High developmental temperature leads to low reproduction despite adult temperature

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Abstract

Phenotypic plasticity can help organisms cope with changing thermal conditions and it may depend on which life-stage the thermal stress is imposed: for instance, exposure to stressful temperatures during development can trigger a positive plastic response in adults. Here, we analyze the thermal plastic response of laboratory populations of *Drosophila subobscura*, derived from two contrasting latitudes of the European cline. We measured reproductive performance through fecundity characters, after the experimental populations were exposed to five thermal treatments, with different combinations of developmental and adult temperatures (14°C, 18°C, or 26°C). Our questions were whether (1) adult performance is changed with exposure to higher (or lower) temperatures during development; (2) flies raised at lower temperatures outperform those developed at higher ones, supporting the "colder is better" hypothesis; (3) there is a cumulative effect on adult performance of exposing both juveniles and adults to higher (or lower) temperatures; (4) there is evidence for biogeographical effects on adult performance. Our main findings were that (1) higher developmental temperatures led to low reproductive performance regardless of adult temperature, while at lower temperatures reduced performance only occurred when colder conditions were persistent across juvenile and adult stages; (2) flies raised at lower temperatures did not always outperform those developed at other temperatures; (3) there were no harmful cumulative effects after exposing both juveniles and adults to higher temperatures; (4) both latitudinal populations showed similar thermal plasticity patterns. The negative effect of high developmental temperature on reproductive performance, regardless of adult temperature, highlights the developmental stage as very critical and most vulnerable to climate change and associated heat waves.

Keywords: phenotypic plasticity; temperature; *Drosophila*; fecundity; history; development

1. Introduction

Phenotypic plasticity can help organisms cope with climate change by allowing for a rapid response to changing thermal conditions. In particular, developmental thermal plasticity may enable beneficial responses through developmental acclimation – *i.e.* higher resistance in adults as a consequence of exposure to stressful temperatures during development (Beaman et al., 2016; Sgrò et al., 2016; Sørensen et al., 2016). Plastic responses have received much attention in recent years, especially those associated with physiological tolerance to either cold or heat extremes (e.g. in insects (Kellermann, 2019; Kellermann and Sgrò, 2018; MacLean et al., 2019; Schou et al., 2017).

An increasing number of experiments are addressing the developmental thermal plasticity associated with adult life-history traits, mainly in ectotherms (Angilletta et al., 2019; Austin and Moehring, 2019; Cao et al., 2018; Iossa et al., 2019; Klepsatel et al., 2019; Klockmann et al., 2017; Manenti et al., 2017; Porcelli et al., 2017; Zamorano et al., 2017 see Kelly, 2019; Mirth et al., 2020 for reviews). This line of research is extremely relevant as traits closely related to fitness, such as fecundity and longevity, are crucial for population persistence and very likely to be affected by climate change (Walsh et al., 2019). In general, evidence indicates a negative impact of high developmental temperature on adult fitness (e.g. Cao et al., 2018; Klepsatel et al., 2019; Klockmann et al., 2017; Porcelli et al., 2017). These results contradict the "hotter is better" hypothesis, which states that higher developmental temperatures lead to enhanced adult performance across different temperatures (Huey et al., 1999). Such detrimental effects will, certainly, depend on the magnitude of the temperature rise. Some studies addressing fecundity support an alternative pattern of developmental acclimation, the "optimal acclimation temperature" hypothesis, where individuals developed at intermediate, "optimal" temperatures show general better adult performance across environments relative to

individuals developed at more extreme temperatures (Klepsatel et al., 2019; Kristensen et al., 2012). Yet, another development acclimation hypothesis – "colder is better" – predicts a better adult performance of individuals raised at lower temperatures (Huey et al., 1999). Evidence for the impact of colder developmental temperatures on fecundity is less conclusive, with positive effects observed in some studies (e.g. (Nunney and Cheung, 1997; Simões et al., 2020; Zamorano et al., 2017), but not in others (Angilletta et al., 2019; Huey et al., 1995; Klepsatel et al., 2019; Kristensen et al., 2012).

Drosophila subobscura is an excellent model organism to study thermal responses. This species shows latitudinal clinal variation for chromosomal inversion frequencies in three distinct continents, likely due to thermal adaptation (Prevosti et al., 1988; Rezende et al., 2010). It, also, clearly responds to thermal challenges both in nature (Balanyá et al., 2006; Rodríguez-Trelles et al., 2013) and in the lab (Fragata et al., 2016; Simões et al., 2020). This species has a range of development temperatures spanning from 6ºC to 26ºC, with extremely low juvenile viability from 27ºC onwards (David et al., 2005; Moreteau et al., 1997; Schou et al., 2017). Optimal viability occurs in the range of 16 ºC to 20ºC (Schou et al., 2017), which overlaps with the temperature optimum estimates of 16-17ºC based on thermal preference assays (Rego et al., 2010).

Developmental thermal plasticity in life-history traits of *D. subobscura* has been associated with lower egg-to-adult viability, fertility, and sperm motility due to high developmental temperatures, thus refuting the "hotter is better" hypothesis (Porcelli et al., 2017; see below). This association is also corroborated by data (in this species) for a decline in the critical thermal maximum (CT_{max}) with increasing developmental temperatures (Schou et al., 2017). In addition, positive effects of developmental plasticity (*i.e.* beneficial acclimation) were found near the lower physiological limits (CT_{min}), but not near the higher limits (CT_{max}) (MacLean et al., 2019; Schou et al., 2017). Still, further

105 evidence is needed on the possible (detrimental or beneficial) effects of either lower or
106 higher temperatures on adult reproductive performance of development, namely testing
107 the “hotter is better” and “colder is better” developmental acclimation hypotheses in
108 fecundity traits.

109 We previously addressed the developmental thermal plasticity patterns in two sets
110 of historically differentiated populations of *Drosophila subobscura* founded from
111 extreme locations of the European cline (Portugal and The Netherlands, see details in
112 (Simões et al., 2017)) after 67 generations of evolution at 18ºC. This was done by
113 subjecting juvenile and adult flies to different temperature combinations of 15 ºC, 18ºC,
114 and 25ºC (Simões et al., 2020). First, we observed that increased temperatures (25ºC)
115 during both juvenile and adult stages led to a poor adult reproductive performance, but
116 higher temperatures in the adult stage only increased it. However, our setup did not allow
117 to distinguish the effect on fecundity of high developmental temperature from high adult
118 temperature, thus not directly testing the “hotter is better” hypothesis, nor the reversibility
119 of the effects of developmental temperature. Second, we found that flies that developed
120 at colder temperature (15ºC) had increased reproductive performance at 15ºC, when
121 compared to those developed at 18ºC (control conditions), indicating cold acclimation.
122 Additional testing is needed to determine if this pattern can be the result of a general
123 better performance of individuals raised at lower temperatures, the “colder is better”
124 hypothesis. Finally, we found some historical effects in thermal plasticity for fecundity,
125 with a higher cold acclimation of the southern populations (Simões et al., 2020).

126 To answer the issues raised by the Simões et al. (2020) study, we report an
127 additional developmental plasticity experiment in the same populations, where we test
128 the adult reproductive performance of individuals exposed to three different temperatures
129 (14ºC, 18ºC, and 26ºC). First, we checked for the enhancement or reduction in

performance of adult flies exposed to higher or lower temperatures during development, testing the hypotheses "colder is better" and "hotter is better" against the "optimal acclimation temperature" (Huey et al., 1999). If "colder is better" is true, then flies raised at lower temperatures will show higher performance than flies raised at higher ones; and the same rationale can be used for the "hotter is better" scenario. Furthermore, a higher performance for flies developed at the intermediate temperature (18ºC) will support the "optimal acclimation temperature" hypothesis. Second, we looked for the existence of a cumulative detrimental effect of life-long exposure to lower or higher temperatures on fecundity, testing the reversibility of its effect. Should that be the case, the performance of flies will be higher when switched to control conditions after development at colder or warmer environments. Finally, we tested for the occurrence of biogeographical effects of temperature on reproduction, by comparing the populations from Portugal and The Netherlands when exposed to distinct thermal conditions. Differences in reproductive performance, if found, will reveal the signature of evolutionary history on the populations' response to these new environments.

2. Material and Methods

2.1 Origin and maintenance of Laboratory Populations

In late August/early September 2013, two natural populations of the palearctic species *Drosophila subobscura* were sampled. The collections were done in Adraga, Portugal (lat. 38º48'N) and Groningen, The Netherlands (lat. 53º13'N). These are two contrasting latitudes of the European cline (which ranges from Scandinavia, ~60ºN, to Northern Africa, ~30ºN – (Prevosti et al., 1988) that experience very different environmental temperatures. Samples were used to establish two sets of laboratory populations: PT, from Adraga, and NL, from Groningen – see details in (Simões et al.,

2017, 2020). Each latitudinal population was three-fold replicated in the lab, originating the PT_{1-3} and NL_{1-3} populations. Population maintenance involved discrete generations with a synchronous 28-day cycle; 12L:12D photoperiod; constant temperature of 18ºC; controlled densities in adults (50 adults per vial) and eggs (70 eggs per vial) in ~30mm^3 glass vials; reproduction for the next generation was around peak fecundity (seven to ten days old imagoes). Census size ranged between 500 and 1200 individuals per population (see also Simões et al., 2017). The thermal plasticity assay was performed when PT and NL populations were at their 71st generation of evolution in the laboratory environment.

Although the physiological responses to temperature in *D. subobscura* can be shaped by environment-by-environment interactions, such as the combined effect of temperature and photoperiod (*e.g.* Moghadam *et al.*, 2019), we have only focused on the plastic response of fly populations in a 12h light:12h dark set up and acknowledge that results under different daylength scenarios might be different.

2.2 Thermal Plasticity assay

To study the effect of different thermal environments on adult performance, we analyzed fecundity in PT and NL populations subjected to five thermal treatments (see Figure 1). In three of the treatments, we exposed the flies to the same developmental and adult temperatures, 14ºC, 18ºC or 26ºC (treatments 14-14, 18-18, and 26-26, respectively). The other two treatments were applied to analyze the impact of (higher or lower) developmental temperature on adult reproductive performance tested in control conditions. This was done by assaying imagoes at 18ºC following their development at either 14ºC or 26 ºC (treatments 14-18 and 26-18, respectively). 20 recently emerged mating pairs (virgin males and females) per population and treatment were formed, with a total of 600 pairs (20 pairs*6 populations*5 temperature treatments). Flies were

transferred to fresh medium every other day, vials were daily checked for the presence of eggs, and the eggs laid by each female were counted in days 7 and 8 since emergence. This procedure allowed to estimate two adult parameters: age of first reproduction (number of days since emergence until the first egg laying) and fecundity (measured as the total number of eggs laid between days 7 and 8). The first parameter addresses the rate of sexual maturity, while the second refers to a period that is close to the age of egg collection for the following generation (seven to ten-day-old imagoes), where selective pressures are likely higher. Previous studies showed that subjecting 18ºC-adapted flies to 13-15ºC highly reduces fecundity and to 23-25ºC enhances it (Fragata et al., 2016; Simões et al., 2020). Also, 27ºC was found to cause ~90% of juvenile mortality (David et al., 2005; Moreteau et al., 1997). These reasons account for the lower and higher temperature choices (14ºC and 26ºC) as we were looking for stressful, but viable conditions.

2.3 Statistical Methods

Thermal plasticity was analyzed by linear mixed models fitted with REML (restricted maximum likelihood). P-values for differences between temperatures, populations (PT or NL), and their interaction were obtained through analyses of variance (Type III Wald F tests, Kenward-Roger degrees of freedom). The model applied was as follows:

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

Y is the trait in study (age of first reproduction or fecundity), Pop is the fixed factor latitudinal population (with categories PT and NL), Rep{Pop} is the random factor replicate population nested in the fixed factor latitudinal population, and Temp is the fixed factor corresponding to the different temperature treatments. Raw data is the mean value for each replicate population and temperature treatment, *e.g.* NL_1 for the 14-14 treatment. Higher fecundity (more eggs) and lower age of first reproduction (faster sexual maturity)

indicate better adult performance. Replicates were derived from each population to analyze how much the differences between the populations (PT and NL) were due to their historical origin and not to random effects. Using the replicate as a random effect in the statistical models is essential to test for consistent differences above the level of the replicate (*i.e.* population level). This applies to both the factor Population and the interaction Population*Temperature (which is the differential plasticity between PT and NL). Given that defining replicates as a random effect means that the average value of each replicate is the raw data used for testing both aforementioned terms, we used the average instead of the individual data.

First, to analyze the effect of higher or lower developmental temperature on adult performance (measured at control conditions, 18ºC), two comparisons were performed: 14-18 *vs.* 18-18, for lower developmental temperature; and 26-18 *vs.* 18-18, for higher developmental temperature. The first comparison allows to test the "colder is better" hypothesis and the second comparison addresses the "hotter is better" hypothesis. Both comparisons allow to address the "optimal acclimation temperature" hypothesis (supported if 18-18 > 14-18 and 18-18 > 26-18). Second, to test the cumulative effect on adult performance of exposing flies to both (lower or higher) development and adult temperature, the following comparisons were done: 26-26 *vs.* 26-18, for higher temperatures; and 14-14 *vs.* 14-18, for lower temperatures. If a negative cumulative effect occurs, we expect that individuals exposed to 18ºC as adults will have a better performance when compared to those kept at the more extreme temperature, *i.e.*, 26-18 (or 14-18) flies will have higher fecundity and lower age of first reproduction than 26-26 (or 14-14) flies. Comparisons with control 18-18 conditions allow to assess the extent to which temperature changes imposed by other thermal treatments are stressful or not, and if these changes are cumulative. The normality and homoscedasticity assumptions for

analysis of variance were checked and were met in our dataset. All statistical analyses were performed in R v3.5.3, with the lme4 (Bates et al., 2015), car (Fox and Weisberg, 2019) and lawstat (Hui et al., 2008) packages.

3. Results

Thermal plasticity was observed for both age of first reproduction and fecundity - when considering all thermal treatments (Figure 2 and Table A1, significant factor Temp). In general, adult performance was lower for individuals developed at the highest temperature tested (26ºC) – Figure 2.

Paired comparisons between developmental temperatures were performed to test for the effects of higher or lower developmental temperature on adult performance (Table 1). On the one hand, there was a significantly lower adult performance (lower fecundity, higher age of first reproduction) of flies developed at 26ºC relative to those developed at 18ºC, when tested in the control 18ºC environment (see Figure 2 and Table 1, 26-18 *vs.* 18-18). On the other hand, individuals developed at 14ºC and kept as adults at 18ºC reached sexual maturity significantly faster than those always kept at control, 18ºC conditions (see Figure 2b and Table 1, 14-18 *vs.* 18-18). No detrimental effect of lower developmental temperature was found for fecundity (see Figure 2a and Table 1, 14-18 *vs.* 18-18).

The test for the cumulative effect of higher developmental temperature showed that switching flies to 18ºC (26-18) did not increase performance when compared to keeping them at 26ºC in both life stages (26-26); but even decreased in the case of age of first reproduction (see Figure 2 and Table 2, 26-18 *vs.* 26-26), so no negative cumulative effect was found. These results suggest that the negative effect of high developmental temperatures could not be reverted.

Conversely, at lower temperatures, performance was reduced only in individuals developed and maintained at such temperatures (see Figure 2, 14-14 *vs.* 18-18). This low performance was reversed when individuals that developed at 14ºC were exposed to 18ºC as adults: flies from the 14-18 thermal treatment showed significantly higher performance than those from the 14-14 treatment (see Figure 2 and Table 2, 14-18 *vs.* 14-14), showing that the combination of lower juvenile and adult temperatures have a negative effect on adult performance.

No significant differences in thermal plasticity between latitudinal populations were observed, either considering all thermal treatments or in the comparisons between them (see Figure 2 and Tables 1 and 2, factor Pop*Temp).

4. Discussion

We report here that higher – but not lower – developmental temperatures led to lower adult performance in *Drosophila subobscura* flies. These effects were permanent, as they could not be reversed or mitigated by exposing the adult flies to a benign environment. In a previous plasticity study with these *D. subobscura* populations, we observed a negative effect of high developmental and adult temperatures on fecundity (Simões et al., 2020). In that experiment, we could not rule out the combined effect of stress in both life stages, as the tested individuals were kept in their whole life cycle at the same, higher temperature. The present results indicate that there is no combined negative effect of high developmental and adult temperature on reproductive performance. In fact, we observed that flies experiencing higher temperatures across life stages had a higher rate of sexual maturity (younger age of first reproduction) relative to those that only experienced high temperatures during the developmental stage. This is likely due to a faster maturation of

279 females (and, eventually, males) in the imago's stage due to faster metabolism at higher
280 temperatures (Clarke and Fraser, 2004). This could, eventually, result in a faster mating
281 speed following emergence, leading to a higher impact on age of first reproduction rather
282 than on fecundity patterns.

283 We found reduced adult fitness in flies exposed to "non-optimal", hotter
284 developmental environments, when compared to those developed at control conditions,
285 which does not support the "hotter is better" hypothesis. These "within-generation",
286 negative carry-over effects have been thoroughly described in *Drosophila* (Kirk Green et
287 al., 2019; Klepsatel et al., 2019; Porcelli et al., 2017). Such effects were, also, found in
288 other ectothermic animals (*e.g.* insects, (Cao et al., 2018; Iossa et al., 2019; Klockmann
289 et al., 2017; Zhang et al., 2015), lizards (*Podarcis muralis*, Van Damme et al., 1992); and
290 starfish (*Parvulastra exigua*, Balogh and Byrne, 2020). Such detrimental effects might
291 result from the irreversible damage of physiological/metabolic pathways and processes,
292 like gametogenesis, brought upon by stressfully high developmental temperatures.
293 Spermatogenesis starts early during embryonic development and the ovary
294 morphogenesis only takes place at the larva-pupa transition (Ashburner and Wright,
295 1980). Spermatogenesis, in fact, has been reported to be particularly vulnerable to heat
296 stress, with male sterility occurring at around 25ºC (David et al., 2005). Thus, the longer
297 maturity rate and reduced fecundity observed here might be (at least, partly) due to lower
298 sperm quality/output in males. Also, Porcelli et al. (2017) found that temperatures ~ 24ºC
299 lead to reduced sperm motility in *D. subobscura*. Future analyses should address the
300 extent to which female and male reproductive performances are (differentially) affected
301 by heat stress and the underlying physiological and metabolic changes in these
302 populations.

303 Furthermore, fly development at lower temperatures did not reduce adult
304 reproductive performance and, in the case of age of first reproduction, it even led to a
305 better performance relative to individuals raised in control conditions; this may be due to
306 a higher ovariole number in individuals raised at lower temperatures (Moreteau et al.,
307 1997). Yet, this positive effect was not observed when flies were more sexually mature,
308 near their peak fecundity. Conversely, performance was reduced when individuals were
309 developed and kept as adults at a lower temperature. The fact that fecundity under control
310 conditions (18ºC) was similar in flies developed at 18ºC or 14 ºC, suggests that the lower
311 adult performance at 14ºC was a result of a reduction in metabolic rate in adults kept at
312 that temperature. This might reduce oogenesis and lead to lower fecundity even if ovariole
313 number increased.

314 Recently, we have found evidence for cold but not heat acclimation in fecundity
315 patterns, with individuals developed at lower temperatures (15ºC) having higher
316 fecundity at 15ºC than those developed in control conditions – 18ºC (Simões et al., 2020).
317 At that point, we could not exclude that such pattern resulted, at least in part, of a general
318 better performance across different environments of flies raised at lower temperatures.
319 The present study indicates that development at lower temperatures does not always lead
320 to improved adult performance, as adult temperature also plays an important role in this
321 case. These results are in contradiction with the "colder is better" hypothesis of
322 developmental plasticity, which posits that individuals developed at colder temperatures
323 always have higher adult performance than individuals raised at other temperatures, i.e.
324 regardless of the test temperature in the adult stage (Huey et al., 1999, see also Zamorano
325 et al., 2017). With this body of data, we can now rule out that hypothesis, at least in the
326 case of fecundity traits, as individuals developed at lower temperatures did not show
327 increased performance when compared to those developed in control conditions. Finally,

these results neither support the "optimal acclimation hypothesis", since flies developed at the intermediate temperature did not show a higher performance (14-18 *vs*. 18-18 comparison; Figure 2).

Adaptation to different thermal environments is expected to result in differential thermal plasticity between populations (Angilletta, 2009; Mathur and Schmidt, 2017; Porcelli et al., 2017, see Kelly, 2019 for a review). Porcelli et al. (2017) reported geographical differentiation in the response to heat stress in *Drosophila subobscura*, with northern populations presenting lower viability and fertility. Previously, we found significant differences between the same latitudinal populations studied here, with a higher reproductive performance of the southern (PT) populations when subjected to lower temperatures in both the development and adult stages (Simões et al., 2020). Here, we did not observe any evidence for historical differences in response to lower temperatures. One explanation might be that in Simões et al. (2020), such differences were detected in early fecundity, *i.e.* fecundity tested during the first week of life, while in this study we focused solely on fecundity patterns near peak reproduction, *i.e.* between days 7 and 8. It is possible that the initial differences in fecundity between populations became diluted with time as exposure to colder conditions in adults might potentiate population differences in the rate of sexual maturation, which will reflect more on the initial amount of laid eggs (*i.e.* early fecundity).

Differences in reproductive performance between thermal treatments could be mediated by body size, since higher developmental temperatures lead to lower body sizes (Kingsolver and Huey, 2008). In a previous plasticity study, four generations earlier, we analyzed whether variation in wing size accounted for the differences in fecundity across populations and treatments. We concluded that wing size (used as a proxy for body size)

352 was unlikely to be an important factor generating such differences in our latitudinal
353 populations.

354 In summary, we here demonstrate that increasing the developmental temperature
355 of *D. subobscura* populations ~8ºC above control conditions leads to an irreversible
356 negative effect on reproductive performance, regardless of which adult temperature these
357 organisms are subjected. As previously noted, these results pinpoint the developmental
358 stage as very critical and vulnerable to climate change and associated heat waves (e.g. see
359 (Kingsolver et al., 2011; Klockmann et al., 2017). The low resilience to increased
360 temperatures during this early stage has likely detrimental consequences to population
361 fitness and persistence. In contrast, we show that this species copes well with colder
362 developmental temperatures, with reduction in performance only occurring when lower
363 temperatures are persistent across life stages.

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Tables

Table 1 – Test statistics for the effect of lower (14ºC) and higher (26ºC) developmental temperature on the thermal response of fecundity characters between populations.

	Trait	Model parameters	$F_{(df1, df2)}$
Higher temperature (26-18 *vs.* 18-18)	Age of First Reproduction (A1R)	Pop	$F_{1,4}$ = 1.792 n.s.
		Temp	$F_{1,4}$ = 331.78 ***
		Pop x Temp	$F_{1,4}$ = 0.599 n.s.
	Fecundity	Pop	$F_{1,4}$ = 0.769 n.s.
		Temp	$F_{1,4}$ = 141.89 ***
		Pop x Temp	$F_{1,4}$ = 1.737 n.s.
Lower temperature (14-18 *vs.* 18-18)	Age of First Reproduction (A1R)	Pop	$F_{1,4}$ = 1.641 n.s.
		Temp	$F_{1,4}$ = 20.891 *
		Pop x Temp	$F_{1,4}$ = 2.630 n.s.
	Fecundity	Pop	$F_{1,4}$ = 0.500 n.s.
		Temp	$F_{1,4}$ = 3.332 n.s.
		Pop x Temp	$F_{1,4}$ = 2.791 n.s.

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

520

521 Table 2 – Test statistics for the reversion of lower (14ºC) and higher (26ºC)
522 developmental temperatures effects on adult performance.

	Trait	Model parameters	$F_{(df1, df2)}$
Higher temperature (26-18 vs 26-26)	Age of First Reproduction (A1R)	Pop	$F_{1,4}$ = 0.017 n.s.
		Temp	$F_{1,4}$ = 15.010 *
		Pop x Temp	$F_{1,4}$ = 1.574 n.s.
	Fecundity	Pop	$F_{1,4}$ = 0.004 n.s.
		Temp	$F_{1,4}$ = 2.507 n.s.
		Pop x Temp	$F_{1,4}$ = 0.661 n.s.
Lower temperature (14-18 vs 14-14)	Age of First Reproduction (A1R)	Pop	$F_{1,4}$ = 0.563 n.s.
		Temp	$F_{1,4}$ = 398.41 ***
		Pop x Temp	$F_{1,4}$ = 0.761 n.s.
	Fecundity	Pop	$F_{1,4}$ = 0.346 n.s.
		Temp	$F_{1,4}$ = 61.787 **
		Pop x Temp	$F_{1,4}$ = 0.037 n.s.

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

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Figure Legends

Figure 1 – Experimental design: combinations of three developmental and three adulthood test temperatures.

Figure 2 – Reproductive performance of flies exposed to the five different thermal treatments (NL-left; PT-right): a) Age of first Reproduction; b) Fecundity (days 7 to 8). Data shows the average value for each replicate x temperature combination and replicates are identified by the respective number.

549 Figure 1

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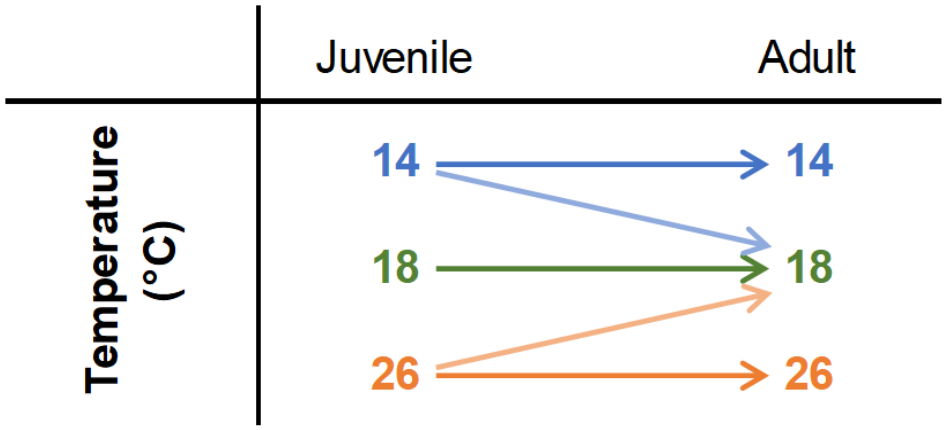

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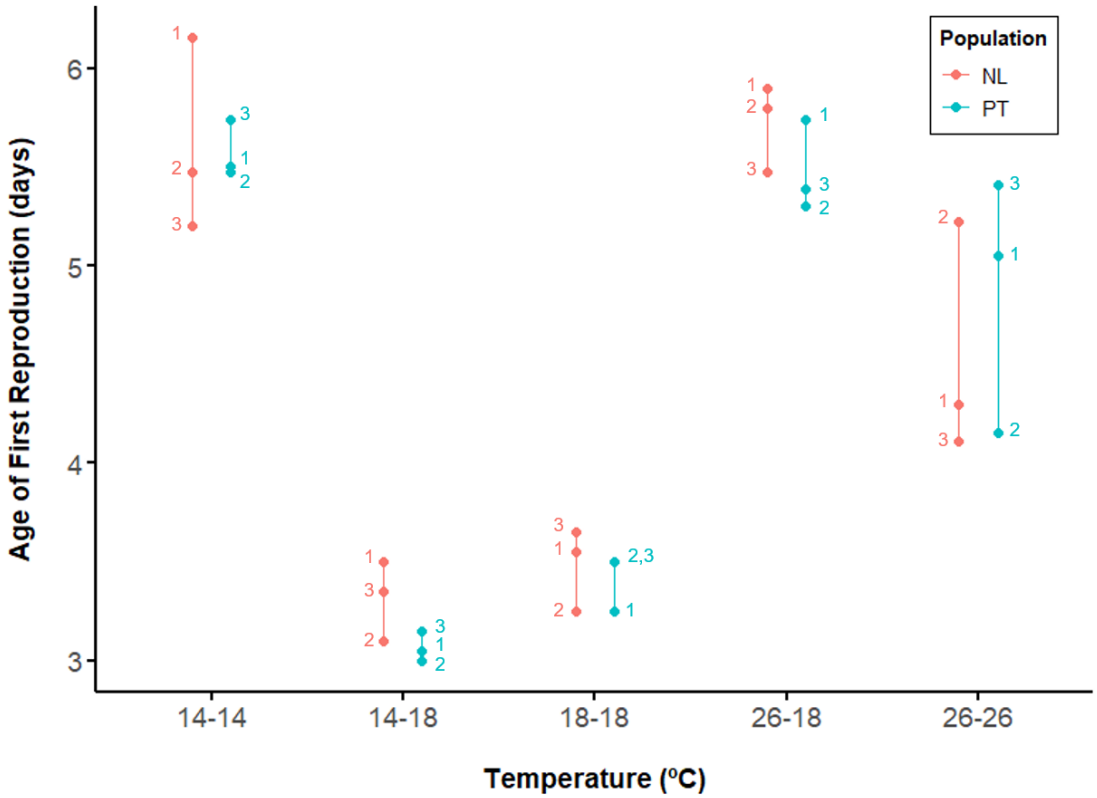
Figure 2A


Population
NL
PT
Age of First Reproduction (days)
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4
3
14-14
14-18
18-18
26-18
26-26
Temperature (°C)

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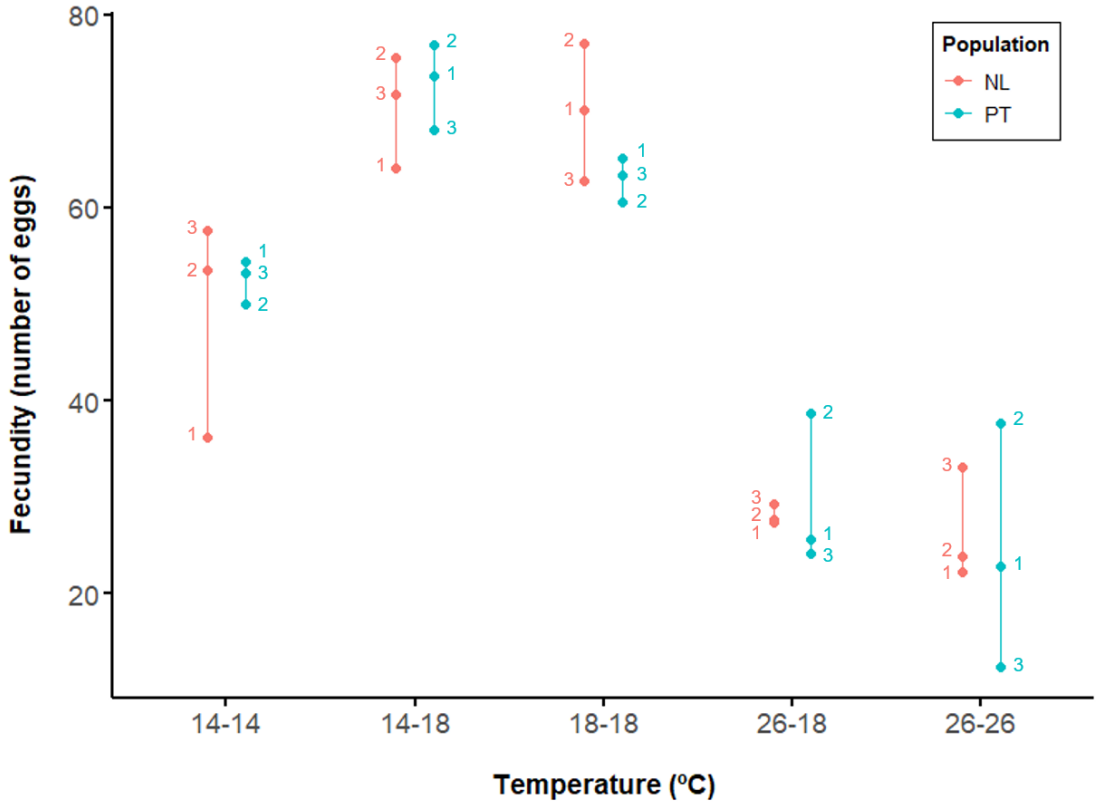
583 Figure 2B



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