations have genetic differences for acclimation to cold temperatures. It
421 remains unknown whether these differences are due to historical differentiation in the
422 genetic background of natural populations and/or to subsequent changes during
423 laboratory evolution.

424 In previous experiments (Seabra et al., 2018), we found a high genomic
425 differentiation of other *D. subobscura* populations sampled in the same geographical
426 locations as those of this study, after 50 generations of lab adaptation. We, therefore,
427 expect that our latitudinal populations also present clear genome-wide differences at this
428 point, with different genetic variants between populations producing similar phenotypic
429 outcomes, as previously observed in past experiments (Fragata et al., 2016, 2014; Simões
430 et al., 2017) and the present one. Future studies of the adaptive dynamics to different
431 thermal regimes will enlighten whether populations differ in their evolutionary potential.

432

433 *4.5 Conclusions*

The ability for cold – but not warm - acclimation response that we observed here suggests that *D. subobscura* is able to cope with lower extreme events, while sudden heat events can be particularly harmful, especially if occurring during the developmental stage. These findings are particularly relevant in the context of adaptation to climate change, as one inevitable effect of global warming with which organisms have to strive is the more extreme lower and higher temperatures – colder winters and hotter summers.

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Tables

Table 1 - Effect of adult temperature on the thermal response for fecundity characters between populations (juvenile temperature of 18ºC and adult temperatures of 15, 18 and 25ºC)

Trait	Model parameters	F(df1, df2)
Age of First Reproduction	Pop	$F_{1,4}$ = 0.866 n.s.
	Temp	$F_{2,8}$ = 156.0 ***
	Pop*Temp	$F_{2,8}$ = 0.242 n.s.
Early Fecundity	Pop	$F_{1,4}$ = 0.169 n.s.
	Temp	$F_{2,8}$ = 139.4 ***
	Pop*Temp	$F_{2,8}$ = 0.038 n.s.
Peak Fecundity	Pop	$F_{1,4.0}$ = 0.567 n.s.
	Temp	$F_{2,8}$ = 17.537 **
	Pop*Temp	$F_{2,8}$ = 0.216 n.s.
Total fecundity	Pop	$F_{1,4.0}$ = 0.430 n.s.
	Temp	$F_{2,8}$ = 191.57 ***
	Pop*Temp	$F_{2,8}$ = 0.365 n.s.

Note: p>0.05 n.s.; 0.05>p>0.01*; 0.01>p>0.001**; p<0.001 ***

669 Table 2 - Effects of lower (15ºC) and higher (25ºC) developmental temperatures on the
670 thermal response for fecundity characters between populations

	Trait	Model parameters	F
Higher temperature (18-25 vs 25-25)	Age of First Reproduction	Pop	0.103 n.s.
		Temp	45.883 **
		Pop*Temp	0.015 n.s.
	Early Fecundity	Pop	0.084 n.s.
		Temp	148.42 ***
		Pop*Temp	0.015 n.s.
	Peak Fecundity	Pop	0.344 n.s.
		Temp	40.355 **
		Pop*Temp	0.226 n.s.
	Total Fecundity	Pop	0.272 n.s.
		Temp	172.27 ***
		Pop*Temp	0.189 n.s.
Lower temperature (18-15 vs 15-15)	Age of First Reproduction	Pop	0.366 n.s.
		Temp	19.129 *
		Pop*Temp	0.304 n.s.
	Early Fecundity	Pop	1.334 n.s.
		Temp	129.01 ***
		Pop*Temp	23.975 **
	Peak Fecundity	Pop	0.052 n.s.
		Temp	35.182 **
		Pop*Temp	0.952 n.s.
	Total Fecundity	Pop	0.466 n.s.
		Temp	71.498 **
		Pop*Temp	5.825 n.s.

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672 Note: p >0.05 n.s.; 0.05>p>0.01*; 0.01>p>0.001**; p<0.001 ***. Degrees of freedom
673 of the Effect and Error are 1 and 4 respectively for all tests.

Figure Legends

Figure 1 - Experimental design applied, with three developmental and three adulthood temperatures.

Figure 2 – Reproductive performance for individuals exposed to the five different thermal treatments. a) Age of first Reproduction; b) Early Fecundity (days 1 to 7); c) Peak Fecundity (days 8 to 10); d) Total Fecundity (days 1 to 10). Error bars represent the standard error of the 3 replicate populations of each latitudinal population.

700 Figure 1

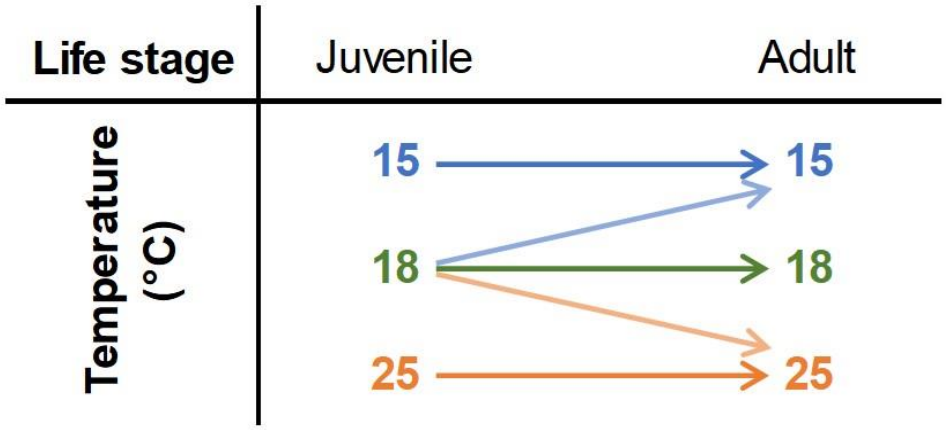

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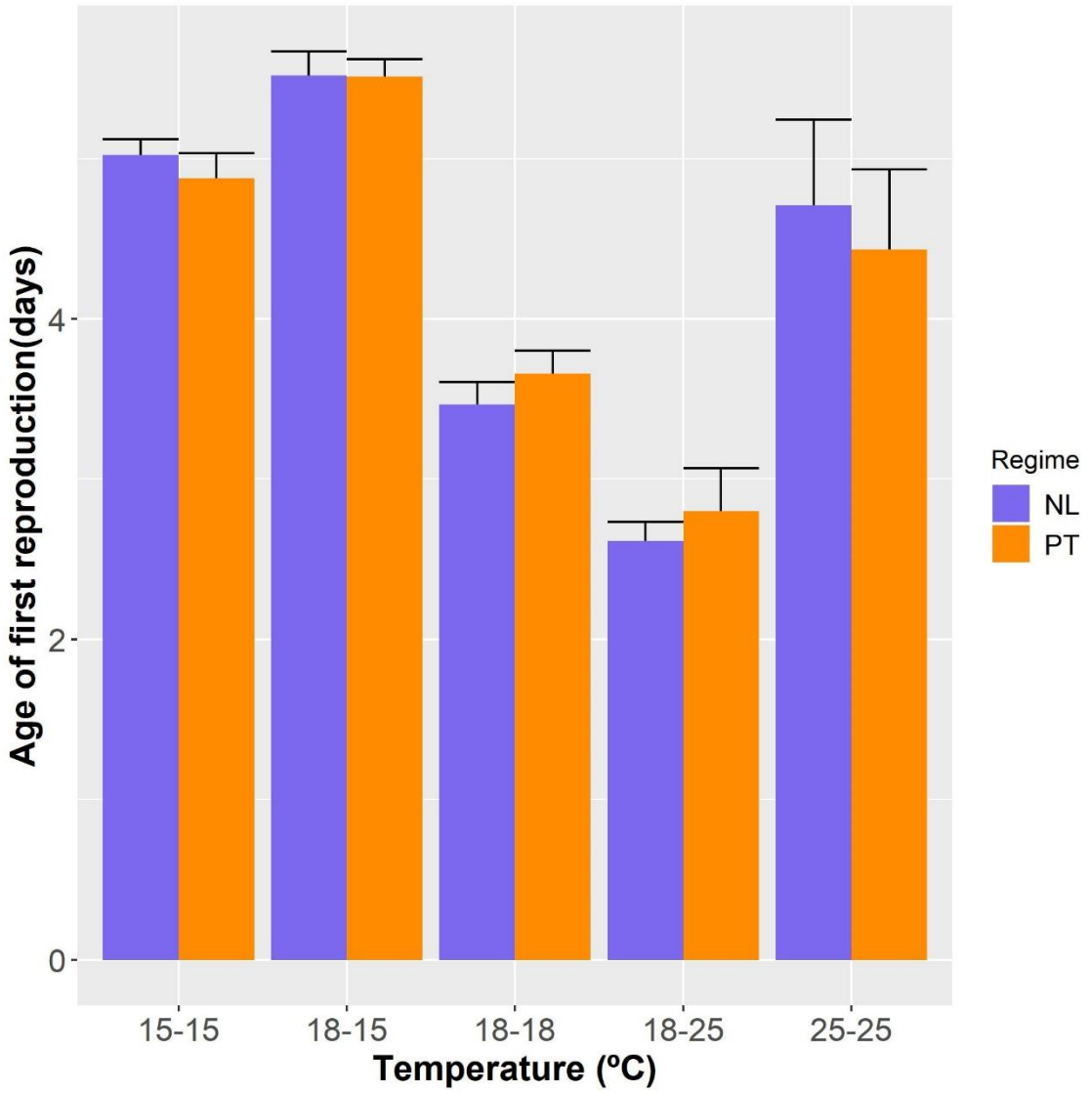
Figure 2A


Age of first reproduction(days)
0
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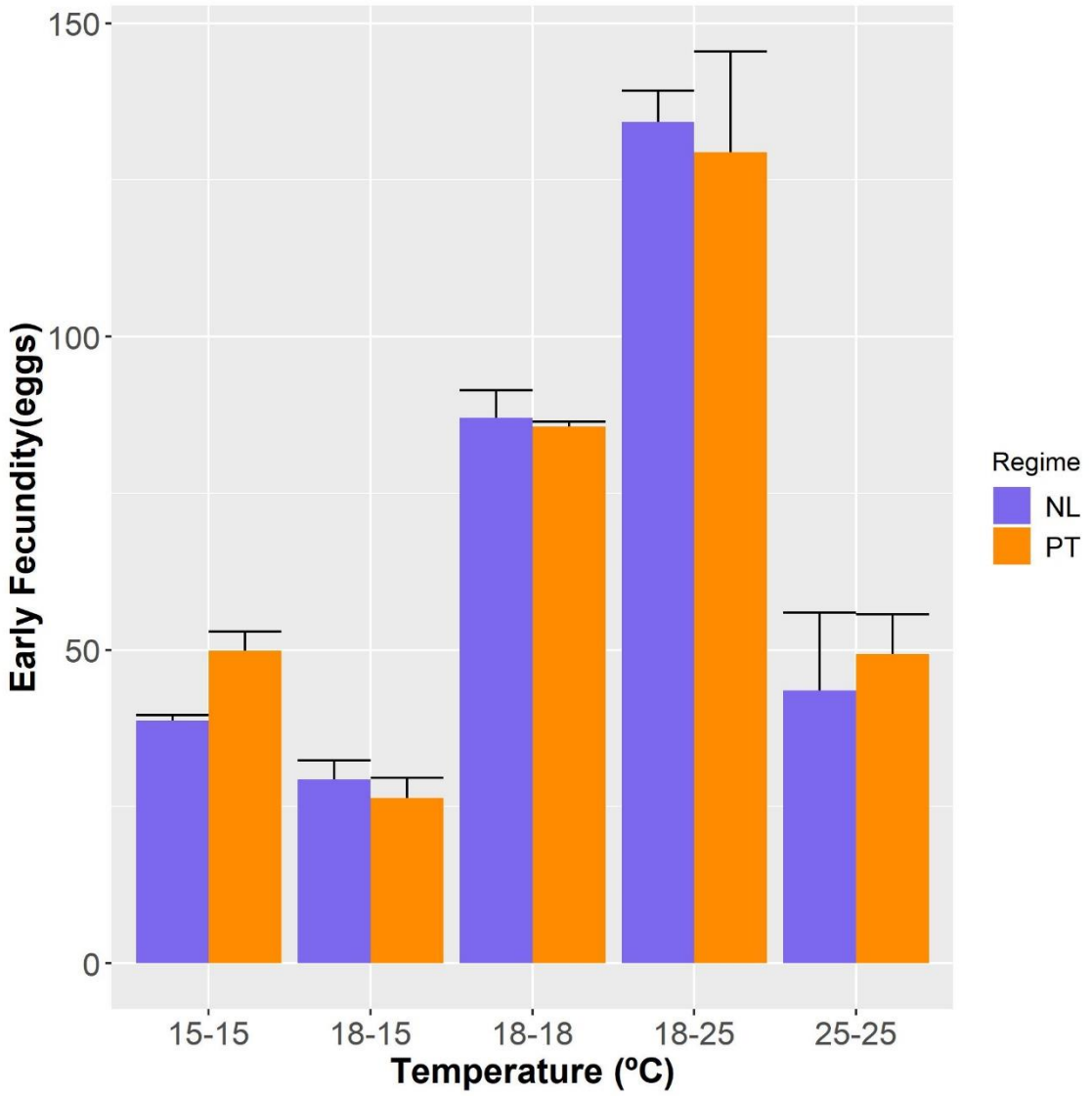
4
15-15
18-15
18-18
18-25
25-25
Temperature (ºC)
Regime
NL
PT

Figure 2B


150
100
50
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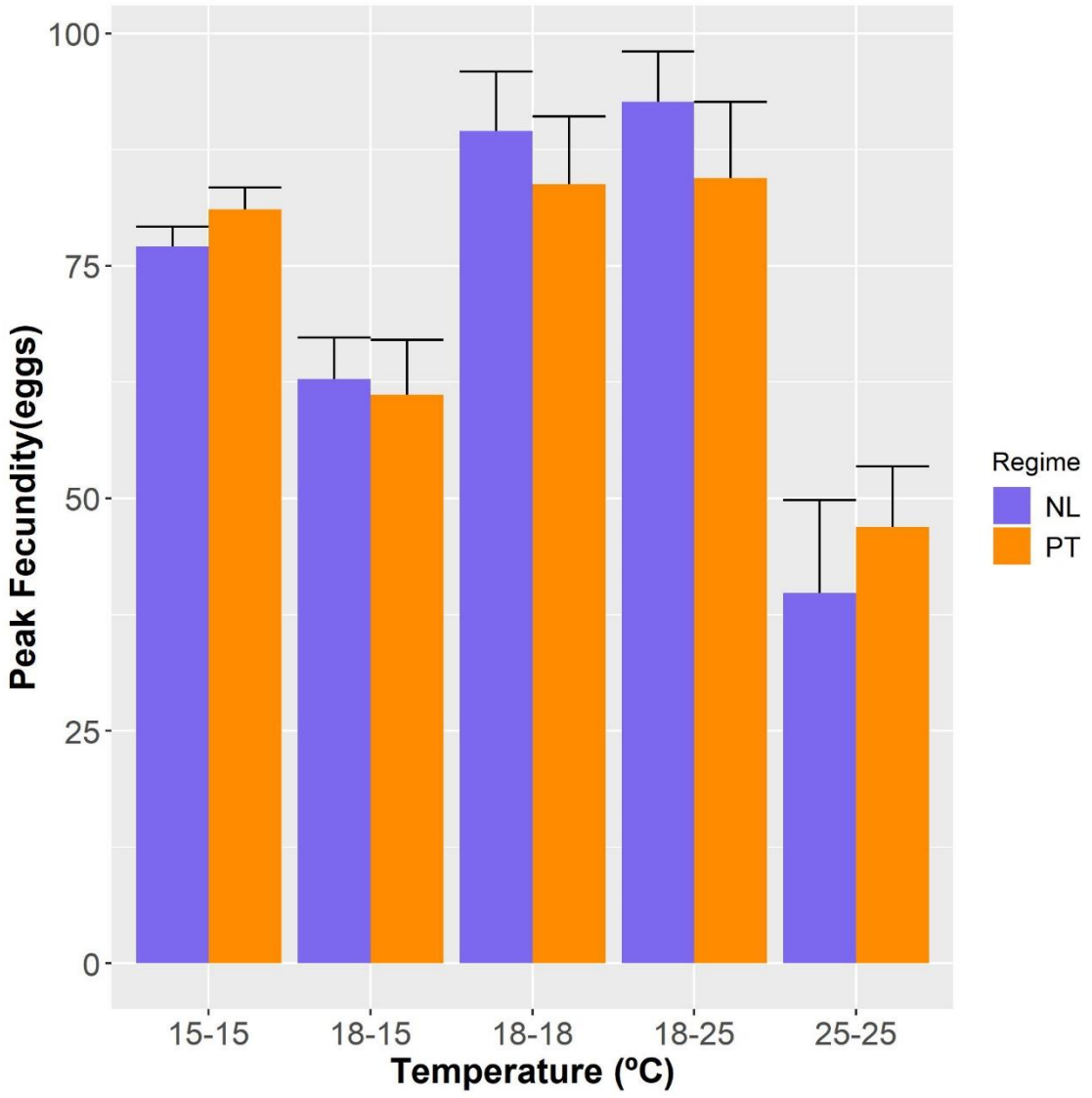
Early Fecundity(eggs)
15-15
18-15
18-18
18-25
25-25
Temperature (ºC)
Regime
NL
PT

741 Figure 2C



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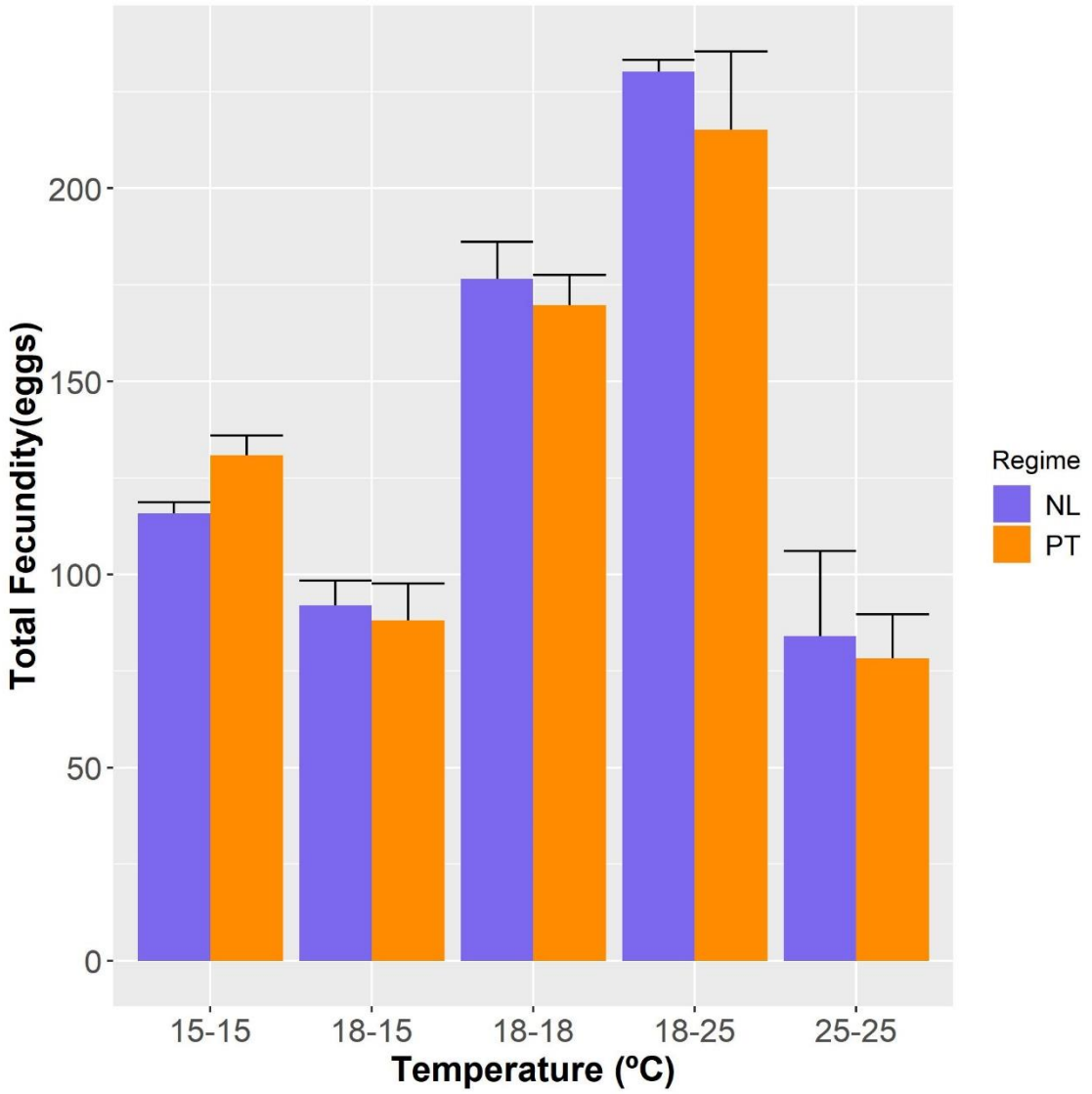
752 Figure 2D



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