Thermal Plasticity of Stage-specific Development Time and Adult Body Size under Temperature Shifts: A Case Study Using *Drosophila melanogaster*

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Abstract

We examined how thermal shifts influence development time and adult body size in *Drosophila melanogaster*. Individual flies were exposed to alternating temperatures of 25°C (optimal) and 17°C (cold), with shifts introduced at key developmental transitions: larval hatching and pupariation. We found while larval-stage temperature is the biggest determinant of thermal plasticity of development time and adult size, the egg-stage temperature also-influences the pace of development and growth throughout pre-adult duration. The effect of low-to-high and high-to-low temperature shifts on development and growth may not be symmetric. When eggs are reared at 25°C and then shifted to 17°C, larval and pupal durations undergo reduction compared to constant 17°C, but it produces slightly larger adults. A higher egg-stage temperature thus seem to exert a carryover effect that accelerates subsequent development and growth when later stages experience colder temperatures. Surprisingly, flies whose egg stage is exposed to 17°C followed by a shift to 25°C also have reduced larval duration and larger size, relative to those developing at constant 25°C. We speculate this could be either because 17°C to 25°C represents a low-to-high temperature shift or a sub-optimal-to-optimal thermal shift that results in metabolic and/or hormonal changes accelerating differentiation and growth. While pupal duration is sensitive to current and to some extent prior thermal environments, it does not contribute substantially to thermal plasticity of size. Development time is longer in males than in females, and this difference seems to start from larval stage while the pupal duration plays a bigger role in creating this sex-specific difference. Overall, employing individual fly rearing, this study helped to unravel the effect of thermal shifts on growth and development in *D. melanogaster* with great precision.

Keywords: thermal plasticity, life stage-specific thermal plasticity, development time, thermal sensitivity, thermal shift, temperature-induced developmental plasticity, body size, *Drosophila*

Introduction

Understanding how climate change affects organisms and shapes their adaptive responses are critical questions in thermal biology. Temperature influences a wide array of biological processes, including development, physiology, metabolism, behaviour, and life history (Huey and Kingsolver, 1989; Johnston and Bennett, 1996; Angilletta, 2009). While natural environments inherently exhibit temperature variability, organisms vary in their susceptibility to thermal changes (Angilletta, 2009). Ectotherms, in particular, are highly vulnerable due to their reliance on ambient temperatures to regulate body function (Addo-Bediako et al., 2000; Angilletta, 2009). Consequently, changes in environmental temperature can have immediate and profound effects on their performance and fitness (Huey and Stevenson, 1979; Pinsky et al., 2019). These effects are manifested through thermal plasticity of biological traits. In recent years, there has been growing interest in understanding mechanistic regulation and adaptive significance of thermal plasticity, as it reflects organismal responses to climate change (Trotta et al., 2006; Cooper et al., 2012; McDonald et al., 2018; Lafuente et al., 2024).

While research on thermal plasticity has traditionally focused on examining how various traits respond to a range of constant temperatures under controlled laboratory conditions (Trotta et al., 2006; Klepsatel et al., 2019; Lafuente et al., 2024), attention has also been paid to understanding how organisms respond to thermal fluctuations, which are intrinsic features of natural environments. Studies have investigated the effects of such fluctuations on development, performance, and life history traits (Al-Saffar 1995, 1996; Petavy et al., 2001; Manenti et al., 2015, 2021; Tobler et al., 2015) However, in addition to daily and seasonal variation, temperature can also differ across life stages, particularly in species with complex life cycles. For example, holometabolous insects go through different life forms during their development, each of which may occupy different microhabitats and thus may experience varying temperature and humidity (Taboada et al., 2013; Woods, 2013; Rolff et al., 2019; Kingsolver and Buckley, 2020; Vives-Ingla et al., 2023). Despite this, we know relatively little about how such stage-specific thermal variability influences developmental plasticity across the ontogeny.

Drosophila, a widely distributed ectothermic holometabolous insect, serves as an excellent model for investigating thermal plasticity. This is due to its broad latitudinal range, which reflects significant natural variation in temperature exposure (James et al., 1997; Trotta et al., 2006; Castañeda et al., 2015), and its experimental tractability, particularly the ease with

which temperature conditions can be precisely manipulated in the laboratory (Huey et al., 1991; Partridge and French, 1996; Ghosh et al., 2013; Kellermann and van Heerwaarden, 2019). Life cycle of *Drosophila* consists of four distinct stages: egg, larva, pupa, and adult. In natural environments, these stages may occupy different microhabitats and, consequently, diverse thermal conditions (Lockwood et al., 2018; Moghadam et al., 2019). The egg stage is immobile and typically laid on the surface of fermenting or rotting fruit. The larval stage is motile but largely confined within the fruit substrate, where temperature and humidity can be buffered (Evans et al., 2017; Schöneberg et al., 2020). As larvae mature, they exit the fruit to search for a suitable pupariation site, often exposing themselves to more variable environmental conditions (Sokolowski et al., 1986; Powell, 1997). In contrast, adults are fully mobile and can actively thermoregulate through behaviour (Reviewed in Dillon et al., 2012). These differences in spatial and behavioural ecology suggest that the thermal environment experienced by each ontogenetic stage can vary substantially due to factors such as substrate differences, daily temperature cycles, or extreme thermal events like heatwaves (Kingsolver et al., 2011; Moghadam et al., 2019; Schöneberg et al., 2020).

Previous research has shown that thermal tolerance and reaction norms can differ across life stages, implying that certain stages may be more thermally sensitive than others (e.g. Kingsolver et al., 2011; Austin and Moehring, 2013; Blanckenhorn et al., 2014; van Heerwaarden et al., 2014;). Building on these findings, we hypothesize that exposure to thermal variation during development will have stage-specific effects, potentially altering the duration of individual pre-adult stages and influencing final adult body size. Understanding these differential effects is critical for uncovering the mechanisms by which thermal environments shape developmental trajectories and life history outcomes in ectothermic organisms.

Thermal plasticity of developmental time has been extensively studied in *Drosophila*, and like most ectotherms, flies reared at lower temperatures exhibit prolonged development (Davidson and Davidson, 1944; Partridge et al., 1994b; Al-Saffar et al., 1995; Ghosh et al., 2013). Developmental events during embryogenesis scale uniformly across temperature, such that the sequence and proportional timing of events remain consistent even as the total duration changes (Kuntz and Eisen, 2014; Chong et al., 2018). Similarly, pre-adult stages including larval instars and the pupal period exhibit a proportionate increase in duration as temperature decreases (Ghosh et al., 2013). The optimal rearing temperature for *Drosophila*

melanogaster is approximately 25°C, whereas development at 17°C takes nearly twice as long. All key developmental stages, that is, egg hatching, larval growth, and pupation, are extended roughly two-fold at 17°C compared to 25°C (Ghosh et al., 2013).

While much is known about developmental plasticity under constant warm or cold temperatures (Huey et al., 1995; Ghosh et al., 2013; Fallis et al., 2014; Klepsatel et al., 2019; Szabla et al., 2024), and under daily temperature fluctuations (Le Vinh Thuy et al., 2016; Saxon et al., 2018; Manenti et al., 2021, 2015), relatively little is understood about how *Drosophila* development responds to thermal shifts occurring across developmental transitions. For example, what happens if temperature changes after larvae hatch from the eggs, or between larval and pupal stages? Does the timing of the shift matter? Does development continue to scale proportionally, or are certain stages more sensitive to changes than others? In light of these questions, we sought to investigate how stage-specific temperature shifts influence the subsequent development of the fly. In this study, we investigated how temperature shifts across different developmental stages, specifically egg, larva, and pupa affect the duration of subsequent stages and the overall development time of *Drosophila melanogaster*. Our goal was to identify the presence and nature of carryover effects, wherein thermal conditions experienced during an earlier developmental stage influence the duration of later stages. We asked: If a developmental stage is reared under relatively cold conditions but preceded by a warmer stage, does the prior exposure to warmer temperature accelerate the current stage's development? Conversely, does exposure to colder temperature during an earlier stage impose a developmental lag, even when the current stage is reared under optimal warm conditions? Additionally, we examined whether these carryover effects vary across the sexes.

Like development time, ectothermic growth is heavily reliant upon ambient temperature (Zuo et al., 2011; Ghosh et al., 2013; Kingsolver et al., 2015; Garrad et al., 2016). Pre-adult development and growth are major determinants of insect body size, as the adults have a hard exoskeleton that limits their capacity to grow (Simpson et al., 1980; Nijhout et al., 2006; Shingleton, 2010; Hanna et al., 2023). While factors like nutrition, age, and reproductive investment may lead to weight fluctuations in an adult insect to some extent (Gerofotis et al., 2019; Poças et al., 2022), size is principally contingent upon the growth achieved during its pre-adult development. 85% ectotherms grow bigger when they develop in colder environments. This inverse relationship between temperature and body size is known as the

temperature size rule (TSR) (Angilletta et al., 2004; Walters and Hassall, 2006; Einum et al., 2021). Temperature size rule is well-documented in *Drosophila* (David et al., 1994; French et al., 1998; Ghosh et al., 2013; McDonald et al., 2018). The possible fitness consequence of TSR and its developmental basis have been topics of intense debate for decades (Atkinson, 1994; Van Der Have and De Jong, 1996; Kingsolver and Huey, 2008). Larger body size in cooler environments may be advantageous because lower surface area-to-volume ratios reduce relative heat loss and enhance thermal retention (Bergmann 1847, Mitchell et al., 2018). However, the fitness consequences of TSR might be more complex than that, and a clear understanding of this phenomenon is still lacking. The developmental basis of TSR has been explored to some extent through empirical studies (Van Der Have and De Jong, 1996; Gibert and De Jong, 2001; Angilletta and Dunham, 2003; Ghosh et al., 2013). Higher temperature generally leads to an increase in both developmental and growth rates (French et al., 1998; Gillooly et al., 2001). Consequently, warmer temperature may make an organism grow faster in size but it would also reduce the duration of growth as the organism completes its development earlier than in colder temperatures (Zuo et al., 2011; Ghosh et al., 2013; Semsar-kazerouni et al., 2022) . This is thought to be the underlying reason for ectotherms developing a smaller body size in warmer temperatures (Van Der Have and De Jong, 1996; Atkinson and Sibly, 1997; Angilletta et al., 2004; Kingsolver et al., 2007). However, the mechanistic basis of TSR may be more nuanced than this and it may vary across species and thermal ranges (Davidowitz and Nijhout, 2004; Ghosh et al., 2013).

Thermal regulation of size in *Drosophila* is often studied either at a range of constant developmental temperatures (Partridge et al., 1994a; Nunney and Cheung, 1997; Ghosh et al., 2013; Lafuente et al., 2018; Klepsatel et al., 2019), or environments with daily thermal fluctuations (Kjærsgaard et al 2012; Manenti et al., 2015, 2021; Saxon et al., 2018). In contrast, how growth and plasticity are regulated when temperature varies across different developmental stages remains largely unexplored. The reason for the same is flies are typically grown in groups cultures, making it difficult to track the effects of changing temperatures on development in a stage-specific manner, because not all flies undergo transition synchronously between stages. In a study by Hoover and Marks, 2021, eggs were reared at 18°C and 28°C and cultures were shifted to alternate temperatures after 24 hours. The study showed that adult size in *Drosophila* is contingent upon early developmental temperature. Nonetheless, a comprehensive understanding of how thermal changes across

developmental stages shape body size plasticity, and whether these effects differ between male and female flies, remains unclear.

In view of the above, we devised a unique way and investigated how the temperature experienced during one stage influences growth during the subsequent stages and how variations in the thermal environment across different pre-adult developmental stages affect the final body size of flies. We employed a novel experimental design in which individual *Drosophila melanogaster* eggs were placed in microcentrifuge tubes containing food shortly after oviposition, then subjected to temperature shifts at key developmental transitions-either at hatching (egg-to-larva), pupariation (larva-to-pupa), or both. We used two contrasting but biologically relevant rearing temperatures: 25°C (optimal) and 17°C (cold), enabling us to implement both low-to-high and high-to-low temperature shifts. By introducing temperature changes at specific developmental transitions, we aimed to disentangle stage-specific versus cumulative effects of thermal conditions on developmental trajectories. This setup allowed precise measurement of stage-specific durations of males and females under each temperature condition and the body weight of individual flies upon emergence.

Materials and Methods

Population ancestry

We used a large, outbred laboratory population of *Drosophila melanogaster* named CO-mix for this study. It is a descendant of the ancestral CO-populations (Rose et al., 1984; Chippindale et al., 1993, 1997). 5 replicates of CO populations were mixed to create the CO-mix population. CO populations and their ancestors are being maintained at 25°C for decades. CO-mix flies are maintained at a population size of above 1200 flies, on a 14-day discrete generation cycle, at 25°C under 24 hours light, and ~70% relative humidity on standard cornmeal medium. Rearing density is controlled at ~70 eggs per 6 ml food in a vial. Twenty-five such vials are reared every generation for growing the flies, and upon eclosion, all flies are transferred to a population cage, and the eggs are collected after 3 days and incubated in vials, to initiate the next generation. At the time of the assay discussed in the paper, CO-mix populations were 15 generations old.

158 *Experimental protocol*

159 For assaying stage specific development time and body size of males and females, individual
160 flies were grown in microcentrifuge tubes (MCTs), each containing 300 µL of cornmeal food.
161 *Drosophila* development includes three major developmental transitions, namely egg to larva
162 (hatching), larva to pupa (pupariation), and pupa to adult (eclosion). For different thermal
163 treatments, thermal shifts between the two treatment temperatures, namely, 17°C and 25°C,
164 were performed either at hatching, or at pupariation, or at both. Total eight thermal
165 combinations were used for the experiment, as shown in Table 1.

166 A small agar piece containing a single egg was placed in each MCT within 2 hours of egg lay
167 from the CO-mix populations. In *D. melanogaster*, eggs appear smooth and swollen when
168 larvae are inside, and after larval hatching, the eggs appear crumpled. This way hatched vs.
169 unhatched eggs can be distinguished clearly under a microscope. For treatments in which
170 eggs were reared at 25°C, eggs were monitored for larval hatching 12 hours onwards post egg
171 collection, at every 2-hour intervals. The time of larval hatching was noted down for each
172 MCT. Hatching check was continued till 26 hours post egg collection, when no new eggs
173 hatched for 3 consecutive checks. Eggs that failed to hatch and became blackened, were
174 discarded. For treatments that required a downshift, individual tubes were shifted from 25°C
175 upon hatching and incubated at 17°C. For the remaining, MCTs were maintained at 25°C.
176 Similar protocol was followed for eggs reared at 17°C, and larval hatching was monitored
177 from 32 hours up to 42 hours post egg collection. For treatments requiring upshifts, MCTs
178 were shifted from 17°C to 25°C upon larval hatching, while for the other treatments, MCTs
179 were maintained at 17°C during the larval stages.

180 Hatched larvae were allowed to grow at specific temperatures and subsequently monitored
181 for pupariation after few days. MCTs were monitored for formation of pupae on inner wall of
182 the tube or lower side of the tube's lid every 4 hours. Upon pupariation, the time was noted,
183 and tubes were retained at 25°C or 17°C, for treatments that involved no shifts at pupariation.
184 Checks were continued till no new pupa was observed for 3 successive checks. Larvae that
185 failed to reach the wandering stage or pupariate, were discarded. For treatments involving
186 shifts at pupariation MCTs with pupae were shifted to specified temperature after noting their
187 pupariation time. As metamorphosis ensued over next few days and pupae became dark
188 indicating development of adult flies within pupal cases, individual tubes were monitored for

189 eclosion. As eclosion started, checks were continued every 4 hours and time was noted along
190 with sex of the flies. Upon eclosion, flies were freeze-killed by keeping them at -20˚C for 40-
191 45 mins and subsequently weighed using a Sartorius Quintix 35 (d = 0.01mg) balance.
192 Eclosion checks were continued till no new flies ecolsed for 3 successive checks.

193 For each thermal treatment, number of MCTs used ranged from 60 to 80, each containing 300
194 µl of cornmeal food. Initially we had anticipated survivorship of flies will be lower at 17°C
195 compared to 25°C as cold temperature has been shown to reduce survivorship in several
196 studies (McKenzie, 1975; Amarasekare and Sifuentes, 2012; Klepsatel et al., 2019).
197 Therefore, we used 60 and 80 eggs in treatments starting with 25°C and 17°C respectively.
198 We observed survivorship above 80% in all four treatments initiated at 17°C and in two of
199 the treatments initiated at 25°C. In the remaining two 25°C treatments, survivorship was
200 lower (72% and 78%), likely due to stochastic variation. The high survivorship in the 17°C
201 treatments may reflect some unknown aspect of fly rearing in MCTs. As a result, sample
202 sizes ranged from 43 to 71 across treatments.

203 *Data analysis*

204 For a given MCT, egg duration was calculated by subtracting the midpoint of the 2 hour-long
205 egg collection window from time of larval hatching. Larval duration for each fly was
206 calculated by subtracting hatching time from the time of pupariation. Pupal duration was
207 calculated by subtracting pupariation time from the time of eclosion. Total egg to adult
208 development time was also noted for individual flies in all 8 thermal treatments. Wet weight
209 of individual flies was noted.

210 All data, except those excluded based on predefined criteria (for example, failed eclosion or
211 incomplete developmental records), were included in the analysis. Prior to statistical testing,
212 each dataset was evaluated for normality using Shapiro-Wilk tests (Shapiro and Wilk, 1965)
213 and visual inspection of Q-Q plots (Supplementary figures S1-S5). Because development
214 time and wet weight measurements consistently violated assumptions of normality and
215 homoscedasticity across multiple treatment groups, all analyses were conducted using
216 nonparametric methods. Traditional nonparametric tests such as Kruskal-Wallis or Friedman
217 test are not suitable for the experimental design used in this study as both tests are limited to
218 single-factor comparisons and cannot model multi-way interactions between independent
219 variables. Because our dataset includes a four-factor full factorial structure (egg, larval, and

pupal temperatures, and sex), along with biologically meaningful interactions among these stages, an analysis framework capable of testing interaction terms was required. Therefore, we employed a full factorial aligned rank transform (ART) ANOVA for the analyses using r-package ARTool (Wobbrock et al., 2011; Elkin et al., 2021; Kay et al., 2025). ART was chosen because it enables nonparametric testing of multi-factorial designs while preserving interpretability of interaction effects. For egg duration effect of egg temperature, sex and their interaction were tested. For larval duration effect of egg and larval temperatures, sex, and interaction of the three factors were tested. For the remaining three variables, namely, pupal duration, egg to adult development time, and wet weight of flies, four-factorial ART ANOVAs were performed with egg, larval and pupal temperatures and sex as factors.

Post hoc pairwise comparisons were performed using estimated marginal means (emmeans) (Lenth and Piaskowski, 2017) computed on ART-transformed data, with p-values adjusted for multiple comparisons. All tests were two-sided unless indicated otherwise. No transformations were applied to the response variables prior to nonparametric testing. ART-ANOVA results including degrees of freedom, sum of squares, F-values, and adjusted p-values for factorial and pairwise analyses are reported in the Results section. Data analysis was performed using custom R scripts written for this study.

Results

Development Time

At 25°C, mean egg duration (hatching time) of flies is 21 h, while at 17°C eggs take 36 h to hatch on average (Supplementary figure S6), reflecting a 1.7-fold change in egg duration. Mean larval development time changes from 95 h to 196 h, and mean pupal development time increases from 96 to 201 h when flies are grown at 17°C as opposed to 25°C. Thus, larval and pupal durations extend by two-fold from 25°C to 17°C.

Egg and larval durations

17°C makes egg and larval durations significantly longer compared to 25°C ($p < 0.0001$). Egg duration does not differ across the sexes (Table 2).

Under constant temperature rearing, average larval duration at 25°C is 95 h, and at 17°C, it is 196 h. Egg and larval-stage temperatures show significant interaction in shaping larval

duration ($p < 0.0001$) (Figures 1a, 1b) (Table 3). Downshifting temperature from 25°Cat egg-stage to 17°C larval temperature (25°Cegg-17°Clarval) significantly reduces mean larval duration (181 h) relative to larval development time observed at constant 17°C (196 h) ($p < 0.0001$). Interestingly, upshifting temperature from 17°C to 25°C from egg to larval stage (17°Cegg-25°Clarval) also causes a small but significant reduction in mean larval duration (90 h) compared to larvae experiencing 25°C during both egg and larval stages (95 h) ($p < 0.0001$). ART ANOVA results indicate significant effect of sex ($p < 0.0001$), egg temperature × sex ($p < 0.0001$), and larval temperature × sex ($p = 0.0268$) interactions. The average difference between male and female larval duration pooled over different treatments is 0.9 h. Males have a slightly longer larval duration (0.5 to 4 h slower) than females across different thermal treatments.

Pupal duration

When pupae are reared at 25°C, their average development time ranges from 96 to 104 h across treatments. At 17°C mean pupal duration ranges from 172 to 201 h across treatments. Pupal duration shows significant effects of all three pre-adult stages and sex ($p < 0.0001$ for each) (Table 4). All two-way interactions involving egg larval and pupal temperatures are significant for pupal duration ($p < 0.001$ or 0.0001). However, the three-way interaction between egg larval and pupal temperatures reveals greater details ($p < 0.0001$) (Table 4).

When pupae are developing at 25°C (Figure 2a), keeping the egg temperature constant (either 25°C or 17°C), but altering the larval temperature, significantly changes the pupal duration. At 25-17-25°C mean pupal duration changes to 100 h from 96 h at 25-25-25°C ($p = 0.0002$). At 17-17-25°C, average pupal duration is 104 h, which is significantly longer than average pupal duration at 17-25-17°C (98 h) ($p = 0.0002$). On the other hand, for pupae developing at 25°C, keeping the larval temperature constant (either 25 or 17°C), but changing the egg temperature also shows significant change in pupal duration. At 17-17-25°C mean pupal duration is 104 h while, at 25-17-25°C it is 100 h, and the difference is significant ($p = 0.0009$). The difference between mean pupal duration at 17-25-25°C and 25-25-25°C is only 2 h (98 *vs.* 96 h), and it as significant ($p < 0.0001$). ART ANOVA can detect small but consistent differences as significant, and we suspect this to be the case for the comparison between these two groups."

When pupae are developing at 17°C (Figure 2b), keeping the egg temperature constant (25°C), and altering the larval temperature decreases mean pupal duration of 201 h at 17-17-

17°C to 195 h at 17-25-17°C. From 25-25-17°C to 25-17-17°C, the mean pupal duration increases from 172 h to 184 h. On the other hand, for 17°C pupal temperature, altering egg temperature while keeping larval temperature constant, changes the mean pupal duration of 201 h 17-17-17°C to 184 h at 25-17-17 h. Average pupal duration at 25-25-17°C is 172 h that extends to 195 h at 17-25-17°C. However, the pairwise comparisons did not detect any of these differences as significant. We suspect this may be due to slightly greater variability in development time at 17°C compared to 25°C, which could reduce the power of nonparametric tests to detect differences between groups.

Average pupal duration in males (148 h) is significantly longer than that of females (142 h) ($p < 0.0001$). The interaction of pupal temperature and sex is significant because females show an 87% increase in average pupal duration from 25°C to 17°C while males show a 93% increase for the same ($p < 0.0001$). The four-way interaction of egg-larval-pupal temperatures and sex is significant ($p = 0.0262$), but post hoc comparisons show no significant difference between different groups.

Egg to adult development time

Total or egg to adult development time is significantly influenced by all developmental stage temperatures and sex ($p < 0.0001$ for each) (Table 5). 17°C temperature during any stage significantly increases total developmental duration compared to 25°C.

All two-way interactions involving different developmental stage temperatures and three-way interaction of egg-larval-pupal temperatures are significant ($p < 0.0001$). Changing developmental temperature from constant 25°C to 17°C increases average egg to adult development time by 105% ($p < 0.0001$). Changing only egg temperature from 25°C to 17°C extends total development time by 6% (17-25-25°C *vs.* 25-25-25°C) ($p < 0.0001$) on average. Changing larval temperature from 25°C to 17°C increases total development time by 43% (25-17-25°C *vs.*25-25-25°C) ($p < 0.0001$) on average. Changing pupal temperature from 25°C to 17°C causes an average increase of 36% in total development time (25-25-17°C *vs.*25-25-25°C) ($p = 0.004$) (Figure 3a)

Changing egg temperature from 17°C to 25°C decreases total development time by 12% (17-17-17°C *vs.* 25-17-17°C) on average. Changing larval temperature from 17°C to 25°C reduces total development time by 26% (17-17-17°C *vs.* 17-25-17°C) on average. The mean reduction in total development time Changing pupal temperature from 17°C to 25°C shortens total development time by 23% (17-17-17°C *vs.*17-17-25°C) (Figure 3b). However, none of

these changes are significant. We suspect that because development time at each stage is longer at 17°C, variability is slightly higher than at 25°C, and as a result, post hoc comparisons on the transformed data do not detect significant differences among these groups.

Males have significantly longer total development time compared to females ($p < 0.0001$) (Figures 3a, 3b). Two-way interaction between egg temperature and sex is not significant, but those between larval temperature and sex, and pupal temperature and sex are significant ($p < 0.0001$ for each). Total development time of males is consistently longer than that of females across all groups. The interactions only reflect the slightly dissimilar degree of difference in thermal sensitivity of development time across sexes in some cases.

Body size (wet weight)

Wet weight of the flies is significantly influenced by larval temperature, pupal temperature and sex ($p < 0.0001$ for each) (Figures 4a, 4b) (Table 6), but egg temperature does not have a main effect on the trait. 17°C rearing temperature during larval stage leads to higher wet weight at eclosion compared to 25°C (1.46 mg *vs.* 1.18 mg per fly). The interaction of egg and larval temperature is significant for wet weight ($p < 0.0001$) (Figures 5a, 5b). Pairwise comparisons reveal that the flies reared at 25°C egg temperature and 17°C larval temperature is significantly bigger than flies having 17°C at both stages (1.50 *vs.* 1.42 mg) ($p = 0.01$). Besides, the other shift temperature treatment, namely, 17°C egg temperature followed by 25°C larval temperature also results in formation of bigger flies than those grown at 25°C egg and larval temperatures (1.23 *vs.* 1.13 mg) ($p < 0.0001$). Flies grown at 17°C egg-larval temperature and 25°C egg-larval temperature are not significantly different (1.42 *vs.* 1.13 mg), and we suspect variability in data and rank transformation lead to lack of significant difference between the two groups. The difference between wet weight of flies having pupal stage temperature of 17°C *vs.* 25°C is small (1.33 mg *vs.* 1.30 mg), but ART ANOVA detects it as significant. We speculate this to be a result of transforming the data to ranks and ART detecting a minute but consistent difference as significant. The three-way interaction of egg-larval temperatures and sex is significant but post hoc comparisons show no significant difference between different groups.

Females are significantly heavier than males (1.47 *vs.* 1.17 mg) (Figures 4a, 4b). Egg temperature × sex, larval temperature × sex, and egg temperature × pupal temperature × sex interactions are significant, but the pairwise comparisons do not show any significant

difference. Other two- and three-way interactions and the four-way interaction are not significant.

Discussion

Effect of thermal shifts on development time

This study provides several important insights into how varying thermal conditions across the ontogeny influence developmental duration and final body size in flies. First, the larval and pupal durations show similar thermal plasticity as both get extended two-fold (100%) from 25°C to 17°C. The egg duration or larval hatching time increases by 70% in the same thermal range.

Both low-to-high and high-to-low temperature shift at egg-larva transition accelerate subsequent larval development

When eggs are reared at 17°C instead of 25°C, average duration of the egg stage is lengthened by 15 hours (Supplementary figure S6). The difference in developmental speed set in by the egg stage temperature is carried onto the subsequent stages. When the egg-stage is at 25°C, but the hatched larvae are shifted to a cooler temperature of 17°C, the carryover effect of warmer egg temperature is observed during larval and development. Larvae at 17°C develop 8% faster on average compared to individuals developing at 17°C from the beginning (Figures 1a, 1b). This supports our *a priori* expectation that early exposure to a warmer temperature would leave an accelerating effect on subsequent development. (French et al., 1998; Gillooly et al., 2001; Zuo et al., 2011)

However, the reverse is not observed for larval development. Contrary to our prediction, exposure to a cold egg-stage temperature of 17°C does not prolong subsequent larval development. Instead, it results in a small but significant reduction in larval duration (Figures 1a, 1b). Thus, both upshifts and downshifts in temperature at the egg-to-larva transition leads to shorter larval development times. This aligns with some previous studies reporting modest acceleration of development under fluctuating thermal conditions (Roltsch et al., 1990, Al-Saffar et al., 1995,1996). Enzyme-catalyzed reaction rates are known to increase exponentially with temperature up to a thermal optimum (Somero, 1995; Hochachka and Somero, 2002). For organisms developing at suboptimal cold temperatures, enzymes likely operate with reduced catalytic efficiency. Studies suggest that when shifted to optimal temperatures, enzyme efficiency and metabolic flux in organisms may increase sharply

(Fields et al., 2001; MacMillan et al., 2012; Chung and Schulte, 2020). We speculate this could be one of the reasons for the larval development becoming faster at 17°Cegg-25°Clarval rearing temperature compared to constant 25°C rearing temperature.-

Effect of egg and larval-stage temperatures on pupal duration

Carryover effects of earlier stage temperatures influence pupal development time in the expected directions.

For pupae developing at 25°C, altering only the larval stage temperature while keeping the egg temperature same, changes the pupal duration significantly reflecting clear carryover effect of larval stage temperature on pupal development time (Figure 2a). Although similar effect of thermal variation of larval temperature is observed for pupae developing at 17°C, and the magnitude of the mean change in pupal duration is much longer (Figure 2b), due to our experimental design and nonparametric tests we could not find a significant change in the latter case. Albeit the data clearly points towards the impact of larval temperature on pupal duration in both directions. Thus, a larval temperature of 17°C extends the subsequent pupal duration while 25°C larval temperature has an accelerating carryover effect on the trait (Figures 2a, 2b).

The effect of 17°C to 25°C egg-larval thermal shift is slightly more complex. This shift causes small but significant acceleration of larval development (as discussed before), while statistically it is found to increase the pupal duration. However, on closer look the mean increase in pupal duration from 25-25-25°C to 17-25-25°C is found to be only 1.3 h. Therefore, we infer while this shift leaves an accelerating effect on larval development, its impact practically does not extend up to the pupal stage (Figure 2a).

The carryover effect of a warm egg-stage temperature on the pupal duration is remarkable. Like larval temperature, the impact of egg temperature on pupal duration at 17°C could not be detected statistically, but it is observed in the data quite clearly as 25°C egg temperature has an overall reducing effect on the pupal duration compared to flies that were kept at 17°C as eggs. The accelerating effect of 25°C egg temperature on pupal duration at 25°C is clear and statistically significant as a thermal shift done for 25-17-17°C reared flies, both larval and pupal durations are significantly reduced compared to that of 17-17-17°C reared flies. This suggests a warm egg stage sets in a higher developmental rate spanning the rest of the ontogeny compared to a warm larval stage. While the thermal plasticity of any given developmental stage and its carryover effect on the subsequent stage was expected, we found

the impact of egg stage temperature spanning the larval stage up to the pupal stage truly surprising. It suggests, for organisms who are exposed to fluctuating weather conditions during their development, developmental processes at a later stage may be contingent upon environmental conditions that occurred days ago and during a much earlier developmental stage (Angilletta et al., 2004; Kingsolver and Huey, 2008).

Contribution of different pre-adult stages to thermal plasticity of total development time

We also found that variation in egg-stage temperature results in the smallest change in overall development time (Figures 3a, 3b), which is expected given that the egg stage is the shortest. In contrast, variability in larval or pupal temperatures has a more pronounced impact on total development time (Figures 3a, 3b). The larval and pupal stages are of comparable length and exhibit similar thermal plasticity when rearing temperature shifts from 25°C to 17°C. However, change in larval temperature has a greater effect on overall development time than change in pupal temperature (Figures 3a, 3b) This contrast arises because the larval stage occurs earlier in development, and thus any temperature induced changes to larval duration can exert a downstream influence on pupal development. While the carryover effect of larval temperature on pupal duration is small and not always statistically significant, the combined impact of altered larval and pupal durations driven by larval temperature shifts results in a substantial change in total development time.

Development time in males vs. female flies

We found males have significantly longer pre-adult development time compared to females, and this is a well-established fact in *Drosophila. D. melanogaster* flies start mating within few hours of eclosion, and much of their sexual development occurs during the process of metamorphosis within pupae. (Casares et al., 1997; Whitworth et al., 2012; Testa et al., 2013) Literature suggests male flies take longer to attain sexual maturity and this causes longer duration of pupal development compared to females (Welbergen and Sokolowski, 1994; Nunney, 2007). However, our study indicates a small amount of delay in male development compared to females starts right from the larval stage. This is a novel finding as it suggests apart from sexual maturity there might be other sex-specific differences in development starting from larval stage that may have a small contribution in lengthening the development of male *Drosophila melanogaster.*

Body size regulation under thermal shifts

The larval stage

Wet-weight data from our study indicate that, similar to constant-temperature conditions, the larval stage plays the most significant role in determining the thermal plasticity of adult body size under temperature-shift scenarios (Ghosh et al., 2013). This is expected because the larval period is relatively long and is the only feeding stage during fly development when most somatic growth occurs.

The pupal stage

The results indicate a main effect of pupal temperature on body size but the mean difference between the flies reared at contrasting temperatures is negligible. Therefore, we suspect this significance appears due to data transformation rather than reflecting a biologically relevant difference. Our previous work has also shown that the pupal stage does not contribute to thermal plasticity of size at eclosion (Ghosh et al., 2013). This is not surprising because the pupal stage is non-feeding and the fly undergoes metamorphosis during this stage within an enclosure, while losing some weight in the process (Ghosh et al., 2013). While the pupal development time is sensitive to temperature, ~~the~~ weight loss during pupal stage is similar at 17°C and 25°C (Ghosh et al., 2013).

The egg stage

There is limited information on how egg-stage temperature contributes to size regulation in flies. In a study by Hoover and Marks, 2021, fly cultures were subjected to single thermal shifts 24 hours after egg laying (from 18°C to 28°C and *vice versa*) and compared with flies reared at constant 18°C and 28°C. Interestingly, flies reared under the 28-18°C regime grew as large as those reared entirely at 18°C. Likewise, flies reared at 18-28°C also showed similar body size to the 18°C group. These results suggest that a brief early exposure to warm temperature does not impact final size if the majority of development occurs in cold. Conversely, early cold exposure followed by warm temperatures throughout the remainder of development significantly influenced adult size, highlighting a lasting effect of early cold condition. The study, therefore, indicates that the early egg-stage temperature may influence adult body size, but the effect may not be symmetric for warm *vs.* cold temperature. However, the experimental design of the study does not allow for precise evaluation of the specific role of egg-stage temperature on adult body size. In our study, as we investigated the effect of thermal upshifts and downshifts exactly at the egg-larva transition, we found that flies reared at 25°C during the egg stage followed by 17°C during the larval stage (25°Cegg-17°Clarval) are larger than those reared at 17°C (17°Cegg-17°Clarval) (Figures 4, 5a, 5b). The possible

474 explanation for body size being bigger at 25-17°C is that a warmer egg-stage temperature
475 presumably initiates a slightly higher early growth rate in the developing insect. This is
476 consistent with our previous findings showing that larvae 24 hours post-hatching are larger at
477 25°C than at 17°C (Ghosh et al., 2013). Furthermore, our development time data demonstrate
478 that a warm egg-stage temperature accelerates the rate of all subsequent stages. Taken
479 together, our findings suggest that early exposure to warm temperatures may not only
480 enhance *developmental rate* but also promote increased *growth rate* in *Drosophila*.
481
482 Regarding the effect of cold temperature during the egg stage, our *a priori* expectation was
483 that exposure to a lower temperature during this period, followed by a warm larval phase,
484 would reduce adult fly size compared to constant warm conditions. This was based on the
485 assumption that early cold exposure would retard growth, which was supported by our earlier
486 observaion that larvae from eggs reared at 17°C are smaller during early larval stages than
487 those from eggs reared at 25°C (Ghosh et al., 2013). However, as mentioned before, our
488 finding is the opposite. 17-25°C treatment not only results in reduced larval duration
489 compared to constant rearing temperature of 25°C but also produces larger adult flies. This
490 suggests that a thermal shift from a cooler egg stage to a warmer larval stage may lead to a
491 modest increase in both developmental and growth rates, which possibly leads to a shorter
492 larval duration and bigger flies compared to flies grown at warm egg and larval stages.
493 Current models about thermal sensitivity of development and growth do not adequately
494 explain such outcomes under changing temperature regimes (Czarnoleski et al., 2013;
495 Manenti et al., 2021). A more detailed analysis of the thermal dynamics underlying
496 differentiation, metabolism, and growth, integrating theoretical modes and empirical research,
497 may help clarify these counterintuitive results.
498
499 Our findings indicate that the effect of egg-stage temperature on final body size is
500 asymmetric when there is a temperature shift at the egg-larva transition. We speculate that a
501 warm egg stage may elevate growth rates slightly, not only during the egg stage but also
502 during the subsequent cold larval stage, compared to constant cold rearing-suggesting that the
503 growth advantage begins *prior* to the thermal shift. Conversely, when the egg stage is cold,
504 the shift to a warmer larval environment may trigger changes in developmental and growth
505 dynamics, resulting in flies that are ultimately larger than those reared under constant warm
506 conditions. In this scenario, the size difference between constant warm and cold egg-warm

larval rearing likely emerges *after* the temperature shift. Taken together, our results suggest that egg-stage temperature can influence not only early larval size but also regulate overall growth trajectories in *Drosophila*, particularly under fluctuating thermal conditions.

Conclusion

The unique experimental design of this study allowed us to (a) investigate how the thermal environment of one pre-adult stage interacts with that of subsequent stages to shape developmental durations, and (b) precisely quantify the contribution of each life stage to the body size plasticity in *Drosophila melanogaster* under variable thermal environments. Together, these findings highlight that under variable temperatures, thermal responses emerge from a network of stage-specific sensitivities, cross-stage carryover effects, and nonlinear interactions between development and growth.

We show that development during any pre-adult stage is shaped not only by the ambient temperature but also by the thermal conditions experienced in the the past including thermal environment experienced several days earlier during an early developmental period like the egg stage can exert its effect on duration of developmental stages that occur much later, like the pupal stage. We found that the larval and pupal durations in flies are of comparable length and exhibit high thermal plasticity, while the relatively shorter egg stage has slightly lower plasticity. Although the larval and pupal stages have similar durations and comparable thermal plasticity, the larval temperature exerts a stronger influence on total developmental time. This is likely because thermal conditions during larval development not only determine larval duration but also leave a carryover effect that extends into and alters pupal duration.

Another important finding from the study is contrary to the simple expectation that a warmer temperature during an earlier stage would accelerate subsequent stages while a colder temperature would cause only a slowdown, our results demonstrate that the interactions between developmental stages and their thermal environments are far more complex. We show that a higher egg stage temperature may reduce developmental duration in subsequent cooler environments, but can paradoxically result in slightly larger flies due to enhanced early growth rates. Interestingly, a cold egg stage followed by a warmer larval stage can also shorten development time yet increase final body size compared to constant warm rearing. The exact mechanism for this phenomenon remains to be explored.

Additionally, while our study supports the established view that differences in development time among male and female flies arise primarily from differences in pupal duration, our results also indicate that males experience a slight developmental delay beginning as early as the larval stage. Another important finding of this study is that although the larval stage remains the primary determinant of adult body size, the egg-stage temperature also contributes to some extent. The contribution of pupal stage temperature on thermal plasticity of body size is negligible.

Several important directions emerge from this work. First, identifying the physiological and molecular mechanisms that mediate cross-stage thermal carryover effects remains a crucial next step. For example, investigating whether thermal shifts alter endocrine signaling, metabolic rates, energy utilization, or nutrient assimilation during development could illuminate why certain combinations of early and late temperatures accelerate development but still allow larger body sizes. Second, future experiments that manipulate temperatures dynamically, such as brief pulses, ramped thermal changes, or ecologically realistic fluctuating regimes, would help determine whether the patterns observed here generalize to more naturalistic conditions. Third, our findings raise intriguing questions about whether similar cross-stage interactions occur in other ectotherms. Comparative work across species with different life histories could clarify whether these carryover effects represent a general feature of ectothermic development or reflect *D. melanogaster* specific physiological responses. Finally, understanding how thermal history shapes body size and developmental timing has implications for predicting organismal responses to climate change. As natural thermal environments become increasingly variable, the ability of early thermal experiences to modulate later developmental outcomes may strongly influence population dynamics, fitness, and thermal adaptation.

Author contributions

AC: design of the study, acquisition of data, analysis of data, validation, drafting the article, revising the article critically for important intellectual content; RR: design of the study, acquisition of data, analysis of data, validation, revising the article critically for important intellectual content; PB: acquisition of data SG: conception and design of the study, analysis and interpretation of data, supervision, drafting the article, revising the article critically for important intellectual content, resources, funding acquisition. All authors approved the version submitted.

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Conflict of interest

We declare we have no competing interests.

Data availability

The raw data used in the study, and custom R scripts written and used for data analyses are available in the supplementary material.

Declaration of generative AI in scientific writing

During the preparation of this work generative AI has not been used.

References

Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. Proc. R. Soc. Lond. B Biol. Sci. 267, 739–745. https://doi.org/10.1098/RSPB.2000.1065

Al-Saffar, Z.Y., Grainger, J.N.R., Aldrich, J., 1996. Temperature and humidity affecting development, survival and weight loss of the pupal stage of *Drosophila melanogaster*, and the influence of alternating temperature on the larvae. J. Therm. Biol. 21, 389–396. https://doi.org/10.1016/S0306-4565(96)00025-3

Al-Saffar, Z.Y., Grainger, J.N.R., Aldrich, J., 1995. Influence of constant and changing temperature and humidity on the development and survival of the eggs and pupae of *Drosophila melanogaster* (Meigen). J. Therm. Biol. 20, 389–397. https://doi.org/10.1016/0306-4565(94)00075-T

Amarasekare, P., Sifuentes, R., 2012. Elucidating the temperature response of survivorship in insects. Funct. Ecol. 26, 959–968. https://doi.org/10.1111/J.1365-2435.2012.02000.X

Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Thermal Adaptation: A Theoretical and Empirical Synthesis 1–302. https://doi.org/10.1093/ACPROF:OSO/9780198570875.001.1

Angilletta, M.J., Dunham, A.E., 2003. The Temperature-Size Rule in Ectotherms: Simple Evolutionary Explanations May Not Be General. American Naturalist 162, 332–342. https://doi.org/10.1086/377187;WEBSITE:WEBSITE:UCHICAGO

Angilletta, M.J., Steury, T.D., Sears, M.W., 2004. Temperature, Growth Rate, and Body Size in Ectotherms: Fitting Pieces of a Life-History Puzzle. Integr. Comp. Biol. 44, 498–509. https://doi.org/10.1093/ICB/44.6.498

Atkinson, D., 1994. Temperature and Organism Size—A Biological Law for Ectotherms? Adv. Ecol. Res. 25, 1–58. https://doi.org/10.1016/S0065-2504(08)60212-3

Atkinson, D., Sibly, R.M., 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends Ecol. Evol. 12, 235–239. https://doi.org/10.1016/S0169-5347(97)01058-6

Austin, C.J., Moehring, A.J., 2013. Optimal temperature range of a plastic species, *Drosophila simulans*. Journal of Animal Ecology 82, 663–672. https://doi.org/10.1111/1365-2656.12041

Blanckenhorn, W.U., Gautier, R., Nick, M., Puniamoorthy, N., Schäfer, M.A., 2014. Stage- and sex-specific heat tolerance in the yellow dung fly *Scathophaga stercoraria*. J. Therm. Biol. 46, 1–9. https://doi.org/10.1016/J.JTHERBIO.2014.09.007

Casares, F., Sánchez, L., Guerrero, I., Sánchez-Herrero, E., 1997. The genital disc of *Drosophila melanogaster*. : I. Segmental and compartmental organization. Dev. Genes Evol. 207, 216–228. https://doi.org/10.1007/S004270050110

Castañeda, L.E., Rezende, E.L., Santos, M., 2015. Heat tolerance in *Drosophila subobscura* along a latitudinal gradient: Contrasting patterns between plastic and genetic responses. Evolution 69, 2721–2734. https://doi.org/10.1111/EVO.12757

Chippindale, A.K., Leroi, A.M., Kim, S.B., Rose, M.R., 1993. Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. J. Evol. Biol. 6, 171–193. https://doi.org/10.1046/J.1420-9101.1993.6020171.X

Chippindale, A.K., Leroi, A.M., Saing, H., Borash, D.J., Rose, M.R., 1997. Phenotypic plasticity and selection in *Drosophila* life history evolution. 2. Diet, mates and the cost of reproduction. J. Evol. Biol. 10, 269–293. https://doi.org/10.1046/J.1420-9101.1997.10030269.X

Chong, J., Amourda, C., Saunders, T.E., 2018. Temporal development of *Drosophila* embryos is highly robust across a wide temperature range. J. R. Soc. Interface 15. https://doi.org/10.1098/RSIF.2018.0304

Chung, D.J., Schulte, P.M., 2020. Mitochondria and the thermal limits of ectotherms. J. Exp. Biol. 223, jeb227801. https://doi.org/10.1242/JEB.227801

Cooper, B.S., Tharp, J.M., Jernberg, I.I., Angilletta, M.J., 2012. Developmental plasticity of thermal tolerances in temperate and subtropical populations of *Drosophila melanogaster*. J. Therm. Biol. 37, 211–216. https://doi.org/10.1016/J.JTHERBIO.2012.01.001

Czarnoleski, M., Cooper, B.S., Kierat, J., Angilletta, M.J., 2013. Flies developed small bodies and small cells in warm and in thermally fluctuating environments. J. Exp. Biol. 216, 2896–2901. https://doi.org/10.1242/JEB.083535

David, J.R., Moreteau, B., Gauthier, J.P., Pétavy, G., Stockel, A., Imasheva, A.G., 1994. Reaction norms of size characters in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis. Genetics Selection Evolution 1994 26:3 26, 229-. https://doi.org/10.1186/1297-9686-26-3-229

Davidowitz, G., Nijhout, H.F., 2004. The Physiological Basis of Reaction Norms: The Interaction Among Growth Rate, the Duration of Growth and Body Size. Integr. Comp. Biol. 44, 443–449. https://doi.org/10.1093/ICB/44.6.443

Davidson, A.J., Davidson, B.J.B., 1944. On the Relationship between Temperature and Rate of Development of Insects at constant Temperatures. Journal of Animal Ecology 13, 26. https://doi.org/10.2307/1326

Dillon, M.E., Liu, R., Wang, G., Huey, R.B., 2012. Disentangling thermal preference and the thermal dependence of movement in ectotherms. J. Therm. Biol. 37, 631–639. https://doi.org/10.1016/J.JTHERBIO.2012.07.004

Einum, S., Bech, C., Kielland, Ø.N., 2021. Quantitative mismatch between empirical temperature-size rule slopes and predictions based on oxygen limitation. Scientific Reports 2021 11:1 11, 23594-. https://doi.org/10.1038/s41598-021-03051-y

Elkin, L.A., Kay, M., Higgins, J.J., Wobbrock, J.O., 2021. An Aligned Rank Transform Procedure for Multifactor Contrast Tests. UIST 2021 - Proceedings of the 34th Annual ACM Symposium on User Interface Software and Technology 754–768. https://doi.org/10.1145/3472749.3474784;PAGE:STRING:ARTICLE/CHAPTER

Evans, R.K., Toews, M.D., Sial, A.A., 2017. Diel periodicity of *Drosophila suzukii* (Diptera: Drosophilidae) under field conditions. PLoS One 12, e0171718. https://doi.org/10.1371/JOURNAL.PONE.0171718

Fallis, L.C., Fanara, J.J., Morgan, T.J., 2014. Developmental thermal plasticity among *Drosophila melanogaster* populations. J. Evol. Biol. 27, 557–564. https://doi.org/10.1111/JEB.12321

Fields, P.A., Wahlstrand, B.D., Somero, G.N., 2001. Intrinsic versus extrinsic stabilization of enzymes: The interaction of solutes and temperature on A4-lactate dehydrogenase orthologs from warm-adapted and cold-adapted marine fishes. Eur. J. Biochem. 268, 4497–4505. https://doi.org/10.1046/J.1432-1327.2001.02374.X;ISSUE:ISSUE:DOI

French, V., Feast, M., Partridge, L., 1998. Body size and cell size in *Drosophila*: The developmental response to temperature. J. Insect Physiol. 44, 1081–1089. https://doi.org/10.1016/S0022-1910(98)00061-4

Garrad, R., Booth, D.T., Furlong, M.J., 2016. The effect of rearing temperature on development, body size, energetics and fecundity of the diamondback moth. Bull. Entomol. Res. 106, 175–181. https://doi.org/10.1017/S000748531500098X

Gerofotis, C.D., Kouloussis, N.A., Koukougiannidou, C., Papadopoulos, N.T., Damos, P., Koveos, D.S., Carey, J.R., 2019. Age, sex, adult and larval diet shape starvation resistance in the Mediterranean fruit fly: an ecological and gerontological perspective. Sci. Rep. 9, 1–12. https://doi.org/10.1038/S41598-019-47010-0;SUBJMETA=1466,158,2455,601,631;KWRD=ECOPHYSIOLOGY,ENTOMOLOGY

Ghosh, S.M., Testa, N.D., Shingleton, A.W., 2013. Temperature-size rule is mediated by thermal plasticity of critical size in *Drosophila melanogaster*. Proceedings of the Royal Society B: Biological Sciences 280. https://doi.org/10.1098/RSPB.2013.0174

Gibert, P., De Jong, G., 2001. Temperature dependence of development rate and adult size in *Drosophila* species: biophysical parameters. J. Evol. Biol. 14, 267–276. https://doi.org/10.1046/J.1420-9101.2001.00272.X

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of size and temperature on metabolic rate. Science (1979). 293, 2248–2251. https://doi.org/10.1126/SCIENCE.1061967;PAGEGROUP:STRING:PUBLICATION

Hanna, L., Lamouret, T., Poças, G.M., Mirth, C.K., Moczek, A.P., Nijhout, F.H., Abouheif, E., 2023. Evaluating old truths: Final adult size in holometabolous insects is set by the end of larval development. J. Exp. Zool. B Mol. Dev. Evol. 340, 270–276. https://doi.org/10.1002/JEZ.B.23165;JOURNAL:JOURNAL:15525015

Hoover, M.M., Marks, C., 2021. Short communication: Context matters: Adult size is contingent on embryonic temperature in *Drosophila melanogaster*. J. Therm. Biol. 95, 102820. https://doi.org/10.1016/J.JTHERBIO.2020.102820

Huey, R.B., Kingsolver, J.G., 1989. Evolution of thermal sensitivity of ectotherm performance. Trends Ecol. Evol. 4, 131–135. https://doi.org/10.1016/0169-5347(89)90211-5

Huey, R.B., Patridge, L., Fowler, K., 1991. Thermal Sensitivity of *Drosophila melanogaster* Responds Rapidly to Laboratory Natural Selection. Evolution (N Y). 45, 751. https://doi.org/10.2307/2409925

Huey, R.B., Stevenson, R.D., 1979. Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. Integr. Comp. Biol. 19, 357–366. https://doi.org/10.1093/icb/19.1.357

Huey, R.B., Wakefield, T., Crill, W.D., Gilchrist, G.W., 1995. Within- and between-generation effects of temperature on early fecundity of *Drosophila melanogaster*. Heredity 1995 74:2 74, 216–223. https://doi.org/10.1038/hdy.1995.30

James, A.C., Azevedo, R.B.R., Partridge, L., 1997. Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. Genetics 146, 881–890. https://doi.org/10.1093/GENETICS/146.3.881

Johnston, I.A., Bennett, A.F., 1996. Animals and Temperature Phenotipic and Evolutionary Adaptation. Seminar series (Society for Experimental Biology (Great Britain)) 59, 436.

Kay, M., Elkin, L.A., Higgins, J.J., Wobbrock, J.O., 2025. Package "ARTool" Title Aligned Rank Transform. https://doi.org/10.1145/1978942.1978963

Kellermann, V., van Heerwaarden, B., 2019. Terrestrial insects and climate change: adaptive responses in key traits. Physiol. Entomol. 44, 99–115. https://doi.org/10.1111/PHEN.12282;SUBPAGE:STRING:ABSTRACT;JOURNAL:JOURNAL:13653032B;ISSUE:ISSUE:DOI

Kingsolver, J.G., Arthur Woods, H., Buckley, L.B., Potter, K.A., MacLean, H.J., Higgins, J.K., 2011. Complex Life Cycles and the Responses of Insects to Climate Change. Integr. Comp. Biol. 51, 719–732. https://doi.org/10.1093/ICB/ICR015

Kingsolver, J.G., Buckley, L.B., 2020. Ontogenetic variation in thermal sensitivity shapes insect ecological responses to climate change. Curr. Opin. Insect Sci. 41, 17–24. https://doi.org/10.1016/J.COIS.2020.05.005

Kingsolver, J.G., Higgins, J.K., Augustine, K.E., 2015. Fluctuating temperatures and ectotherm growth: Distinguishing non-linear and time-dependent effects. Journal of Experimental Biology 218, 2218–2225. https://doi.org/10.1242/JEB.120733/262086/AM/FLUCTUATING-TEMPERATURES-AND-ECTOTHERM-GROWTH

Kingsolver, J.G., Huey, R.B., 2008. Size, temperature, and fitness: three rules.

Kingsolver, J.G., Massie, K.R., Ragland, G.J., Smith, M.H., 2007. Rapid population divergence in thermal reaction norms for an invading species: breaking the temperature–size rule. J. Evol. Biol. 20, 892–900. https://doi.org/10.1111/J.1420-9101.2007.01318.X

Klepsatel, P., Girish, T.N., Dircksen, H., Gáliková, M., 2019. Reproductive fitness of *Drosophila* is maximised by optimal developmental temperature. J. Exp. Biol. 222. https://doi.org/10.1242/JEB.202184

Kuntz, S.G., Eisen, M.B., 2014. *Drosophila* Embryogenesis Scales Uniformly across Temperature in Developmentally Diverse Species. PLoS Genet. 10, e1004293. https://doi.org/10.1371/JOURNAL.PGEN.1004293

Lafuente, E., Duneau, D., Beldade, P., 2024. Genetic basis of variation in thermal developmental plasticity for *Drosophila melanogaster* body pigmentation. Mol. Ecol. 33. https://doi.org/10.1111/MEC.17294

Lafuente, E., Duneau, D., Beldade, P., 2018. Genetic basis of thermal plasticity variation in *Drosophila melanogaster* body size. PLoS Genet. 14, e1007686. https://doi.org/10.1371/JOURNAL.PGEN.1007686

Le Vinh Thuy, J., VandenBrooks, J.M., Angilletta, M.J., 2016. Developmental plasticity evolved according to specialist-generalist trade-offs in experimental populations of *Drosophila melanogaster*. Biol. Lett. 12. https://doi.org/10.1098/RSBL.2016.0379/62483

Lenth, R. V., Piaskowski, J., 2017. emmeans: Estimated Marginal Means, aka Least-Squares Means. CRAN: Contributed Packages. https://doi.org/10.32614/CRAN.PACKAGE.EMMEANS

Lockwood, B.L., Gupta, T., Scavotto, R., 2018. Disparate patterns of thermal adaptation between life stages in temperate vs. tropical *Drosophila melanogaster*. J. Evol. Biol. 31, 323–331. https://doi.org/10.1111/JEB.13234;ISSUE:ISSUE:DOI

MacMillan, H.A., Williams, C.M., Staples, J.F., Sinclair, B.J., 2012. Reestablishment of ion homeostasis during chill-coma recovery in the cricket *Gryllus pennsylvanicus*. Proceedings of the National Academy of Sciences 109, 20750–20755. https://doi.org/10.1073/PNAS.1212788109

Manenti, T., Kjærsgaard, A., Schou, T.M., Pertoldi, C., Loeschcke, V., Moghadam, N.N., 2021. Responses to developmental temperature fluctuation in life history traits of five *Drosophila* species (Diptera: Drosophilidae) from different thermal niches. Insects 12. https://doi.org/10.3390/INSECTS12100925/S1

Manenti, T., Loeschcke, V., Moghadam, N.N., Sørensen, J.G., 2015. Phenotypic plasticity is not affected by experimental evolution in constant, predictable or unpredictable fluctuating thermal environments. J. Evol. Biol. 28, 2078–2087. https://doi.org/10.1111/JEB.12735

McDonald, J.M.C., Ghosh, S.M., Gascoigne, S.J.L., Shingleton, A.W., 2018. Plasticity through canalization: The contrasting effect of temperature on trait size and growth in Drosophila. Front. Cell Dev. Biol. 6, 423798. https://doi.org/10.3389/FCELL.2018.00156/BIBTEX

McKenzie, J.A., 1975. The Influence of Low Temperature on Survival and Reproduction in Populations of *Drosophila Melanogaster*. Aust. J. Zool. 23, 237–247. https://doi.org/10.1071/ZO9750237

Mitchell, D., Snelling, E.P., Hetem, R.S., Maloney, S.K., Strauss, W.M., Fuller, A., 2018. Revisiting concepts of thermal physiology: Predicting responses of mammals to climate

change. Journal of Animal Ecology 87, 956–973. https://doi.org/10.1111/1365-2656.12818;CTYPE:STRING:JOURNAL

Moghadam, N.N., Ketola, T., Pertoldi, C., Bahrndorff, S., Kristensen, T.N., 2019. Heat hardening capacity in *Drosophila melanogaster* is life stage-specific and juveniles show the highest plasticity. Biol. Lett. 15, 20180628. https://doi.org/10.1098/RSBL.2018.0628

Nijhout, H.F., Davidowitz, G., Roff, D.A., 2006. A quantitative analysis of the mechanism that controls body size in *Manduca sexta*. J. Biol. 5. https://doi.org/10.1186/JBIOL43

Nunney, L., 2007. Pupal period and adult size in *Drosophila melanogaster*: a cautionary tale of contrasting correlations between two sexually dimorphic traits. J. Evol. Biol. 20, 141–151. https://doi.org/10.1111/J.1420-9101.2006.01214.X

Nunney, L., Cheung, W., 1997. The effect of temperature on body size and fecundity in female *Drosophila melanogaster*: Evidence for adaptive plasticity. Evolution (N Y). 51, 1529–1535. https://doi.org/10.1111/J.1558-5646.1997.TB01476.X

Partridge, L., Barrie, B., Fowler, K., French, V., 1994a. Thermal evolution of pre-adult life history traits in *Drosophila melanogaster*. J. Evol. Biol. 7, 645–663. https://doi.org/10.1046/J.1420-9101.1994.7060645.X

Partridge, L., Barrie, B., Fowler, K., French, V., 1994b. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. Evolution (N Y). 48, 1269–1276. https://doi.org/10.1111/J.1558-5646.1994.TB05311.X

Partridge, L., French, V., 1996. Thermal evolution of ectotherm body size: why get big in the cold? Animals and Temperature 265–292. https://doi.org/10.1017/CBO9780511721854.012

Petavy, G., David, J.R., Gibert, P., Moreteau, B., 2001. Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. J. Therm. Biol. 26, 29–39. https://doi.org/10.1016/S0306-4565(00)00022-X

Pinsky, M.L., Eikeset, A.M., McCauley, D.J., Payne, J.L., Sunday, J.M., 2019. Greater vulnerability to warming of marine versus terrestrial ectotherms. Nature 569, 108–111. https://doi.org/10.1038/s41586-019-1132-4

Poças, G.M., Crosbie, A.E., Mirth, C.K., 2022. When does diet matter? The roles of larval and adult nutrition in regulating adult size traits in *Drosophila melanogaster*. J. Insect Physiol. 139, 104051. https://doi.org/10.1016/J.JINSPHYS.2020.104051

Powell, J.R., 1997. Progress and Prospects in Evolutionary Biology: The *Drosophila* Model. Progress and Prospects in Evolutionary Biology. https://doi.org/10.1093/OSO/9780195076912.001.0001

Rolff, J., Johnston, P.R., Reynolds, S., 2019. Complete metamorphosis of insects. Philosophical Transactions of the Royal Society B: Biological Sciences 374. https://doi.org/10.1098/RSTB.2019.0063/42344

Roltsch, W.J., Mayse, M.A., Clausen, K., 1990. Temperature-Dependent Development Under Constant and Fluctuating Temperatures: Comparison of Linear Versus Nonlinear Methods for Modeling Development of Western Grapeleaf Skeletonizer (Lepidoptera: Zygaenidae). Environ. Entomol. 19, 1689–1697. https://doi.org/10.1093/EE/19.6.1689

Rose, M.R., Dorey, M.L., Coyle, A.M., Service, P.M., 2011. The morphology of postponed senescence in *Drosophila melanogaster*. https://doi.org/10.1139/z84-230 62, 1576–1580. https://doi.org/10.1139/Z84-230

Saxon, A.D., O'Brien, E.K., Bridle, J.R., 2018. Temperature fluctuations during development reduce male fitness and may limit adaptive potential in tropical rainforest *Drosophila*. J. Evol. Biol. 31, 405–415. https://doi.org/10.1111/JEB.13231

Schöneberg, T., Arsenault-Benoit, A., Taylor, C.M., Butler, B.R., Dalton, D.T., Walton, V.M., Petran, A., Rogers, M.A., Diepenbrock, L.M., Burrack, H.J., Leach, H., Van Timmeren, S., Fanning, P.D., Isaacs, R., Gress, B.E., Bolda, M.P., Zalom, F.G., Roubos, C.R., Evans, R.K., Sial, A.A., Hamby, K.A., 2020. Pruning of small fruit crops can affect habitat suitability for *Drosophila suzukii*. Agric. Ecosyst. Environ. 294, 106860. https://doi.org/10.1016/J.AGEE.2020.106860

Semsar-kazerouni, M., Siepel, H., Verberk, W.C.E.P., 2022. Influence of photoperiod on thermal responses in body size, growth and development in *Lycaena phlaeas* (Lepidoptera: Lycaenidae). Current Research in Insect Science 2. https://doi.org/10.1016/j.cris.2022.100034

Shapiro, S.S., Wilk, M.B., 1965. An Analysis of Variance Test for Normality (Complete Samples). Biometrika 52, 591. https://doi.org/10.2307/2333709

Shingleton, A.W., 2010. The regulation of organ size in *Drosophila*. Organogenesis 6, 76–87. https://doi.org/10.4161/ORG.6.2.10375

Simpson, P., Berreur, P., Berreur-Bonnenfant, J., 1980. The initiation of pupariation in *Drosophila* : dependence on growth of the imaginal discs. Development 57, 155–165. https://doi.org/10.1242/DEV.57.1.155

Sokolowski, M.B., Bauer, S.J., Wai-Ping, V., Rodriguez, L., Wong, J.L., Kent, C., 1986. Ecological genetics and behaviour of *Drosophila melanogaster* larvae in nature. Anim. Behav. 34, 403–408. https://doi.org/10.1016/S0003-3472(86)80109-9

Somero, G.N., 1995. Proteins and temperature. Annu. Rev. Physiol. 57, 43–68. https://doi.org/10.1146/ANNUREV.PH.57.030195.000355

Szabla, N., Maria Labecka, A., Antoł, A., Sobczyk, Ł., Angilletta, M.J., Czarnoleski, M., 2024. Evolution and development of *Drosophila melanogaster* under different thermal conditions affected cell sizes and sensitivity to paralyzing hypoxia. J. Insect Physiol. 157, 104671. https://doi.org/10.1016/J.JINSPHYS.2024.104671

Taboada, A., von Wehrden, H., Assmann, T., 2013. Integrating Life Stages into Ecological Niche Models: A Case Study on Tiger Beetles. PLoS One 8, e70038. https://doi.org/10.1371/JOURNAL.PONE.0070038

Testa, N.D., Ghosh, S.M., Shingleton, A.W., 2013. Sex-Specific Weight Loss Mediates Sexual Size Dimorphism in *Drosophila melanogaster*. PLoS One 8. https://doi.org/10.1371/JOURNAL.PONE.0058936

Tobler, R., Hermisson, J., Schlötterer, C., 2015. Parallel trait adaptation across opposing thermal environments in experimental *Drosophila melanogaster* populations. Evolution (N Y). 69, 1745–1759. https://doi.org/10.1111/EVO.12705

Trotta, V., Calboli, F.C.F., Ziosi, M., Guerra, D., Pezzoli, M.C., David, J.R., Cavicchi, S., 2006. Thermal plasticity in *Drosophila melanogaster*: A comparison of geographic populations. BMC Evolutionary Biology 2006 6:1 6, 67-. https://doi.org/10.1186/1471-2148-6-67

Van Der Have, T.M., De Jong, G., 1996. Adult Size in Ectotherms: Temperature Effects on Growth and Differentiation. J. Theor. Biol. 183, 329–340. https://doi.org/10.1006/JTBI.1996.0224

van Heerwaarden, B., Lee, R.F.H., Overgaard, J., Sgrò, C.M., 2014. No patterns in thermal plasticity along a latitudinal gradient in *Drosophila simulans* from eastern Australia. J. Evol. Biol. 27, 2541–2553. https://doi.org/10.1111/JEB.12510

Vives-Ingla, M., Sala-Garcia, J., Stefanescu, C., Casadó-Tortosa, A., Garcia, M., Peñuelas, J., Carnicer, J., 2023. Interspecific differences in microhabitat use expose insects to contrasting thermal mortality. Ecol. Monogr. 93, e1561. https://doi.org/10.1002/ECM.1561;WEBSITE:WEBSITE:ESAJOURNALS;JOURNAL:JOURNAL:15577015;WGROUP:STRING:PUBLICATION

Walters, R.J., Hassall, M., 2006. The temperature-size rule in ectotherms: May a general explanation exist after all? American Naturalist 167, 510–523. https://doi.org/10.1086/501029

Welbergen, P., Sokolowski, M.B., 1994. Development time and pupation behavior in the *Drosophila melanogaster* subgroup (Diptera: Drosophilidae). J. Insect Behav. 7, 263–277. https://doi.org/10.1007/BF01989734/METRICS

Whitworth, C., Jimenez, E., Doren, M. Van, 2012. Development of sexual dimorphism in the *Drosophila* testis. Spermatogenesis 2, 129. https://doi.org/10.4161/SPMG.21780

Wobbrock, J.O., Findlater, L., Gergle, D., Higgins, J.J., 2011. The Aligned Rank Transform for nonparametric factorial analyses using only ANOVA procedures. Conference on Human Factors in Computing Systems - Proceedings 143–146. https://doi.org/10.1145/1978942.1978963;PAGE:STRING:ARTICLE/CHAPTER

Woods, H.A., 2013. Ontogenetic changes in the body temperature of an insect herbivore. Funct. Ecol. 27, 1322–1331. https://doi.org/10.1111/1365-2435.12124;PAGEGROUP:STRING:PUBLICATION

Zuo, W., Moses, M.E., West, G.B., Hou, C., Brown, J.H., 2011. A general model for effects of temperature on ectotherm ontogenetic growth and development. Proceedings of the Royal Society B: Biological Sciences 279, 1840. https://doi.org/10.1098/RSPB.2011.2000

Tables

Table 1: Schematic of the 8 thermal treatments

Treatment	Egg	Larva	Pupa	Sample size (N)
1	25°C	25°C	25°C	48
2	25°C	17°C	25°C	43
3	25°C	17°C	17°C	53
4	25°C	25°C	17°C	47
5	17°C	17°C	17°C	68
6	17°C	17°C	25°C	69
7	17°C	25°C	17°C	70
8	17°C	25°C	25°C	71

Table 2: ANOVA table from aligned rank transform (ART) analysis showing nonparametric factorial effects of egg temperature and sex on egg duration

Effect	*df*	*df.res*	*SS*	*F*	*P*
Egg temperature	1	465	6213590	1543.12	<0.0001
Sex	1	465	14851.5	0.86047	0.35409
Egg temperature × Sex	1	465	4395.58	0.24968	0.61753

Table 3: ANOVA table from aligned rank transform (ART) analysis showing nonparametric factorial effects of egg temperature, larval temperature and sex on larval duration

Effect	*df*	*df.res*	*SS*	*F*	*P*
Egg temperature	1	461	620849	38.4675	<0.0001
Larval temperature	1	461	6114328	1323.02	<0.0001
Sex	1	461	463047	27.5745	<0.0001
Egg temperature × Larval temperature	1	461	2147225	166.063	<0.0001
Egg temperature × Sex	1	461	678796	42.2873	<0.0001
Larval temperature × Sex	1	461	85342.5	4.93496	0.026804
Egg temperature × Larval temperature × Sex	1	461	16796.2	1.07689	0.299938

Table 4: ANOVA table from aligned rank transform (ART) analysis showing nonparametric factorial effects of egg temperature, larval temperature, pupal temperature and sex on pupal duration.

Effect	*df*	*df.res*	*SS*	*F*	*P*
Egg temperature	1	453	2271449	172.548	<0.0001
Larval temperature	1	453	2532872	200.069	<0.0001
Pupal temperature	1	453	6058874	1312.78	<0.0001
Sex	1	453	907893	57.9492	<0.0001
Egg temperature × Larval temperature	1	453	604683	36.0436	<0.0001
Egg temperature × Pupal temperature	1	453	2140315	159.691	<0.0001
Larval temperature × Pupal temperature	1	453	299457	17.1511	<0.0001
Egg temperature × Sex	1	453	191251	10.6788	0.001166
Larval temperature × Sex	1	453	820118	48.8803	<0.0001
Pupal temperature × Sex	1	453	444640	26.7229	<0.0001
Egg temperature × Larval temperature × Pupal temperature	1	453	382196	22.6091	<0.0001
Egg temperature × Larval temperature × Sex	1	453	90393.9	5.01351	0.02563
Egg temperature × Pupal temperature × Sex	1	453	34886.9	1.9032	0.1684
Larval temperature × Pupal temperature × Sex	1	453	7956.81	0.49468	0.48221
Egg temperature × Larval temperature × Pupal temperature × Sex	1	453	90729.3	4.97527	0.026201

Table 5: ANOVA table from aligned rank transform (ART) analysis showing nonparametric factorial effects of egg temperature, larval temperature, pupal temperature and sex on egg to adult development time

Effect	*df*	*df.res*	*SS*	*F*	*P*
Egg temperature	1	453	5223961	758.663	<0.0001
Larval temperature	1	453	6020071	1302.97	<0.0001
Pupal temperature	1	453	6120664	1387.88	<0.0001
Sex	1	453	1938412	140.633	<0.0001
Egg temperature × Larval temperature	1	453	1380870	92.5074	<0.0001
Egg temperature × Pupal temperature	1	453	1336785	92.2378	<0.0001
Larval temperature × Pupal temperature	1	453	187510	11.3818	0.000805
Egg temperature × Sex	1	453	62754.1	3.56971	0.059481
Larval temperature × Sex	1	453	636798	40.3336	<0.0001
Pupal temperature × Sex	1	453	846499	53.8916	<0.0001
Egg temperature × Larval temperature × Pupal temperature	1	453	75627.6	4.53923	0.033665
Egg temperature × Larval temperature × Sex	1	453	37064.7	2.43229	0.119557
Egg temperature × Pupal temperature × Sex	1	453	7659.8	0.43298	0.510863
Larval temperature × Pupal temperature × Sex	1	453	119593	8.47546	0.003777
Egg temperature × Larval temperature × Pupal temperature × Sex	1	453	232648	13.5555	0.00026

Table 6: ANOVA table from aligned rank transform (ART) analysis showing nonparametric factorial effects of egg temperature, larval temperature, pupal temperature and sex on wet weight of flies

Effect	*df*	*df.res*	*SS*	*F*	*P*
Egg temperature	1	453	1496.06	0.07957	0.778
Larval temperature	1	453	3825454	402.21	<0.0001
Pupal temperature	1	453	294177	16.3024	<0.0001
Sex	1	453	4044877	445.393	<0.0001
Egg temperature × Larval temperature	1	453	472308	26.6533	<0.0001
Egg temperature × Pupal temperature	1	453	16423.3	0.87377	0.3504
Larval temperature × Pupal temperature	1	453	37295.1	1.99581	0.1584
Egg temperature × Sex	1	453	103906	5.59182	0.0184
Larval temperature × Sex	1	453	72646.5	3.90096	0.0488
Pupal temperature × Sex	1	453	131.576	0.007	0.9333
Egg temperature × Larval temperature × Pupal temperature	1	453	58989.3	3.16659	0.0758
Egg temperature × Larval temperature × Sex	1	453	1758.68	0.09366	0.7597
Egg temperature × Pupal temperature × Sex	1	453	148197	8.03298	0.0048
Larval temperature × Pupal temperature × Sex	1	453	43245.1	2.33958	0.1268
Egg temperature × Larval temperature × Pupal temperature × Sex	1	453	27.0376	0.00145	0.9696

Figures

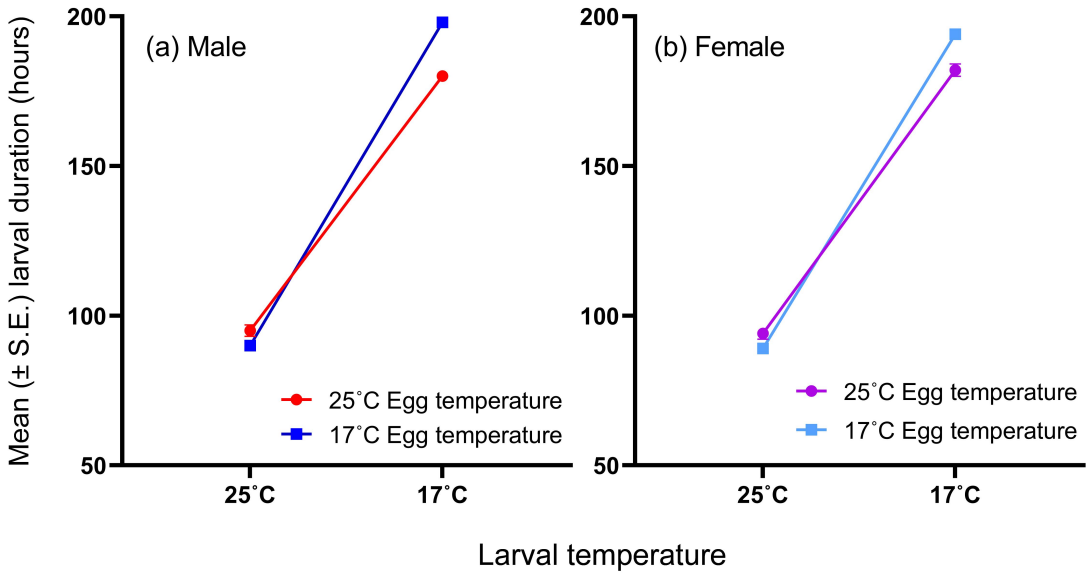

Figure 1: Reaction norm of mean larval duration across larval temperatures for a given egg stage temperature in (a) male and (b) female flies. The error bars in both plots represent standard error across replicate MCTs.

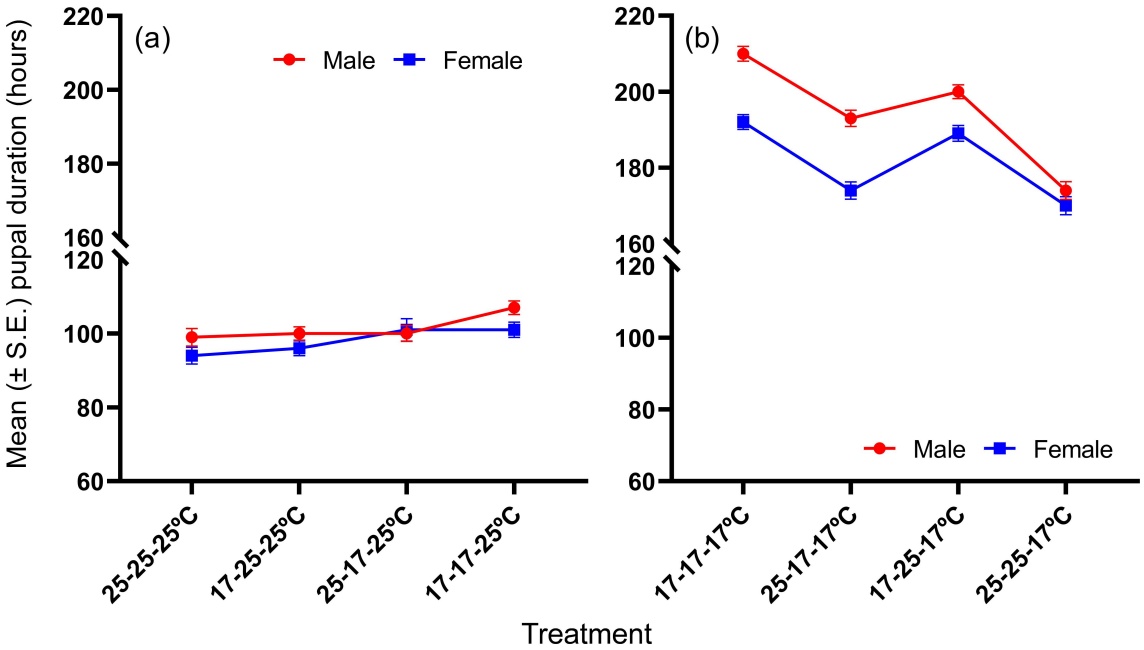

Figure 2a: Mean pupal duration at 25°C across different thermal pre-treatments. The error bars represent standard error across replicate MCTs.
Figure 2b: Mean pupal duration at 17°C across different thermal pre-treatments. The error bars represent standard error across replicate MCTs.

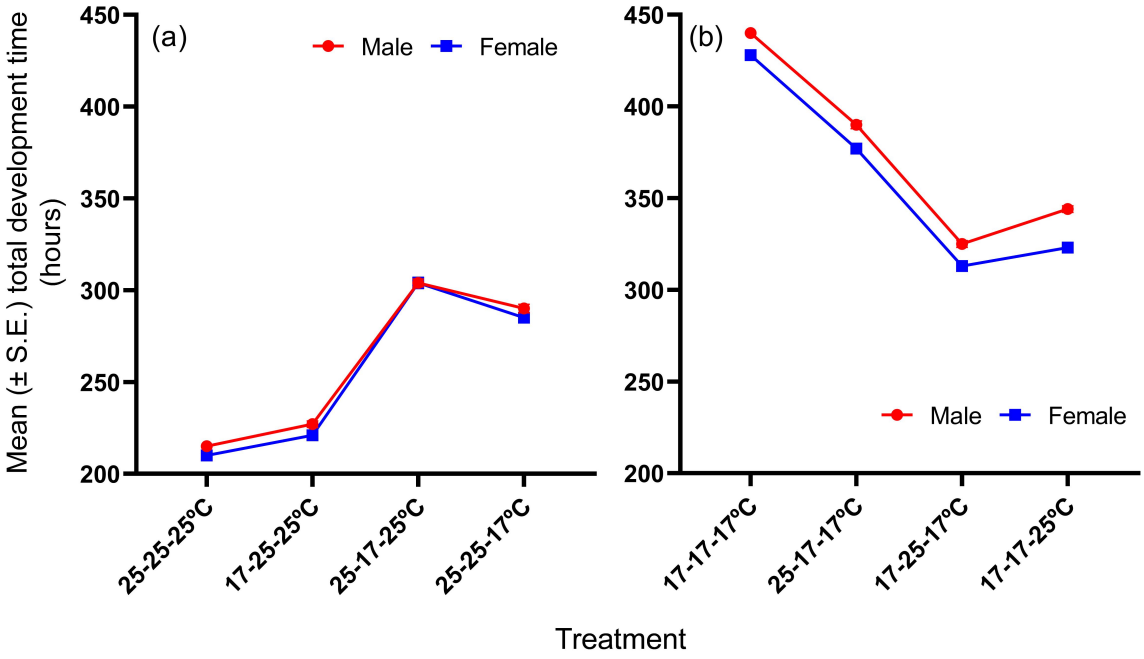

Figure 3a: Mean sex-specific egg to adult development time across thermal treatments in which temperature is gradually changed from 25°C to 17°C across different life stages (first egg, then larva, then egg-larva both). The error bars represent standard error across replicate MCTs.

Figure 3b: Mean sex-specific egg to adult development time across thermal treatments in which temperature is gradually changed from 17°C to 25°C across different life stages (first egg, then larva, then egg-larva both). The error bars represent standard error across replicate MCTs

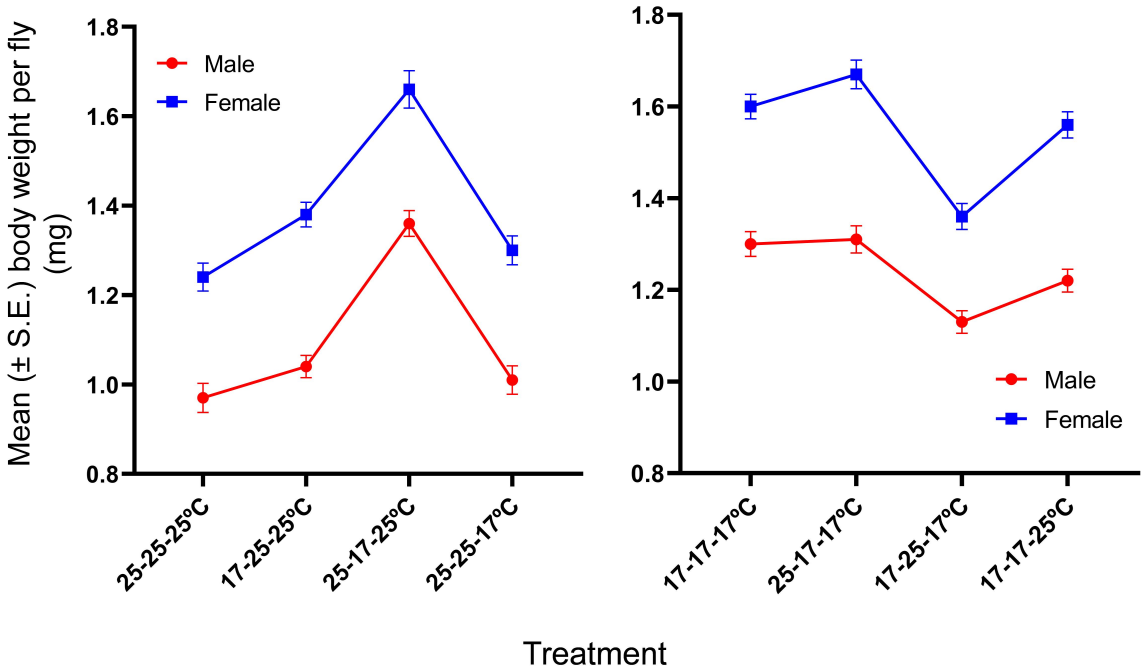

Figure 4a: Mean sex-specific wet weight of flies across thermal treatments in which temperature is gradually changed from 25°C to 17°C across different life stages (first egg, then larva, then egg-larva both). The error bars represent standard error across replicate MCTs.

Figure 4b: Mean sex-specific wet weight of flies across thermal treatments in which temperature is gradually changed from 17°C to 25°C across different life stages (first egg, then larva, then egg-larva both). The error bars represent standard error across replicate MCTs.

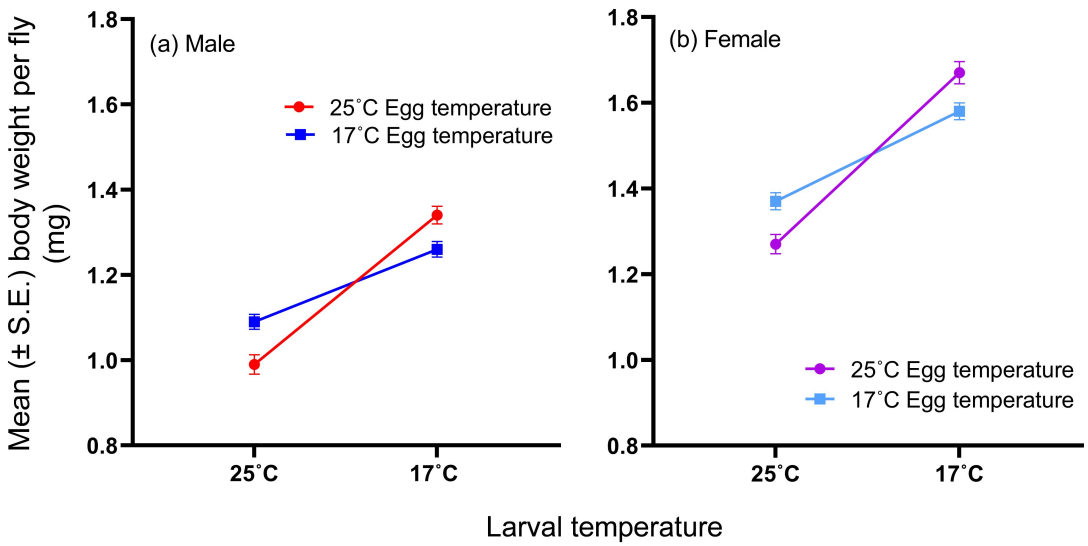

Figure 5a: Mean wet weight of male flies for combination of egg and larval stage temperatures. The error bars represent standard error across replicate MCTs.

Figure 5b: Mean wet weight of female flies for combination of egg and larval stage temperatures. The error bars represent standard error across replicate MCTs.