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Thermal Preference Plasticity in Ectotherms: Integrating Temperature Affinity and Thermoregulation Precision

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ABSTRACT: Thermal preference (Tp) prevents ectotherms from encountering sublethal temperatures. Its plasticity likely modulates the importance of behavioral thermoregulation under changing conditions. While it has been widely recognized that Tp varies across ontogeny, the plasticity level of this trait across life stages is poorly understood. We propose a novel conceptual framework relating two plastic components of Tp: its mean, which indicates temperature affinity, and its variance, which informs on the precision of behavioral thermoregulation. We tested this framework at the population scale by measuring Tp variations across life stages of an insect model after several generations under contrasting developmental temperatures. Tp plastic responses differed among life stages. Generally, we obtained a bell-shaped relationship between temperature affinity and precision of thermoregulation, indicating a strategy to avoid suboptimal and supraoptimal temperatures in *Drosophila melanogaster*, but not in all life stages. We highlight the need to change the paradigm underlying the study of Tp plasticity beyond the use of a single metric (median or range) to better comprehend thermoregulatory strategies.

Keywords: Drosophila melanogaster, behavioral thermoregulation, phenotypic plasticity, selected temperature.

Introduction

The thermal biology of organisms provides crucial information to assess the potential impacts of climate change on the physiology, ecology, and distribution of ectotherms. Although thermal limits (critical or lethal temperatures) have been widely used to quantify the vulnerability of ectotherms to climate warming (Sunday et al. 2011, 2014; García-Robledo et al. 2016; Pincebourde and Casas 2019), species can use behavioral thermoregulation to exploit particular microclimates in their habitat (Huey et al. 2012; Caillon et al. 2014; Sunday et al. 2014; Leith et al. 2024). Indeed, species that exhibit precise thermoregulation are not expected to have evolved particularly high or plastic tolerance limits (Logan et al. 2019; Munoz and Bodensteiner 2019). Therefore, behavioral thermoregulation ability and its plasticity could be more relevant than thermal limits when assessing responses to environmental changes (Sears et al. 2016; Leith et al. 2024; Pincebourde 2024).

Thermal preference (Tp) is a comprehensive trait that directly reflects an organism's ability to thermoregulate behaviorally (Angilletta 2009). In its simplest definition, Tp is the body temperature (set point or range) an ectotherm selects when provided with a large range of temperatures, in the absence of constraints that apply in the field (e.g., predation, competition, pathogens). This trait, usually measured under controlled conditions in the laboratory (Dillon et al. 2009), is often used together with field body temperature patterns and the distribution of operative temperatures to assess the efficiency of behavioral thermoregulation (Hertz et al. 1993; Angilletta 2009). Thus, a large body of literature has focused on the Tp of ectotherms to determine its adaptive value, for instance with warm-adapted species displaying greater Tp than cold-adapted species. In general, the Tp matches relatively well the optimal temperature of various performance metrics, although this is not always the case (Martin and Huey 2008; Angilletta 2009; Huey et al. 2012). Tp also varies along environmental gradients, indicating adaptation of optimal temperature to local conditions (Levinton 1983; Trochet et al. 2018), a countergradient pattern of Tp (Freidenburg and Skelly 2004), or no variation, indicative of a Bogert effect (Logan et al. 2019). In addition, numerous factors influence Tp, including infection by *Wolbachia* (Truitt et al. 2019; Hague et al. 2021), circadian rhythm (Goda et al. 2014), social behavior (Stapley 2006), and water constraints (Rozen-Rechels et al. 2019). Globally, however, the level of plasticity of the Tp remains understudied and poorly conceptualized (Gvoždík 2012).

Tp is potentially very plastic at various levels. Seasonal plasticity was demonstrated in the Tp of newts and lizards, increasing the effectiveness of thermoregulation (Hadamová

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and Gvoždík 2011; Ortega et al. 2016). Similarly, in a montane horned lizard, one year of warmer temperatures was enough to induce an increase in preferred temperature (Domínguez-Guerrero et al. 2021). Tp plasticity resulting from acclimation or previous (intra- or transgenerational) thermal history was also demonstrated in several *Drosophila* species (Rajpurohit and Schmidt 2016; MacLean et al. 2019). In addition, the level of plasticity may vary according to age and life stage, the latter reflecting the ontogenetic variation that is often observed in thermal adaptation (Bowler and Terblanche 2008; Kingsolver et al. 2011; Colinet et al. 2013; Pincebourde and Casas 2015; Lockwood et al. 2018). The degree of plasticity in thermoregulation likely deviates between life stages with different mobility. For instance, adult insects might rely more on behavioral thermoregulation to manage thermal extremes, reducing their need for physiological plasticity, while less mobile insect larvae might depend more on physiological acclimation because of their reduced behavioral options (Marais and Chown 2008). In *Drosophila* species, both the Tp and its level of plasticity generally differ between larvae and adults (MacLean et al. 2019). Nonetheless, studies reporting information on the variation and level of plasticity of Tp across life stages remain rare.

Tp can be divided into two components, its mean (or median) and its variance (or range), either at the within-group level or across time at the individual level. Most studies focus on the mean Tp (e.g., Labra et al. 2009; Takeuchi et al. 2009) because it represents the central tendency of the temperature range targeted by a population in the context of behavioral thermoregulation (Camacho and Rusch 2017). Within populations, interindividual variability in the Tp is quantified by the variance component. This interindividual variation may reflect a bet-hedging strategy, where different individuals within the population adopt diverse Tp, thus increasing the chance of survival in fluctuating environmental conditions (i.e., spreading the risk strategy). This strategy could, however, constrain adaptive strategies where Tp evolves in response to environmental pressures (Kain et al. 2015). The relationship between mean and variance of the Tp is rarely investigated. The level of covariation between the two metrics can inform on the ability to perform behavioral thermoregulation with high precision in challenging thermal environments. In newts, seasonal acclimation resulted in an inverse relationship between the mean and range of Tp: higher mean Tp was associated with a decreased range of Tp (Hadamová and Gvoždík 2011), although this result was not directly interpreted. Both metrics can also respond differently, and independently, to environmental change, as reported in adult versus juvenile *Pardosa* spiders (Cabon et al. 2023). This complexity may result from differential levels of plasticity of the two metrics across life stages or aging.

In this study, we propose a conceptual framework that considers both the mean and the variance of Tp for any active thermoregulator species. This framework examines plastic changes in thermoregulatory capacities by addressing both the precision of thermoregulation and the thermal needs (fig. 1). The variance or range in Tp inversely reflects the precision of thermoregulation: higher variance or range indicates lower precision (fig. 1*A*). The mean or median Tp informs on the thermal need (or affinity): a high or low thermal need corresponds to a globally warm or cold preference, respectively (fig. 1*A*). While this framework can be applied to determine thermoregulatory strategies either at the within-group level or across time at the individual level, hereafter we draw conceptual hypotheses mostly in the context of changes in populational Tp by considering interindividual Tp variations. The Tp is expected to change in both variance and mean in response to many factors and according to the optimal temperature (i.e., temperature that produces the best overall performance for the relevant trait under study). Several hypotheses are plausible.

i. *Precision at extreme hypothesis*. The need for precise thermoregulation is high at suboptimal or supraoptimal temperatures and relatively lower near the optimal temperature. When the mean Tp is close to the optimum, the variance in Tp is high (i.e., the organism does not need to be very precise in temperature selection). However, at more extreme temperatures, precise temperature selection (lower variance) is required to avoid lethal temperatures (fig. 1*B*).

ii. *Precision at optimum hypothesis*. Conversely, the need for precise thermoregulation is low at suboptimal or supraoptimal temperatures and relatively high near the optimal temperature (fig. 1*C*).

iii. *Asymmetric precision hypothesis*. The constraints for precise thermoregulation apply only at one end of the temperature range, either at low (fig. 1*D*) or high (fig. 1*E*) temperature.

iv. *Stable precision hypothesis*. The thermal need does not impact the thermoregulation precision, which can be either low (fig. 1*F*) or high (fig. 1*G*) according to physiological requirements. Globally, multiple thermal optima may exist (Sinclair et al. 2016), and thus the Tp we are referring to corresponds to the average temperature that best supports overall (physiological) performance as a potential compromise between different thermal optima. These physiological optima may include metabolic rate, digestion rate, or rate of egg maturation, for instance. Another interpretation is that this thermal need (mean Tp) is at, or near, the optimal temperature for the intrinsic growth rate of the population (i.e., the temperature adults should select to maximize the population growth rate).

Various examples exist in the literature that can be interpreted as providing indirect support for one or more of these hypotheses. For example, because of the Jensen inequality

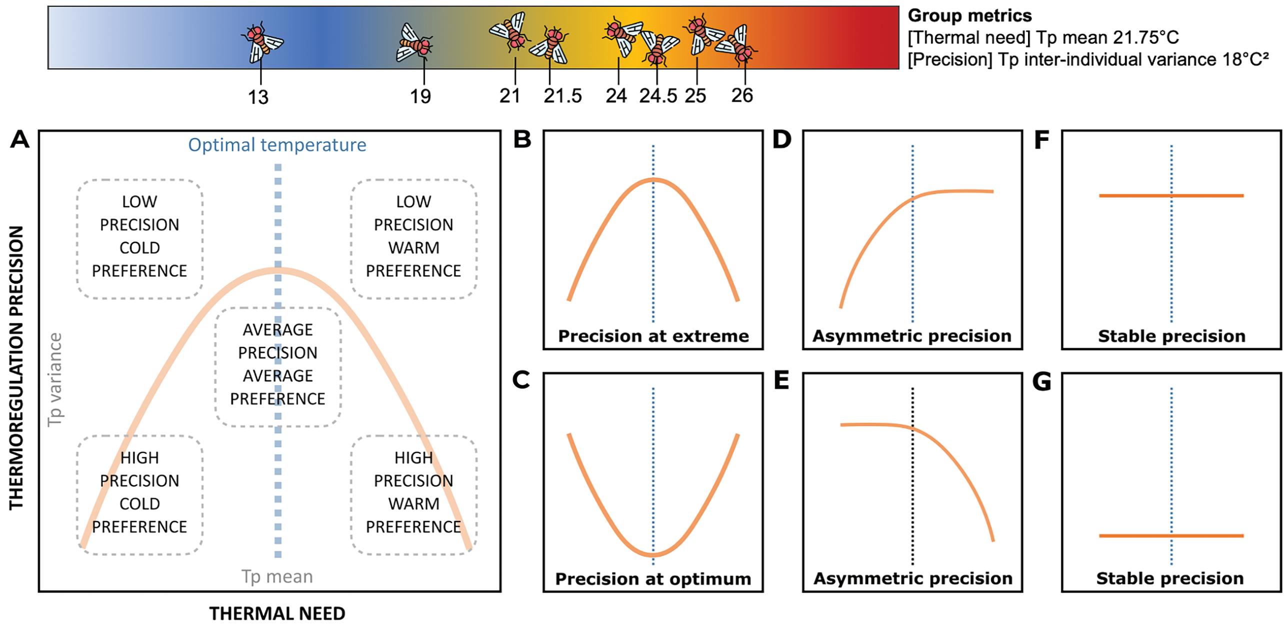

Figure 1: Conceptual view of plastic changes in thermal preference (Tp) metrics. *A*, Variation of the thermoregulation precision (Tp variance) according to the thermal need (mean Tp) of a population. The orange line represents a hypothetical relationship between the two variables. The dashed line represents a hypothetic optimal temperature. The five dashed cases indicate the thermoregulation precision and the thermal preference in each area of the plot. Plastic responses can modulate the thermoregulation precision and not change the thermal need, modulate the thermal need and not change the thermoregulation precision, or modulate both the thermal need and the thermoregulation precision. *B*–*G*, Hypothetical responses of plasticity-induced changes in Tp metrics. *B*, Precision at extreme hypothesis. The requirements for thermoregulation precision are high at both suboptimal or supraoptimal temperatures and relatively lower at the optimal temperature. *C*, Precision at optimum hypothesis. The requirements for thermoregulation precision are low at both suboptimal and supraoptimal temperatures and relatively higher at the optimal temperature. *D*, *E*, Asymmetric precision hypothesis. The requirements for precise thermoregulation apply only at one end of the temperature range, either at low (*D*) or high (*E*) temperature. *F*, *G*, Stable precision hypothesis. The thermal need does not impact the thermoregulation precision, which is either low (*F*) or high (*G*). The banner on top of these diagrams represents one technical aspect of Tp tackled in this study, that is, a linear thermal gradient with the position of multiple individuals providing their Tp after a waiting time, producing the following metrics for this group: the average of all individual Tp (thermal need) and the interindividual variability in Tp (thermoregulatory precision of the group).

(Martin and Huey 2008), it is riskier for organisms to be close to their critical thermal maximum, leading to a prediction of low variance when mean Tp is high (asymmetric precision hypothesis). A mygalomorph spider had the same mean Tp across different acclimation temperatures, but the Tp variance was lower when reared at high temperature (Alfaro et al. 2013). Another indirect line of evidence for a negative covariation between mean and variance of Tp was reported for several drosophilid species at the egg-laying stage (MacLean et al. 2019). Contrasting results, with either positive or negative covariation between the mean and variance of Tp, were reported in two marine snails (Lah et al. 2017). Additionally, acclimation to various temperatures can induce shifts in mean and variance of Tp independently (Reiser et al. 2014), producing a favorable context for a mix of hypotheses i and iv. Nevertheless, none of these studies set out to correlate changes in mean and variance of Tp, impeding any interpretation as to how plasticity in Tp components plays out to modulate the overall thermoregulatory strategy.

We tested this new framework with the fly *Drosophila melanogaster*. We measured the mean and variance of Tp throughout the life stages of *D. melanogaster* by focusing on the interindividual variability in temperature selection. We stimulated plastic responses by applying several treatments and factors (developmental temperatures, mating status: mature vs. young virgin adults), across different generations (G1 to G3), to obtain the largest overall range of Tp mean and variance as possible. Our aim was to assess the Tp at the population/group level to better analyze how plasticity can flex the overall thermoregulation response to various factors. We did not intend to estimate the variation of Tp at the individual level in this work; indeed, the thermoregulatory precision is hard to quantify at the individual level because the movement behavior becomes noisy at this level due to exploratory behaviors that may be independent of true Tp (Dillon et al. 2009). Instead, our reasoning at the population level allows us to conceptualize (plastic) Tp variations in a pragmatic manner: (1) the mean or median Tp of a given group/population provides a proxy of the temperature set point or range targeted by individuals (the thermal need), and thus we can analyze how plastic responses modulate this thermal need; and (2) we expect individuals to spread largely across the thermal gradient when there is only weak selective pressure to thermoregulate with precision (i.e., high interindividual variance), and by contrast we expect all individuals in a group to converge toward close temperatures when there is a high selective pressure to thermoregulate precisely (e.g., when they developed under elevated temperature).

We had various levels of comparison to test these expectations on *D. melanogaster*: (1) across life stages for a given developmental temperature (ontogenic or age effect), (2) among thermal treatments for a given life stage (level of plasticity), and (3) between generations to assess any intergenerational plasticity influence. We predicted that adults display more plasticity than larvae, with Tp more responsive to the temperature treatments in terms of both mean and variance. Adult flies are more likely to have more opportunity for behavioral thermoregulation than larvae, which are confined to the fruit where eggs were deposited. We found that the strength of covariation between mean and variance of Tp is lower in larvae than adults and that life stages followed different strategies for behavioral thermoregulation (see fig. 1). The level of plasticity varied widely and independently across the two components of Tp.

Material and Methods

Origin and Maintenance of Experimental Flies

We used a laboratory population of *Drosophila melanogaster* from the ECOBIO laboratory in Rennes, France. It was initially established from >100 flies captured in Rennes and Plancoët (Brittany, France) in 2010. Every year, new wild individuals collected in September are added to the mass population to limit inbreeding (Colinet et al. 2012). Therefore, this line has a wild phenotype and contained a natural level of genetic variability at the time the experiments were conducted in 2017. Several hundred individuals were reared at 25°C, 12L∶12D photoperiod, and 75% relative humidity (typical conditions for optimal population growth in this species), in 200-mL bottles containing 25 mL of standard fly medium consisting of sugar (saccharose, 50 g L^{-1}), brewer's yeast (80 g L^{-1}), agar (15 g L^{-1}), propionic acid (3 mL L^{-1}), and a fungicide solution (methyl-4-hydroxybenzoate-ethanol 95% solution, 8 mL L^{-1}). To generate flies for the experiments, groups of 15 mated females were allowed to lay eggs in 200-mL bottles during a 6-h period, to avoid overcrowding (Colinet et al. 2013).

Population Generations and Developmental Temperature Treatments

From the population reared at 25°C, about 500 adults were collected to constitute five subpopulations distributed among five climatic chambers (temperature precision ±1°C; Strader, Angers, France). Groups of 4–5-day-old adults were placed in 200-mL bottles containing medium for females to oviposit during a 6-h period. Adults were then removed, and bottles with eggs were randomly assigned to one of five thermal treatments: three constant treatments (C22: 22°C; C25: 25°C; C28: 28°C) and two fluctuating regimes differing in their thermal variance (F1: 23.5°C–26.5°C; F2: 22°C–28°C; both with a mean of 25°C), all under a 12L∶12D photoperiod and 75% relative humidity. In the fluctuating regimes, the coldest and warmest temperatures were reached at midnight and midday, respectively, and were maintained for almost 11.5 h (the transition lasted about 30 min). We measured the Tp of virgin young flies (V), which were isolated at emergence and left to age for 2 days, and of mature adults (A) of 12 days of age, as well as those of third-instar larvae (L3) and wandering third-instar larvae (L3W). To assess whether the responses to thermal treatments shift over generations, these measurements were repeated in generations one (G1) and three (G3). For adults, both sexes were tested.

Thermal Gradient Apparatus

Tp was measured by placing the insects in a linear thermal gradient encompassing temperatures from 10°C to 35°C. Our system was similar to the apparatus used in previous studies of Tp in *Drosophila* (Takeuchi et al. 2009; Goda et al. 2014). Tp assays were performed using a custom linear thermal gradient involving an aluminum slab (400 × 200 × 2 mm) heated on one side by an auto-adhesive resistance (40W) and cooled on the other side by a Peltier device (45W; fig. S1; figs. S1–S3 are available online). The system was placed in a climatically controlled laboratory at a constant air temperature of 22°C. A system of closed lanes was used to allow flies—both adults and larvae—to move along this temperature gradient without escaping and to easily determine the temperature experienced at each point from the insect's position along the gradient. For larvae, the lanes were filled with a homogeneous nutritional gel (composed of saccharose [50 g L^{-1}] and agar [7 g L^{-1}]), which allowed the displacements of larvae and prevented them from desiccation. Finally, the displacements of the insects inside the lanes of the thermal gradient were recorded with a camera (HDC-SD200, Panasonic) positioned above the system. The thermal gradient was calibrated before the experiments to determine the temperature at any position.

Tp Measurement

The setup described above was used to measure the Tp of L3, L3W, V, and A. For each trial, 20 individuals were introduced inside each lane to assess a populational Tp (total sample size = 120 individuals—i.e., 20 individuals × 6 lanes). The six lanes were considered as independent replicates because no communication or contact was possible among individuals from different lanes and the thermal gradient was perfectly reproducible from run to run. The individuals were filmed over 60 min. In all instances the movements were much less frequent after about 20 min for both larvae and adults. Therefore, we considered the Tp of every individual to be the temperature selected after 40 min—this is in line with the time point used in most studies of the Tp of fruit flies (see table S1; tables S1–S4 are available online). This period gave plenty of time for the flies to first explore their lane and second to make their choice as a resting temperature, such that any difference in locomotor ability between individuals is unlikely to affect the Tp measurement. The experimental assay started slightly differently between adults and larvae. For adults, the thermal gradient was off (i.e., 22°C across lanes) when introducing the flies into each lane, such that flies were randomly distributed inside each lane. The system was switched on after 10 min, and the gradient took another 10 min to stabilize. We measured males and females separately to detect any influence of sex. For larvae, we used the same approach as described by Takeuchi et al. (2009): larvae were deposited in the agar gel at the position corresponding to 28°C on each lane. The lane system containing larvae was then applied to the aluminum plate with the thermal gradient already active. All Tp assays were run within the same time window (between 10:00 and 18:00) to minimize any influence of the circadian rhythm of Tp. The light environment was homogeneous to avoid any directional attraction (Dillon et al. 2009).

Statistical Analyses

All data were analyzed in R (R Core Team 2022). Tp values below 13°C were removed from the analyses (7.9% of all data), as they corresponded to flies entering a quasi-comatose state (i.e., cold stupor; Vannier 1994) when encountering the cold end of the gradient during exploratory behavior (see also Dillon et al. 2009). Final sample sizes varied typically between 95 and 117 individuals per modality except in a few instances (see summary in table S2). Tp was analyzed using a maximum likelihood approach by fitting a generalized linear mixed model (GLMM) using the lmer function in the lme4 package (Bates et al. 2015). Tp values were square root transformed to get them near normality. GLMM included developmental temperature, life stage, sex, generation, and their interactions as fixed factors and the thermal gradient lane as a random factor. Model selection was done using the Akaike information criterion, resulting in the model including all interactions. We used Q-Q plots of the residuals to determine whether the assumptions of the models were met. For post hoc analyses, we ran separate GLMMs for each combination of life stage and generation, to determine the effect of developmental temperature on Tp. Pairwise differences between estimated marginal means were calculated using the R package emmeans (Lenth et al. 2024). To infer the relationship between Tp mean and variance, we estimated the mean Tp and the Tp variance for each group based on the position of individuals measured after 40 min in each lane of the gradient (males were removed from the analysis to exclude sex effect). Differences between groups in Tp mean were tested using GLMM on square-root-transformed Tp with developmental temperature, life stage, and their interactions as fixed factors and the thermal gradient lane as a random factor, followed by pairwise comparisons using the R package emmeans. Differences between groups in Tp variance were tested using ANOVA on residuals (equivalent to the Levene test), followed by Tukey's multiple comparison test. Despite a correlation between TP variance and TP range (fig. S2), we chose to use variance rather than range because variance provides a more accurate measure of dispersion around the mean. Finally, we applied a LOESS procedure (with tension 10) that allowed us to retrieve the pattern without a priori assumption of shape. The shape of the LOESS was visually compared with the hypothetical relationships listed in figure 1 to interpret the thermoregulation strategies.

Results

Tp Variations: General Patterns

Drosophila melanogaster's Tp was influenced by all explanatory variables and most of their interactions (table 1). In larvae, Tp increased with constant developmental temperatures, except for L3W in G3 (fig. 2). For instance, L3 larvae in G1 had a Tp of 23.6°C ± 0.3°C (mean ± SE) when developing at C22 and a Tp of 26.5°C ± 0.4°C at C28. Conversely, in virgin adults (V), Tp decreased from 23.9°C ± 0.4°C to 21.7°C ± 0.6°C when reared at C22 and C28 (in G1), respectively (fig. 2). In mature adults (A), Tp increased with developmental temperature in G1 but remained constant in G3 (fig. 2). Sex had inconsistent

Table 1: Summary of the linear mixed effect regression model of the influence of developmental temperature (DevT), life stage, sex, generation, and their interactions on thermal preference of *Drosophila melanogaster*

Explanatory variable	df	χ^2	P
DevT	4	63.2969	<.0001
Life stage	3	634.1703	<.0001
Sex	1	5.20041	.0226
Generation	1	21.6493	<.0001
DevT × life stage	12	256.4587	<.0001
DevT × sex	4	51.1238	<.0001
Life stage × sex	1	5.6220	.0177
DevT × generation	4	17.3841	.0016
Life stage × generation	3	119.9243	<.0001
Sex × generation	1	1.3188	.2508
DevT × life stage × sex	4	67.2771	<.0001
DevT × life stage × generation	12	83.3001	<.0001
DevT × sex × generation	4	11.0390	.0261
Life stage × sex × generation	1	1.6947	.1930
DevT × life stage × sex × generation	4	56.1484	<.0001

influence, with no differences, females having higher Tp than males, or vice versa.

Life Stage Plasticity in Tp Components

In G1, mean Tp decreased while Tp variance increased across life stages in all temperature treatments (fig. 3; tables S3, S4). Mean Tp increased with the constant temperature treatment in all life stages, with the exception of virgin adults (V) reared at C28 having the lowest mean Tp. Meanwhile, the Tp variance increased with constant temperature treatments in all life stages, with the exception of mature adults (A) reared at C28 being intermediate. The mean Tp of individuals that experienced fluctuating temperature regimes followed a similar relationship as at C25. Their Tp variance, however, differed. The Tp variance of L3 and L3W was similar to that at C22 and C25, respectively. For virgin (V) and mature adults (A), their Tp variances were lower compared with constant treatments, with mature adults (A) having the lowest Tp variance.

In G3, the decrease in mean Tp across life stages remained but the relationship was much steeper than in G1 (fig. 3; tables S3, S4), with an average decrease of 7.7°C between L3 and A adults (compared with 2.8°C in G1). Individuals at C28 had the steepest drop in mean Tp, from 30.8°C in L3 to 17.1°C in mature adults (A). The relationship between constant temperature treatments also changed relative to G1: in L3, the mean Tp was the lowest at C25 and the highest at C28. In mature adults (A), conversely, it was the lowest at C28 and the highest at C25. In fluctuating regimes, the mean Tp patterns were similar to those of C22, except for L3 in the F2 regime (22°C–28°C), which had the lowest mean Tp. The Tp variance differences among life stages were also much more pronounced than in G1. In constant temperature regimes, the Tp variance increased from L3 to virgin adults (V) and then dropped steeply in mature adults (A). The Tp variance of individuals in the F1 regime (23.5°C–26.5°C) followed the same pattern as that of C25 until virgin adult stage (V) but did not drop in mature adults (A), while individuals from the F2 regime (22°C–28°C) followed the same pattern as those from C22, with the exception of virgin adults (V).

The Relationship between Tp Variance and Mean

Overall, the relationship between Tp mean and variance was modulated by the plastic responses to all temperature treatments, both at intra- and intergenerational levels (LOESS curves, fig. 4). The relationships between Tp variance and mean differed between life stages and changed after two generations (fig. 4). The Tp variance of L3 from G1 increased with the mean Tp, corresponding to the asymmetric precision hypothesis (fig. 1*D*), indicating a requirement for precise thermoregulation behavior at low temperature. This relationship was reversed in L3 from G3, with a requirement for precise thermoregulation at high temperature (fig. 1*E*). L3W from G1 had a higher precision of thermoregulation behavior around the thermal optimum, corresponding to the precision at optimum hypothesis (fig. 1*C*). For G3 L3W, the thermoregulation precision was relatively high overall, corresponding to the stable precision hypothesis (fig. 1*G*). Nevertheless, the mean Tp of L3W displayed the lowest plasticity, impeding the assignment to any hypothesis. G1 and G3 virgin adults (V) had the same plastic response: the need for thermoregulation precision was high at suboptimal or supraoptimal temperatures and low around the thermal optimum, corresponding to the precision at extreme hypothesis (fig. 1*B*). Older mature adults (M) from G1 also showed this response but shifted in G3 to the asymmetric precision hypothesis (fig. 1*D*).

Discussion

The Tp provides crucial information on the ability of ectotherms to thermoregulate behaviorally and therefore to survive and/or reproduce in heterogeneous environments (Leith et al. 2024). The level of plasticity of Tp, if adaptive, should reflect an ectotherm's propensity to buffer against the negative effects of challenging or nonoptimal thermal conditions. However, in contrast with widely used traits such as thermal limits, the level of plasticity of Tp is poorly documented (Gvoždík 2012), and the factors that shape this plasticity remain uncertain. Our data illustrate that Tp

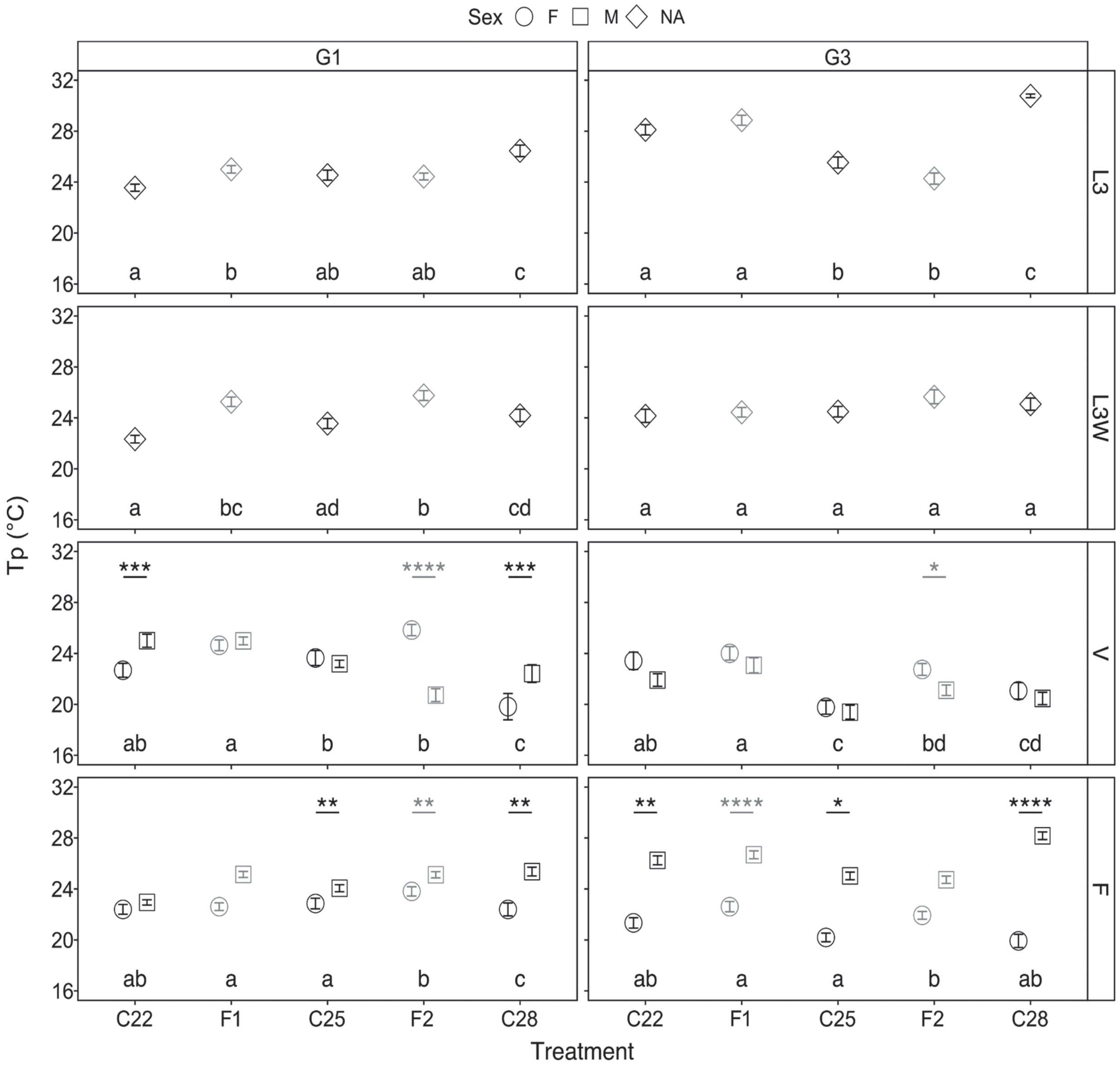

Figure 2: Plastic responses of thermal preference (Tp). Shown is the Tp of *Drosophila melanogaster* ($n = 6{,}173$) according to developmental temperature treatment (constant regimes C22 [22°C], C25 [25°C], and C28 [28°C] in black; fluctuating regimes F1 [23.5°C–26.5°C] and F2 [22°C–28°C] in gray). Panels show mean Tp with respect to generation (G1, G3) vertically and life stage (L3 = third larval stage; L3W = wandering larval stages; V = 2-day-old virgin adults; A = 12-day-old adults) horizontally. Each symbol represents the mean Tp for a replicate of a given sex (circles = females; squares = males; diamonds = undetermined sex, larvae). The error bars represent standard errors for mean Tp at a given temperature across all replicate experiments carried out for each life stage, generation, and sex. Significance of differences between temperature treatments and sex are represented by different letters (a, b, c, d; bottom of each panel) and asterisks ($^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$; $^{****}P < .0001$; top of each panel), respectively.

can display high levels of plasticity, at both intra- and intergenerational levels and across different life stages with variation of up to 15°C in the mean Tp—a level that is at least threefold higher than the plasticity of thermal limits in insects (compare with values provided in Gunderson et al. 2017). At the population or group level, both mean Tp (a metric of thermal affinity or need) and Tp variance (or any other metric of interindividual variations, as a proxy of thermoregulation precision) change depending on the current and previous thermal environment, suggesting that the ability to adjust thermoregulation precision is as plastic as the mean Tp itself. This highlights the need for a change in

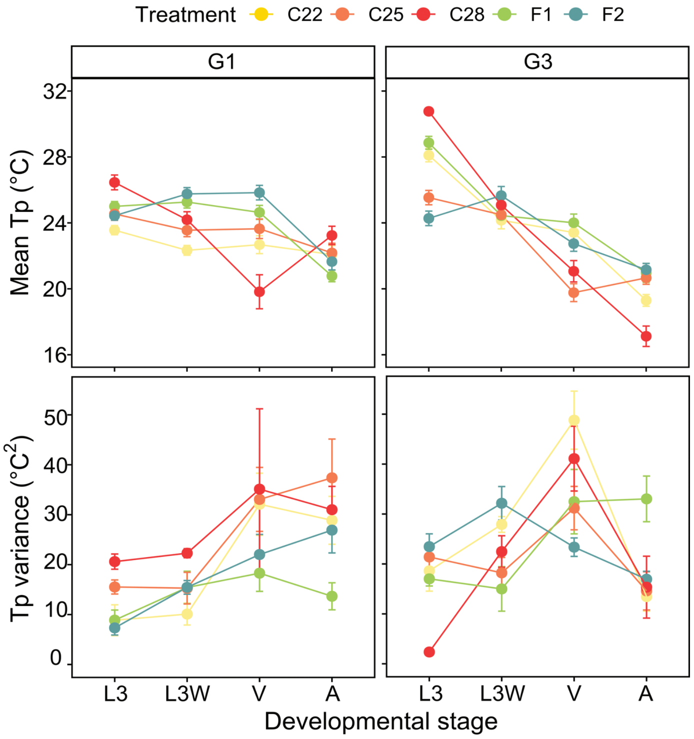

Figure 3: Life stage specificity of plasticity in thermal preference (Tp) components. Shown are mean Tp (*top panels*) and variance (*bottom panels*) of female *Drosophila melanogaster* ($n = 4{,}216$; mean ± SE) according to life stage (L3 = third larval stage; L3W = wandering larval stage; V = 2-day-old virgin adults; A = 12-day-old adults). Panels show averaged Tp mean and variance with respect to generation (G1, G3) vertically. Each symbol represents the averaged Tp for a replicate at a given factor level of temperature treatment (constant regimes C22 [22°C], C25 [25°C], and C28 [28°C] in yellow, orange, and red, respectively; fluctuating regimes F1 [23.5°C–26.5°C] and F2 [22°C–28°C] in green and blue, respectively).

the paradigm underlying the study of Tp beyond the use of a single metric, such as mean or median Tp, to integrate both metrics of thermal affinity and thermoregulation precision (see fig. 1). Below we consider (i) how the plastic Tp shifts can be interpreted based on the ecology of the species across both life stages and generations, (ii) the possible adaptive value of hyperplasticity in Tp, and (iii) avenues for the further development of our conceptual framework.

Interpreting Tp across Life Stages

The high plasticity of Tp complicates the interpretation of any change driven by multiple factors. The conceptual framework we propose simplifies this process by integrating all Tp responses along only two axes, the thermal need (Tp mean) and the thermoregulatory precision (Tp variance). For instance, virgin V adults (at G1) follow the precision at extreme hypothesis, according to which any plastic response should induce an increase in thermoregulatory precision when the thermal need approaches sublethal temperatures. Our data highlight that the different life stages have different thermoregulatory strategies (see fig. S3) and also that the strategy can vary due to transgenerational acclimation. Now the priority is to investigate the mechanisms determining the Tp strategy (fig. 1). Indeed, adults tend to display more comparable Tp across developmental temperatures in G1 than in G3: in G1, the Tp is higher and lower than rearing temperature when developed under constant 22°C and 28°C, respectively, likely because flies target their optimal temperature range. By contrast, after three generations under these conditions, thermal stress induced by exposure to sublethal temperature (28°C is below the lethal temperature, which is above 38°C in *D. melanogaster*; Manenti et al. 2018) probably overtakes this basal response, since the Tp diverged more widely.

Differences in mobility between life stages may contribute to disparate thermoregulatory mechanisms. In contrast to flying adults, which may rely more on behavioral thermoregulation than physiological plasticity to manage suboptimal or supraoptimal temperatures, the less mobile larvae may rely more on physiological acclimation due to reduced behavioral options within their decomposing fruit (Feder and Krebs 1998; Marais and Chown 2008). Larval stages are therefore expected to have evolved less precise thermoregulation and broader thermal needs than adults, and probably also less plastic Tp (Marais and Chown 2008).

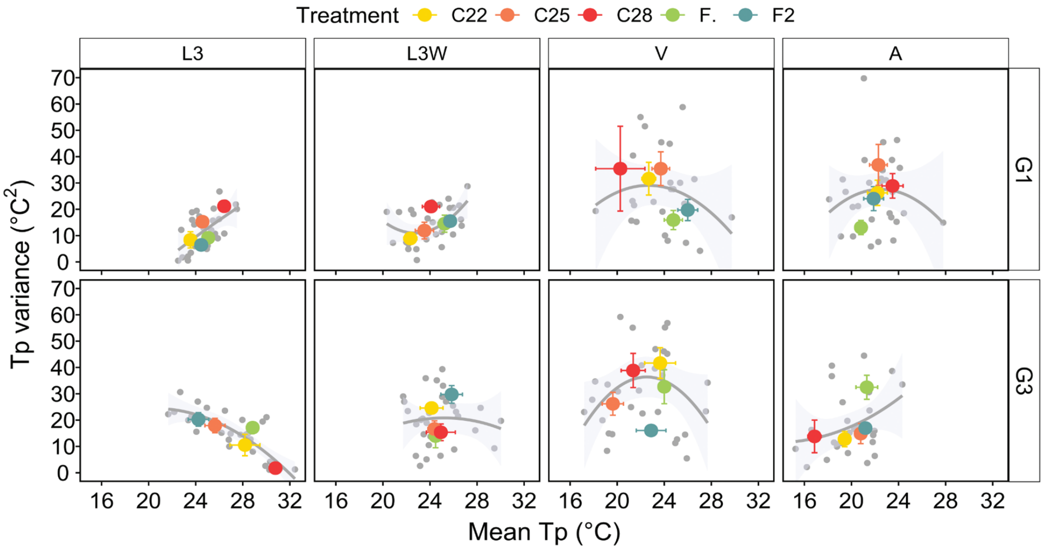

Figure 4: Relationship between thermal preference (Tp) variance and mean. Shown is the variance of *Drosophila melanogaster* female Tp according to mean Tp. The figure demonstrates significant differences in the type of relationship (shape of the LOESS) between Tp variance and mean across life stages (by comparing graphs from the same row) as well as changes in this relationship after two generations (by comparing graphs from same column), indicating the influence of transgenerational plasticity. Panels show averaged Tp mean and variance ($n = 6$) with respect to life stage (L3 = third larval stage; L3W = wandering larval stage; V = 2-day-old virgin adults; A = 12-day-old adults) vertically and generation (G1, G3) horizontally. Each symbol represents the averaged Tp for a replicate at a given level of temperature treatment (constant regimes C22 [22°C], C25 [25°C], and C28 [28°C] in yellow, orange, and red, respectively; fluctuating regimes F1 [23.5°C–26.5°C] and F2 [22°C–28°C] in green and blue, respectively). The error bars represent standard errors for both mean and variance of Tp. The gray points are the raw data for each replicate, and the gray curves are drawn using LOESS procedure (with 95% confidence intervals).

In agreement with this hypothesis, we found that Tp plasticity (Tp differences between treatments) was lower in larvae than in adults overall (see also fig. S3). Overall, however, larvae (L3) showed higher precision (lower Tp variance) than adults, especially when the mean Tp was high (e.g., larvae at C28 in G3). This counterintuitive finding is consistent with previous observations in several *Drosophila* species (MacLean et al. 2019). This is unlikely to be related to mobility differences between larvae and adults, since both had largely enough time to explore their lane in the thermal gradient (see references in table S1). A plausible explanation is that fly larvae evolved the ability to exploit fine-scale temperature gradients that can occur across small structures such as fruit. In apples, for instance, thermal gradients of up to 15°C can occur across a single fruit (Saudreau et al. 2007), and codling moth larvae use this gradient for behavioral thermoregulation (Kührt et al. 2005). The ability of larvae to utilize fine-scale thermal gradients within their microhabitats could explain their higher precision in thermoregulation, despite their overall lower Tp plasticity. The wandering stage L3W was intermediate between L3 and adults. This is the stage at which larvae leave their food source to pupate within the soil or on the plant surface (Sokolowski et al. 1984), and this stage can be expected to have evolved thermoregulatory abilities to exploit microhabitats that are optimal for the development of pupae. The role of fine-scale microclimatic variation in the evolution of Tp is likely to be underestimated and underappreciated (Pincebourde et al. 2016; Convey et al. 2018).

In adult flies, the thermal need and the range of permissive temperatures differ according to their nutritional or reproductive status, and these shifts help explain our results. Virgin adults had the highest Tp variance (i.e., low thermal precision), especially in G3. Virgin adults can loosely thermoregulate around the optimal temperature with little or no cost to their future reproduction because they are viable across a broad temperature range (10°C–33°C), and only temperatures above 29°C are deleterious for reproduction (Hoffman 2010). In contrast, fertile females are more restricted in their options because they have to find suitable oviposition sites for both the hatching of their eggs (Huey et al. 1995) and the development of their offspring (Fogleman 1979; MacLean et al. 2019). Indeed, oviposition site preference has a narrow temperature range (Fogleman 1979) and fertility is more constrained than viability, with a temperature range of 14°C–29°C (Hoffman 2010). This thermal constraint would explain the high level of thermoregulation precision in G3 fertile females. The narrower temperature range for fertility would also explain the drop in mean Tp of fertile females in G3, especially in the constant

28°C temperature regime. After three generations being exposed to temperatures (28°C) close to that at which fertility starts to be compromised (around 29°C), choosing lower temperatures would not only protect the females from harmful temperatures but also favor physiological repair processes (Ørsted et al. 2022). The production of offspring is known to be more sensitive to elevated temperatures than other fitness-related traits in *Drosophila* and other insects (Parratt et al. 2021; van Heerwaarden and Sgrò 2021; Ørsted et al. 2024). Therefore, adjustments of Tp similar to those we describe here for *D. melanogaster* may be widespread across insects.

Tp Plasticity as a Means to Counteract the Bogert Effect?

Our data demonstrate significant transgenerational plasticity in Tp with, for instance, an increase of more than 4°C in Tp mean under C28 and F2 regimes from G1 to G3. Even though thermoregulatory behavior generally exhibits a low heritability (Logan et al. 2018; Castañeda et al. 2019), we found that three generations under C28 were sufficient to produce larvae with a more pronounced preference for elevated temperature and with high thermoregulation precision. This rapid change, likely due to transgenerational plasticity or to selection, contrasts with the relatively slow rates of evolution of Tp across 20,000 years in lizards (Ibargüengoytía et al. 2021), although among classical thermal traits selection pressure may be greatest on Tp (Gilbert and Miles 2017). It remains unclear from our study to what extent this transgenerational plasticity is adaptive, but we speculate that high plasticity across very few generations may obstruct the Bogert effect. The Bogert effect explains how behavior (e.g., selecting the same narrow temperature range whenever it is available) can generate physiological inertia limiting the evolution of higher tolerance or warmer Tp under changing environmental conditions (Huey et al. 2003; Logan et al. 2019; Muñoz and Bodensteiner 2019; Muñoz 2022). However, the transgenerational plasticity of Tp opens the potential for the thermal physiology of the organism to respond rapidly when novel thermal environments are experienced. Future studies combining experimental approaches and modeling of selection pressures are required to help address this question.

Tp Plasticity as a Means to Escape Sublethal Effects under Fluctuating Conditions?

The partitioning of thermal need (Tp mean) and thermoregulatory precision (Tp variance) allows us to unravel responses that are otherwise unclear (i.e., complexity to extract information from fig. 2). The fluctuating temperature treatments that we applied with the same temperature average of 25°C were informative. In general, the fluctuating thermal regimes induced larger changes in Tp variance than in Tp mean compared with C25. Therefore, daily temperature fluctuations can modulate the thermoregulation precision of the organism, but not necessarily its thermal need. This is consistent with other studies showing how thermal traits (mainly CT_{max} or metrics of the thermal performance curve) can vary with the amplitude of daily fluctuations while mean temperature remains identical (Paaijmans et al. 2013; Foray et al. 2014; Sinclair et al. 2016). By contrast, a shift in the thermal need in response to thermal fluctuations was reported in species living in highly fluctuating environments (Podrabsky et al. 2008). The influence of fluctuating temperature on the mean Tp should depend on the abiotic context (Denny 2017). Surprisingly, our data indicate that increased amplitude of daily fluctuation improved the precision of thermoregulation after several generations, but very little after the first generation. Higher precision should increase the probability of avoiding high temperatures that can induce sublethal effects, including physiological stress and decreased survival (Koussoroplis et al. 2017). Therefore, this response is likely adaptive but is based mostly on transgenerational plasticity instead of developmental plasticity.

Our inferences are necessarily bounded by the experimental treatments that we applied. The temperatures in our treatments were all within the permissive temperature range for the development of *D. melanogaster*. The thermal limits of *D. melanogaster* are well beyond these temperature treatments (Hoffman 2010), and fluctuating treatments could include short exposures to sublethal/near-lethal temperatures to possibly increase the amplitude of plastic responses in Tp (Colinet et al. 2015; Koussoroplis et al. 2017). A broader range of plasticity should help to better delineate the response curves of the relationships between mean Tp and Tp variance. Similarly, we assumed that the optima, taken as a global trait, remains at or near 25°C, but we recognize that optimal temperature for life history traits such as growth rate or egg laying can also change in response to environmental temperature changes (Fischer et al. 2011; Buckley et al. 2022). Future analysis of Tp plasticity should be integrated with assessment of thermal performance curves, or combined with estimates of optimal temperature and limits for specific traits (Huey et al. 2012), to be compared with the actual microclimatic temperatures experienced by each life stage (Pincebourde and Casas 2015), to refine the extent to which the plasticity we describe is adaptive.

Future Perspectives for This Novel Conceptual Framework of Tp

Our framework analyses the plastic levels of the Tp at the group level (interindividual variations), which is relevant for evaluating general population trends in response to environmental changes. This framework could also be used to quantify the level of plasticity in the Tp responses at the individual level, although measuring individual data can be challenging in experiment setups requiring a large number of individuals. Such analysis at the individual level could help infer the thermoregulatory strategies of individuals at fine temporal scales and the extent to which plasticity can play a role in modulating flies' behavior depending on endogenous and environmental factors. The framework would need modification of the metric used to infer the thermal need, since the optimal temperature for an individual largely depends on the performance trait that is considered (Sinclair et al. 2016).

The framework proposed here generates at least four testable predictions on the Tp adaptation according to different life histories and factors. First, we predict that locomotor abilities influence the relative importance of Tp adaptation and plasticity in less mobile stages (typically larvae in many insects) that display a high thermoregulation precision with low plasticity to exploit optimally the fine-scale microclimate heterogeneity. Second, the impact of warming on Tp is expected to be larger after several generations, highlighting the key role of intergenerational plasticity in the response of thermoregulation strategies to warming. Third, daily temperature fluctuations can modulate Tp variance more than the mean Tp, suggesting that fluctuating environments improve thermoregulation precision and may allow optimal exploitation of the microclimate mosaic. Fourth, the highest thermoregulatory precision and adaptive plasticity level should be met in fertile adults, particularly in the sex with the lowest thermal limit for fertility. Future works challenging these predictions would significantly advance our understanding of the evolution of Tp plasticity in ectotherms.

The application of the framework to *D. melanogaster* unravels the use of a strategy following the precision at extreme hypothesis in which thermoregulation is less precise within the permissive temperature range than near the thermal limits in adults. This is consistent with recent studies revealing that behavioral thermoregulation in herbivorous insects primarily serves to escape overheating and improve survival rather than to approach an optimal temperature for reproduction (Leith et al. 2024). Nevertheless, this may not be the case for all ectotherms. For instance, reptiles rely on behavioral thermoregulation to approach their optimum for reproduction-related traits as much as to escape extreme temperatures (Ladyman et al. 2003; Taylor et al. 2021). The application of our conceptual framework in the context of a comparative approach across ectotherms should provide deeper knowledge of how interactions between reproduction and thermoregulation strategies translate into Tp plasticity.

Finally, we manipulated the level of plasticity by focusing on a few key variables (developmental/adult temperature, life stage, age, sex). Our framework can now be extended by integrating more variables of interest with the aim of exploring the maximum level of Tp plasticity for a given species. In ectotherms, Tp variation depends on numerous factors, including body mass (Shepherd et al. 2008; Cabon et al. 2023), reproductive status (MacLean et al. 2019), short-term acclimation (Mascaró et al. 2019), water status (Rozen-Rechels et al. 2019), diet quality or quantity (Hardison and Eliason 2024), altitude/latitude (Hodgson and Schwanz 2019), pathogens/disease (Beukema et al. 2021), the presence of symbionts such as *Wolbachia* (Arnold et al. 2019), and the presence of prey/predator cues (Smolinský and Gvoždík 2012). The precise sensory and neuronal pathways controlling Tp are known in *Drosophila* (Hamada et al. 2008), paving the way to comparisons in Tp plasticity across genotypes differing at this level. We anticipate that the amplitude of Tp responses to these factors modulate to a large extent the ecological responses to climate warming.

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Statement of Authorship

Conceptualization: S.P., H.C., G.D.; funding acquisition: S.P., H.C.; methods development/experimental design: S.P., H.C.; data collection: N.M.; data analysis: G.D.; data validation: G.D., S.P.; data visualization: G.D.; supervision: S.P., H.C.; writing—original draft: G.D., S.P., H.C.; writing—review and editing: G.D., S.P., H.C.

Data and Code Availability

All data supporting the results and the code used to generate the figures is freely available in Zenodo (https://doi.org/10.5281/zenodo.15087756; Deconninck et al. 2025).

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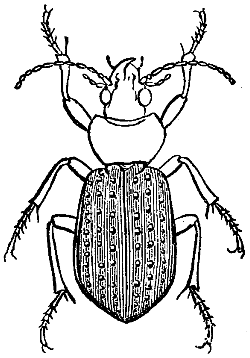

"We introduce to our readers a large family of ground-beetles (*Carabidæ*, from *Carabus*, the name of the typical genus) which prey on those insects largely injurious to crops." Figured: "*C*[*alosoma*] *calidum* Fabr." From "Entomological Calendar" (*The American Naturalist*, 1868, 2:110–111).