Plastic shifts in thermal preference and thermoregulation strategy across ontogeny in an invasive fly

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Conflict of interest

The authors have nothing to disclose.

Author contributions

GD, VF and SP conceived the ideas and designed methodology; GD collected and analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

The data supporting the results and the code used are freely available on Zenodo.org (https://doi.org/10.5281/zenodo.19649949). The DOI represents all versions and will always resolve to the latest one.

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ABSTRACT

Behavioural thermoregulation allows ectotherms to escape extreme or seek optimal temperatures. Its precision can impact survival and fitness under changing conditions and its plasticity can be an adaptive strategy when the plasticity of thermal limits is insufficient to buffer against warming. We explore the developmental and intergenerational plasticity of behavioural thermoregulation strategies using the invasive fly *Drosophila suzukii*, including *Wolbachia*-infected individuals. We measured the plasticity of its thermal preference (Tp) and used its mean (thermal need) and variance (thermoregulation precision) to assess thermoregulation strategies. Typically, mean Tp increased with developmental temperature as well as the precision in temperature selection. Tp differed between life stages (higher in larvae and females than in males), reflecting different thermal needs. *Wolbachia* infection was associated with a reduction of Tp in adults but an increase in larvae, associated with a shift of the thermoregulation strategy with higher precision at intermediate Tp. Modulation of Tp may represent a mechanism for coping with changing or novel environmental conditions, opening new perspectives as to whether plasticity in Tp is adaptive under natural conditions, whether such plasticity facilitates colonisation or persistence during range expansion, and whether Tp plasticity itself evolves during range expansion.

Keywords: Behavioural thermoregulation, *Drosophila suzukii*, phenotypic plasticity, selected temperature

INTRODUCTION

Ectotherms are particularly vulnerable to changes in temperature as their body temperature often matches the ambient or surface temperature with direct effect on their physiology (Angilletta 2009). Altered temperature regimes such as those induced by climate change are already impacting species leading to shifts in distribution and extinctions (Pecl et al. 2017; Renner and Zohner 2018; Harvey et al. 2020). Ectotherms are threatened by temperatures exceeding their thermal limits across all latitudes (Sunday et al. 2014), especially during heat waves (Morley et al. 2019). Plastic responses constitute the main process by which ectotherms can adjust their tolerance level to heat waves in the short term (Kingsolver and Buckley 2018; Buckley and Kingsolver 2021). However, the level of plasticity of thermal limits (e.g., Gunderson et al. 2017; Fey et al. 2019), and of other thermal performance traits such as optimal temperature (Sinclair et al. 2016; Kingsolver and Buckley 2017; Johnson et al. 2023), typically are insufficient to buffer against warming (Burton and Einum 2025).

Ectotherms can, alternatively, use behavioural thermoregulation to escape overheating (Leith et al. 2024) or to find optimal temperatures within heterogeneous habitats (Martin and Huey 2008), which makes them more likely to survive (Danks 1978; Ma and Ma 2022). The precision of thermoregulation could, therefore, impact survival and fitness under changing conditions (Pincebourde and Woods 2020; Ma et al. 2021; Deconninck et al. 2025). For example, a thermoregulation strategy that allows an organism to be highly precise when selecting a temperature that is optimal for a physiological process (e.g., development, egg maturation) can increase fitness directly. Alternatively, a strategy that allows an organism to be highly precise at selecting a temperature, when it approaches potentially deleterious temperature, can increase survival, and hence fitness, indirectly. In ectotherms, these thermoregulation strategies have been traditionally considered to be fixed at the species level (May 1979; Lahondère 2023), but some plasticity has been reported in various traits related to

behavioural thermoregulation (Gvoždík 2012; Llewelyn et al. 2017). These levels of plasticity can also vary across ontogeny and may be linked to shifts in thermoregulation strategies, as reported in *D. melanogaster* (Deconninck et al. 2025). For example, thermoregulation precision may vary with temperature depending on the costs associated with sublethal temperature avoidance and the benefits of reaching the optimal temperature. This recent conceptual development of behavioural thermoregulation plastic responses (Deconninck et al. 2025) remains to be applied more broadly to infer the extent to which behavioural plasticity may help ectotherms to adapt rapidely to varying temperature conditions, including resistance or tolerance to extreme heat.

In this study, we explore the developmental and intergenerational plasticity of thermoregulation strategies in the invasive fly *Drosophila suzukii* (Mastumura 1931). This pest species of soft fruit shows various seasonal adaptations (Tait et al. 2020, 2021), exploits different habitats (e.g., crops, woodland, urban areas; Ulmer et al. 2024), has invaded temperate and tropical areas (Walsh et al. 2011), has plastic thermal limits (Raynaud-Berton et al. 2024) and is an important example of a successful invader that is benefiting from climate change (Ward and Masters 2007; Diez et al. 2012; Cornelissen et al. 2019; Little et al. 2020; Robinson et al. 2020; Skendžić et al. 2021; Deconninck et al. 2026). We used thermal preference (Tp) mean and variance as proxies of behavioural thermoregulation strategies using the framework developed by Deconninck et al. (2025). In this framework, the mean Tp reflects the thermal need (higher Tp likely reflecting a shift of optimum temperature of physiological processes to warmer temperature; Martin and Huey 2015), the variance of Tp reflects the precision (the lower the variance, the higher the precision) and their relationship allows deciphering of behavioural thermoregulation strategies (Deconninck et al. 2025). We measured the Tp of *D. suzukii* across ontogeny (larvae, young and old adults, including males, virgin and fertile females), including individuals infected by the endosymbiont *Wolbachia*, under constant and

fluctuating temperature regimes across four generations. Temperature regimes were used to induce strong plastic responses allowing assessment of their impact on the behavioural strategy, particularly on thermoregulation precision. Contrasting results have been reported in the literature on the direction of the effects of temperature, life stage and *Wolbachia* infection on Tp (Yamamoto and Ohba 1984; Krstevska and Hoffmann 1994; McDaniel et al. 1995; Rajpurohit and Schmidt 2016; MacLean et al. 2019; Truitt et al. 2019; Strunov et al. 2023). We hypothesised that life stage, sex and age would have a strong influence on Tp, predicting that larvae and females would seek higher temperatures to accelerate their development or egg maturation (Cohet and David 1978; Gandara and Drummond-Barbosa 2022; Grainger and Levine 2022), and males would have lower Tp because of lower sterility threshold (Jørgensen et al. 2006; van Heerwaarden and Sgrò. 2021; Ørsted et al. 2024). We explored the intergenerational plasticity of Tp and its consequence on the behavioural strategy, to assess the extent to which plastic changes in thermoregulation behaviour across ontogeny are transmitted to next generation via maternal effects, a link that has been investigated only rarely (Gilbert and Warner 2026). Nevertheless, generation was not expected to have an important effect on Tp within only few generations (Paranjpe et al. 2013; Castañeda et al. 2019) and within the permissive range of temperatures.

MATERIALS AND METHODS

Origin and maintenance of experimental flies

Drosophila suzukii flies originated from a field collection of infested raspberries in Rennes, France (48°7'2.158"N, 1°40'40.053"W), in October 2020, by ECOBIO Laboratory (University of Rennes). One hundred and ninety isofemale lines were produced and nine of them were infected by *Wolbachia* as assessed by specific PCR assays with *Wolbachia*-specific primers targeting the *wsp* gene (81F/691R) following Braig et al. (1998). These infected lines were

mixed in cages, and the population amplified. Tetracycline treatment was used to obtain separate lines differing by the presence (W+) or absence (W–) of *Wolbachia* following Hague et al. (2021). The microbiota of W– flies was restored by rearing W– flies on a substrate inoculated with the faeces of W+ flies for three generations following Strunov et al. (2022), as used by Deconninck et al. (2024). The two lines were reared in 100 mL plastic bottles containing ~30 mL of standard cornmeal diet and incubated at 20°C, LD 12:12 h and 75% RH (STRADER, EV1300, Angers, France). At least 30 bottles of 50–200 flies per line were used for continuous maintenance. The *Wolbachia* infection status of the two lines was verified prior to the experiments using diagnostic PCR (Braig et al. 1998).

Temperature regimes

Groups of around a hundred 4-to-5-day-old adults from the source lines were placed for 5 d at 20°C in 100 mL bottles containing oviposition medium. Adults were then removed and bottles with eggs were randomly distributed between five temperature treatments: three constant regimes (16, 20, 24°C) and two fluctuating regimes differing in their thermal variance (18–22 and 16–24°C, both with mean temperature of 20°C) under L:D 12:12 h and 75% RH (climatic chambers, STRADER, Angers, France) (Figure 1A). In the fluctuating regimes, the coldest and warmest temperatures were reached at midnight and midday, respectively, and maintained for almost 11.5 hours (the transition between the two temperatures occurred over 30 min). Populations were maintained for at least four generations and up to 15 generations for some regimes (see thermal preference measurement section, below). A subsample of ~500 adult flies from each temperature treatment, ranging from the first to the last emergences, were distributed in eight bottles with fresh oviposition medium to produce the next generation.

Thermal gradient apparatus

153 Thermal preference (Tp) was measured by placing the insects in a linear thermal gradient
154 encompassing temperatures from 13 to 28°C. Our system was similar to the apparatus used in
155 previous studies on Tp of *Drosophila* (Takeuchi et al. 2009; Goda et al. 2014; Deconninck et
156 al. 2025). The apparatus consisted of an aluminium slab (500 × 250 × 20 mm) heated at one
157 end and cooled at the other, resulting in a linear temperature gradient. The high thermal
158 conduction of aluminium guarantees a stable gradient when the heating and cooling devices
159 are set at a constant level. Peltier devices (45W, Quick-cool®, Germany) coupled with two
160 Arduino controllers (UNO R3), a temperature probe and a display device were used to control
161 the temperatures at both ends of the gradient. A hand-made dissipater device was used to avoid
162 overheating of the Peltier elements: cool water continuously circulated within an aluminium
163 block placed against the warm side of the Peltiers. This cooling system was connected to a
164 large water tank (about 50 L) using a water pump. The entire system was placed in a
165 climatically controlled laboratory at a constant air temperature of 22°C.
166 In the aluminium slab, 10 lanes (400 × 10 × 5 mm) were drilled out and covered with a
167 plastic (Makrolon®) strip to allow the flies (adults and larvae) to move along the gradient
168 without escaping. The plastic strips were transparent to enable determination of the insects'
169 position in the gradient. The plastic strip had holes for the introduction of adult flies into the
170 lanes, that were subsequently covered during the runs. For larvae, the lanes were filled with a
171 homogeneous nutritional gel (solution composed of distilled water, saccharose 50 $g.L^{-1}$ and
172 agar $7g.L^{-1}$) which permitted the movement of larvae. The movement of insects within the lanes
173 was recorded using a camera (Kodak PixPro AZ422) positioned above the system, which took
174 an image every 5 min.
175 Using HOBO devices (UX120-014M, ONSET) connected to thermocouples (T-type
176 copper Constantan, diameter 0.2 mm, TC Direct, Dardilly, France), we calibrated the gradients
177 before experiments allowing the determination of temperature at any position in each lane.

178 Plastic strips were designed with multiple holes each separated by 4 cm to introduce
179 thermocouples that recorded the temperatures along the lanes every 30 sec for 60 min. For each
180 lane, we applied linear regression models with spatial coordinates and the mean temperature
181 record over the experimental hour. The thermal gradient was strongly linear in all lanes (all R^2
182 > 0.99), stable over time and between assays, allowing the accurate prediction of temperature
183 from the position along the lane.
184
185 **Thermal preference measurement**
186 We use two units of the gradient apparatus described above, with all electrical parameters kept
187 constant throughout the study, to measure the Tp of larvae (L3), young adults (3 day-old) and
188 old adults (13 day-old), including males, virgin and fertile females and W– and W+ individuals.
189 The life stage, sex and reproductive status of adults were grouped under 'Life stage' (LifeS)
190 for analyses and included seven levels: larvae (L3), 3- and 13-day-old virgin females
191 (Fem_V_3d; Fem_V_13d), 3- and 13-day-old fertile females (Fem_F_3d; Fem_F_13d), and 3-
192 and 13-day-old males (Male_3d; Male_13d) (Table 1; Figure 1B). Measurements were made
193 on individuals from generations 1 to 4 (G1–G4; Figure 1A). Extra measurements were made
194 at G10 and G15 for some temperature regimes (Figure S2).
195 For each trial (adults or larvae), 10 individuals (with a unique combination of variables)
196 were introduced into each lane to assess the range of Tp of that source population (Figure 1C).
197 For each unique combination of variables, 50 individuals were measured (50 × 7 life stages ×
198 5 temperature regimes × 4 generations = 7,000 individuals). The 10 lanes were considered as
199 independent replicates because no communication or contact was possible among individuals
200 from different lanes. Before starting the assays, the gradient was switched on 15 min to allow
201 stabilisation. Adult flies or larvae were placed in the centre of the lane at a temperature of
202 ~20°C (through the hole). The lanes were photographed every 5 min over 60 min to document

individual positions. Initial trial runs indicated that the movements of individuals were much less frequent after about 20 min. Therefore, we considered the Tp of every individual to be the temperature along the thermal gradient at their position after 45 min, according to common practice in *Drosophila* Tp assessment (position after 37 ± 14 min; see Deconninck et al. 2025 for review). All Tp assays were run within the same daily time window (between 0900 and 1200), to minimise any influence of circadian rhythm on Tp, and light was homogeneous to avoid any attraction toward a given direction (Dillon et al. 2009). All flies were handled with a mouth aspirator to limit stress due to manipulation.

Statistical analyses

Data were analysed in R version 4.5.3 (R Core Team, 2021). Thermal preference (Tp) was analysed using linear mixed-effects models fitted using the lmer function from the *lme4* package (Bates et al. 2015). The model included temperature treatment, generation (G1 to G4), *Wolbachia* infection status, life stage, and all two-way interactions among these factors as fixed effects. Gradient and lane nested within gradient were included as random effects to account for the experimental design.

Conditional effect sizes were derived from the mixed-effects model using estimated marginal means (EMMs) computed with the *emmeans* package (https://CRAN.R-project.org/package=emmeans). Pairwise contrasts were calculated using treatment-versus-control comparisons, using 20 °C (temperature treatment), L3 (life stage), or *Wolbachia*-uninfected (W–) individuals as reference levels depending on the factor analysed. Effect sizes (ΔTp relative to the reference level) and their associated standard errors were extracted and visualised as point estimates with 95% confidence intervals.

To identify the thermoregulation strategies, we quantified the mean (reflecting thermal need) and variance (reflecting precision of selection) of Tp for each combination of variables

(method by Deconninck et al. 2025). We calculated the mean and variance of Tp for the groups of 10 individuals (sharing a unique combination of variables) within each lane. We then averaged these lane-level means and variances across the five replicate lanes corresponding to each temperature treatment combination, to retain the replicate as a random factor for lane in our analyses (see below). The patterns of the relationship between the mean and variance of Tp were first explored using a LOESS smoothing procedure (span = 10) within each life stage × *Wolbachia* combination, following the method in Deconninck et al. (2025). This non-parametric approach allowed visual identification of potential thermoregulation strategies within each life stage, without imposing a predefined functional form, and considering the important variation of Tp induced by developmental temperature treatments. To statistically test these patterns and compare relationship shapes among life stages and *Wolbachia* infection status, we then fitted second-order polynomial linear models including Tp mean, *Wolbachia*, life stage, and their interactions as fixed factors. Linear (β_1) and quadratic (β_2) components were tested for each *Wolbachia* × life stage combination to determine whether relationships were U-shaped, inverted U-shaped, linear or non-significant. These analyses on thermoregulation strategies were performed on pooled data from generations G1–G4 to increase the power of the test, and because generation had only weak effect. We were mostly interested in the variation induced by temperature treatments to assess thermoregulation strategies of each life stage, but the thermoregulation strategies can also be assessed within each temperature treatment following the same procedure to explore the variation induced by ontogeny (Figure S3).

RESULTS

Important Tp plasticity

All variables and most of their interactions had significant effects on Tp, with temperature treatment, life stage and *Wolbachia* having the strongest effects (all $p < 0.0001$; Table 2; Figure S1). Temperature treatment effect depended on life stage with larval Tp being much less influenced (Figure 2A) but most adults selecting lower temperatures when developed at 16 °C and higher temperatures when developed at 24 °C (mean ± se: 19.0 ± 0.1 and 20.5 ± 0.1 °C, respectively; Figure 2A). Individuals that developed under the low fluctuating regime (18–22 °C) had Tp similar to those developed at constant 20 °C (both 19.9 ± 0.1 °C) but selected lower Tp under the high fluctuating regime (16–24 °C), similar to the constant 16 °C regime (19.1 ± 0.1 °C; Figure 2A). Regarding life stage, males had the lowest Tp (18.8 ± 0.1 °C both 3 d and 13 d), virgin females had intermediate Tp (3 d: 20.2 ± 0.1; 13 d: 19.2 ± 0.1 °C), and larvae and fertile females had the highest Tp (L3: 20.6 ± 0.1; 3 d: 20.9 ± 0.1; 13 d: 20.5 ± 0.1 °C, respectively) (Figure S1, 2A, 2B). *Wolbachia*-infected individuals generally had reduced Tp compared to non-infected individuals (W–: 20.1 ± 0.0°C; W+: 19.6 ± 0.0 °C), but the effect was driven by adults having lower Tp when infected, while larvae had a higher Tp when infected (Figure 2C). Finally, Tp decreased from 20.0 ± 0.1 °C in G1 to 19.6 ± 0.1 °C in G3 but further returned to values equivalent to those in G1 (G4: 19.8 ± 0.1; Figure 2D).

Distinct behavioural thermoregulation strategies among life stages

Only a subset of life stages showed significant relationships between Tp variance (reflecting precision) and Tp mean (reflecting thermal need), and *Wolbachia* infection appeared to change the shape of this relationship (Figure 3, Table S1). W– larvae, old fertile females and old males had a lower Tp variance at high mean Tp, suggesting a ‘precision at high temperature’ strategy (Deconninck et al. 2025). W– young males and W+ old males had a low Tp variance at low and high mean Tp, suggesting a ‘precision at extreme’ strategy (inverted U-shape; Deconninck

et al. 2025). W+ larvae had a high Tp variance at low and high mean Tp, suggesting a 'precision at optimum' strategy (U-shape; Deconninck et al. 2025).

DISCUSSION

Behavioural thermoregulation can buffer environmental changes, before ectotherms reach their thermal limits. While Tp can inform on thermoregulation abilities, an integrative approach interconnecting different sources of plastic responses is crucial to determine the adaptive role of behavioural thermoregulation (Gvoždík 2012). Here, we show that the Tp of *D. suzukii* is highly plastic, responding to temperature treatment, life stage and *Wolbachia* infection, with strong interactions between factors. These plastic responses however translated into relatively conserved behavioural thermoregulation strategies across ontogeny, with dominance of 'precision at extreme' strategies (low variance of Tp when approaching deleterious temperatures). The contrasting effects of *Wolbachia* infection on Tp and behavioural thermoregulation strategies in larvae and adults is notable and could reflect different mechanisms driving the response in different life stages. Our results suggest that the prediction of thermoregulation behaviour of field individuals requires detailed knowledge of their thermal history, ontogeny and infection status.

Multiple drivers of thermal preference plasticity

The Tp of *D. suzukii* was highly plastic, responding to all variables tested, with temperature, life stage (including ontogeny, sex and age) and *Wolbachia* having the strongest effects. Generation had the lowest effect on Tp and, even if intergenerational dynamics of Tp has been rarely explored, Tp has been shown to have a low heritability and be influenced mostly by maternal effects (Paranjpe et al. 2013; Castañeda et al. 2019).

Temperature treatment was one of the most important factors influencing Tp, consistent with previous studies on fruit flies (MacLean et al. 2019). Increasing environmental temperature mostly resulted in an increase in Tp. Analogous positive correlations have been reported in several ectotherms, including seahorses, shrimps, killifish and spiders (Podrabsky et al. 2008; Alfaro et al. 2013; Reiser et al. 2014; Mascaró et al. 2019). Some studies in *Drosophila* have also shown positive effects of temperature on Tp (Yamamoto and Ohba 1984; McDaniel et al. 1995), but others have reported either no effect on adult Tp (MacLean et al. 2019) or a decrease in Tp when temperatures are above thermal optima (Krstevska and Hoffmann 1994). Our results suggest that the inconsistency between studies could be linked to differences in sex, age and reproductive status of the flies, since all of these modulated the strength, and sometimes the direction, of the effect of temperature. Beyond thermal optima, exposure to stressful temperatures and the hardening process are also expected to decrease Tp (McDaniel et al. 1995; Dillon et al. 2009).

The Tp of *D. suzukii* responded differently to constant and fluctuating temperature regimes. Low amplitude of daily fluctuations (18-22°C) gave the same outcomes as an absence of fluctuations with similar mean (constant 20 °C), consistent with results in *D. melanogaster* (Deconninck et al. 2025). However, larger amplitude of daily fluctuations (16-24 °C) resulted in a reduction of Tp similar to that seen with development at constant low temperature (16 °C). One possible adaptive explanation for this is that a comparatively low Tp can protect from potentially harmfully high temperature, as reported in killifish (Podrabsky et al. 2008), although *D. suzukii* can tolerate temperatures well above 30 °C (Enriquez and Colinet 2017). Alternatively, because *D. suzukii* that developed under warm temperature (24 °C) selected higher Tp than under the high fluctuating regime, the lower end of the fluctuation could be driving the reduced Tp in flies that develop in fluctuating temperature regimes. This is consistent with recent findings reporting that the thermal optimum for survival shifts from

around 25 to 18 °C when *D. suzukii* develop under constant versus fluctuating temperatures (of similar amplitude to our treatment; Raynaud-Berton et al. 2024). Because temperature fluctuations improve performance at lower (mean) temperatures, the shift in Tp therefore appears to be adaptive by tracking this physiological effect.

In *Drosophila*, both the Tp and its level of plasticity generally differ between larvae and adults (MacLean et al. 2019). In *D. suzukii*, larvae had higher Tp than adults overall, although equivalent to that of fertile females. Choosing warmer temperature to allow faster development could give a competitive advantage to larvae and reduce their risk of predation before emergence (Nunney 1990; Grainger and Levine 2022; but see also 'grow now, pay later' hypothesis of Metcalfe and Monaghan (2001)). Females had higher Tp than males, and this difference was particularly pronounced after females had mated. Fertile females might seek higher temperatures to accelerate oogenesis (Cohet and David 1978; Gandara and Drummond-Barbosa 2022) while virgin females may select relatively lower temperatures to save energy (by lowering their metabolism) and to maximise their longevity (Marshall and Sinclair 2010). Old adults also selected relatively low Tp, which could indicate similar strategies (energy saving) as virgin females (Marshall and Sinclair 2010). Males had the lowest Tp, consistent with reports that male fertility is more sensitive to elevated temperatures than that of females (Jørgensen et al. 2006; van Heerwaarden and Sgrò 2021; Ørsted et al. 2024).

Wolbachia-infected adults exhibited a lower Tp. This is consistent with findings in *D. melanogaster*, where multiple *Wolbachia* strains caused significant reductions in adult Tp ranging from 2 to 8 °C (Truitt et al. 2019), converse to the hypothesis that *Wolbachia* effects on Tp are attributable to confounding effects (Strunov et al. 2023). A reduced Tp could be a self-medicating behaviour to reduce proliferation of the bacteria ('behavioural chill' hypothesis; Fedorka et al. 2016) and attenuate any deleterious effects and fitness costs associated with over-proliferation of these endosymbiotic bacteria (Strunov et al. 2013a,b).

Surprisingly, however, we found the reverse in larvae. *Wolbachia*-infected larvae had an increased Tp, albeit of lower amplitude than in adults (by up to 0.8°C), which has not been reported before and could be linked to *Wolbachia* manipulation of host behaviour (see thermoregulation strategy section, below).

Thermoregulation strategy: Be precise when approaching thermal extremes

The increase in mean Tp associated with warmer developmental temperatures generally resulted in higher precision in temperature selection (low Tp variance) in most life stages ('precision at extremes' and 'precision at high temperature', as described by Deconninck et al. (2025)). This thermoregulation strategy is consistent with studies of herbivorous insects demonstrating that behavioural thermoregulation is particularly important for escaping overheating and improving survival, rather than achieving optimal temperatures for reproduction (Leith et al. 2024). Our results contrast, however, with studies on spider mites, that seek optimal temperature by moving on the leaf surface under mild conditions (Caillon et al. 2014) but likely rely on physiological mechanisms, rather than behaviour, to tolerate heat extremes (Ma et al. 2021), as their microhabitat (leaf surface) does not provide sufficient thermal heterogeneity to provide thermal refuges. Fruit flies, by contrast, can easily move and fly when reaching extremes, leading to behavioural strategies to escape the heat. Moreover, adults must avoid sublethal thermal stress, particularly at high temperatures, because fertility is impaired at temperatures lower than those that compromise survival (Jørgensen et al. 2006; Parratt et al. 2021; van Heerwaarden and Sgrò 2021; Ørsted et al. 2024). *Drosophila suzukii* could also show precise thermoregulation under any circumstances. In our study, some life stages did not display obvious behavioural thermoregulation strategies, but had generally low Tp variance (high precision). The Tp variance reported in this study was also generally much lower than that reported in *D. melanogaster* (Deconninck et al. 2025), suggesting that *D. suzukii*

is overall more precise than *D. melanogaster*. The absence of obvious relationship between Tp mean and variance may also be a result of a limited range of plastic responses in *D. suzukii* or to the permissive temperature regimes we used.

Globally (regardless of *Wolbachia* infection status), fly larvae preferred warmer temperatures than any other life stages. The larval stage is certainly better adapted to warm microenvironments than adults as larvae can be exposed to temperature well above ambient air temperature, in particular when fruit are exposed to solar radiation (Woolf and Ferguson 2000; Saudreau et al. 2009). Optimal temperature for larval development is around 28 °C (Sánchez-Ramos et al. 2019; Raynaud-Berton et al. 2024), while optimal temperature for adults is usually lower (e.g., temperature that maximises egg production in females is around 24-26.7 °C; Winkler et al. 2021; Baser et al. 2025). Even if larvae are physically constrained within the fruit they infest, they may still benefit from within-fruit thermal heterogeneity to escape overheating and / or seek the optimum temperature for their development (Kührt et al. 2005; Saudreau et al. 2007, 2009, 2011). Notably, *Wolbachia* not only increased the Tp of larvae (i.e., in the opposite direction to that in adults) but it also induced a shift in their thermoregulation strategy: they were more precise (i.e. lower Tp variance) when choosing intermediate temperatures ('precision at optimum' strategy as described by Deconninck et al. (2025)), a strategy that was not observed in non-infected flies. Locomotion behaviour in the thermal gradient could have differed between infected and non-infected individuals. This unlikely caused the observed differences given that all individuals had sufficient time to explore entirely the gradient lanes within the 45 min trials.. In our analysis, we assumed that mean Tp reflects an optimum temperature for performance and that a shift in mean Tp indicates a shift in optimum temperature (Martin and Huey 2008). Precise thermoregulation would allow infected larvae to select temperatures that optimise their foraging and accelerate their development, which is the main function of thermoregulation behaviour reported in reptiles (Ladyman et al.

2003; Taylor et al. 2021). Another possible explanation is that *Wolbachia* may manipulate larval thermoregulation to its own benefit. By promoting the selection of warmer microhabitats, the symbiont could optimise its intracellular proliferation (Fedorka et al. 2016; Strunov et al. 2013a,b). This hypothesis remains speculative at this stage and warrants further investigation. This contrasts with the hypothesis that infected adults prefer lower temperature to limit *Wolbachia* proliferation (see above), posing questions about neuro-ethological mechanisms in the *Wolbachia*-fly interaction that could differ between larval and adult stages.

CONCLUSIONS

Drosophila suzukii exhibited marked plasticity of Tp, with all tested variables influencing its expression. Although our study does not permit direct assessment of the fitness consequences associated with this plasticity, modulation of Tp may represent a potential mechanism for coping with extreme or novel environmental conditions (Gvoždík 2012; Ma et al. 2021). In *D. suzukii*, and other Drosophilidae, this behavioural thermoregulation plasticity might have contributed to range expansion and invasive success (Deconninck et al. 2026). Whether the Tp measured in artificial thermal gradient translates into actual behaviour in more complex situations, including in the field, remains extremely challenging to answer. Nevertheless, our conceptual approach of inferring thermoregulation strategies from the mean and variance of Tp may allow more realistic inferences. Future research should aim to determine (i) whether plasticity in Tp is adaptively relevant under natural conditions, for example by modulating exposure to deleterious thermal extremes, (ii) whether such plasticity facilitates colonisation or persistence during range expansion, and whether (iii) Tp plasticity itself evolves during range expansion, as predicted by theoretical models (Eriksson and Rafajlović 2022; Usui et al. 2023; Swaegers et al. 2024).

REFERENCES

Alfaro C, Figueroa DP, Torres H, Veloso C, Venegas F, Canals L, Canals M. 2013 Effect of thermal acclimation on preferred temperatures in two mygalomorph spiders inhabiting contrasting habitats. *Physiol. Entomol.* **38**, 20–25. (doi:10.1111/j.1365-3032.2012.00853.x)

Angilletta Jr. MJ. 2009 *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press. (doi:10.1093/acprof:oso/9780198570875.001.1)

Baser N, Rossini L, Anfora G, Temel KM, Gualano S, Garone E, Santoro F. 2025 Thermal development, mortality, and fertility of an Apulian strain of *Drosophila suzukii* at different temperatures. *Insects* **16**, 60. (doi:10.3390/insects16010060)

Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48. (doi:10.18637/jss.v067.i01)

Braig HR, Zhou W, Dobson SL, O'Neill SL. 1998 Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont Wolbachia pipientis. *J. Bacteriol.* **180**, 2373–2378. (doi:10.1128/JB.180.9.2373-2378.1998)

Buckley LB, Kingsolver JG. 2021 Evolution of thermal sensitivity in changing and variable climates. *Annu. Rev. Ecol. Evol. Syst.* **52**, 563–586. (doi:10.1146/annurev-ecolsys-011521-102856)

Burton T, Einum S. 2025 High Capacity for Physiological Plasticity Occurs at a Slow Rate in Ectotherms. *Ecol. Lett.* **28**, 1–9. (doi:10.1111/ele.70046)

Caillon R, Suppo C, Casas J, Arthur Woods H, Pincebourde S. 2014 Warming decreases thermal heterogeneity of leaf surfaces: Implications for behavioural thermoregulation by arthropods. *Funct. Ecol.* **28**, 1449–1458. (doi:10.1111/1365-2435.12288)

448 Castañeda LE, Romero-Soriano V, Mesas A, Roff DA, Santos M. 2019 Evolutionary
449 potential of thermal preference and heat tolerance in *Drosophila subobscura*. *J. Evol. Biol.*
450 **32**, 818–824. (doi:10.1111/jeb.13483)
451 Cohet Y, David J. 1978 Control of the adult reproductive potential by preimaginal thermal
452 conditions - A study in *Drosophila melanogaster*. *Oecologia* **36**, 295–306.
453 (doi:10.1007/BF00348055)
454 hCornelissen B, Neumann P, Schweiger O. 2019 Global warming promotes biological
455 invasion of a honey bee pest. *Glob. Chang. Biol.* **25**, 3642–3655. (doi:10.1111/gcb.14791)
456 Danks H V. 1978 Modes of seasonal adaptation in the insects: I. Winter survival. *Can.*
457 *Entomol.* **110**, 1167–1205. (doi:10.4039/Ent1101167-11)
458 Deconninck G, Larges J, Henri H, Beaugeard L, Foray V, Pincebourde S. 2024. *Wolbachia*
459 improves the performance of an invasive fly after a diet shift. *J. Pest Sci. (2004).* **97**, 2087–
460 2099. (doi:10.1007/s10340-023-01739-w)
461 Deconninck G, Meyer N, Colinet H, Pincebourde S. 2025. Thermal preference plasticity in
462 ectotherms: integrating temperature affinity and thermoregulation precision. *Am. Nat.*
463 Deconninck G *et al.* 2026 A roadmap to key traits of invasive Drosophilidae. *Biol. Rev.*
464 (doi:10.1002/brv.70148)
465 Diez JM *et al.* 2012 Will extreme climatic events facilitate biological invasions? *Front. Ecol.*
466 *Environ.* **10**, 249–257. (doi:10.1890/110137)
467 Dillon ME, Wang G, Garrity PA, Huey RB. 2009 Thermal preference in *Drosophila*. *J.*
468 *Therm. Biol.* **34**, 109–119. (doi:10.1016/j.jtherbio.2008.11.007)
469 Enriquez T, Colinet H. 2017 Basal tolerance to heat and cold exposure of the spotted wing
470 drosophila, *Drosophila suzukii*. *PeerJ* **5**, e3112. (doi:10.7717/peerj.3112)
471 Eriksson M, Rafajlović M. 2022 The role of phenotypic plasticity in the establishment of
472 range margins. *Philos. Trans. R. Soc. B* **377**. (doi:10.1098/rstb.2021.0012)

Fedorka KM, Kutch IC, Collins L, Musto E. 2016 Cold temperature preference in bacterially infected *Drosophila melanogaster* improves survival but is remarkably suboptimal. *J. Insect Physiol.* **93**–**94**, 36–41. (doi:10.1016/j.jinsphys.2016.08.005)

Fey SB *et al.* 2019 Opportunities for behavioral rescue under rapid environmental change. *Glob. Chang. Biol.* **25**, 3110–3120. (doi:10.1111/gcb.14712)

Gandara ACP, Drummond-Barbosa D. 2022 Warm and cold temperatures have distinct germline stem cell lineage effects during *Drosophila* oogenesis. *Development* **149**. (doi:10.1242/dev.200149)

Gilbert AL, Warner DA. 2026 Transgenerational plasticity and climate change: phenotypic responses across ectothermic animals. *J. Exp. Biol.* 229. (doi:10.1242/jeb.250304)

Goda T, Leslie JR, Hamada FN. 2014 Design and analysis of temperature preference behavior and its circadian rhythm in *Drosophila*. *J. Vis. Exp.* , 1–8. (doi:10.3791/51097)

Grainger TN, Levine JM. 2022 Rapid evolution of life-history traits in response to warming, predation and competition: A meta-analysis. *Ecol. Lett.* **25**, 541–554. (doi:10.1111/ele.13934)

Gunderson AR, Dillon ME, Stillman JH. 2017 Estimating the benefits of plasticity in ectotherm heat tolerance under natural thermal variability. *Funct. Ecol.* **31**, 1529–1539. (doi:10.1111/1365-2435.12874)

Gvoždík L. 2012 Plasticity of preferred body temperatures as means of coping with climate change? *Biol. Lett.* **8**, 262–265. (doi:10.1098/rsbl.2011.0960)

Hague MTJ, Woods HA, Cooper BS. 2021 Pervasive effects of *Wolbachia* on host activity. *Biol. Lett.* **17**, rsbl.2021.0052. (doi:10.1098/rsbl.2021.0052)

Harvey JA, Heinen R, Gols R, Thakur MP. 2020 Climate change-mediated temperature extremes and insects: From outbreaks to breakdowns. *Glob. Chang. Biol.* **26**, 6685–6701. (doi:10.1111/gcb.15377)

498 Johnson CA, Ren R, Buckley LB. 2023 Temperature sensitivity of fitness components across
499 life cycles drives insect responses to climate change. *Am. Nat.* **202**, 753–766.
500 (doi:10.1086/726896)
501 Jørgensen KT, Sørensen JG, Bundgaard J. 2006 Heat tolerance and the effect of mild heat
502 stress on reproductive characters in *Drosophila buzzatii* males. *J. Therm. Biol.* **31**, 280–
503 286. (doi:10.1016/j.jtherbio.2005.11.026)
504 Kingsolver JG, Buckley LB. 2017 Quantifying thermal extremes and biological variation to
505 predict evolutionary responses to changing climate. *Philos. Trans. R. Soc. B Biol. Sci.* **372**,
506 20160147. (doi:10.1098/rstb.2016.0147)
507 Kingsolver JG, Buckley LB. 2018 How do phenology, plasticity, and evolution determine
508 the fitness consequences of climate change for montane butterflies? *Evol. Appl.* **11**, 1231–
509 1244. (doi:10.1111/eva.12618)
510 Krstevska B, Hoffmann AA. 1994 The effects of acclimation and rearing conditions on the
511 response of tropical and temperate populations of *Drosophila melanogaster* and *D.*
512 *simulans* to a temperature gradient (Diptera: Drosophilidae). *J. Insect Behav.* **7**, 279–288.
513 (doi:10.1007/BF01989735)
514 Kührt U, Samietz J, Dorn S. 2005 Thermoregulation behaviour in codling moth larvae.
515 *Physiol. Entomol.* **30**, 54–61. (doi:10.1111/j.0307-6962.2005.00431.x)
516 Lahondère C. 2023 Recent advances in insect thermoregulation. *J. Exp. Biol.* **226**.
517 (doi:10.1242/jeb.245751)
518 Ladyman M, Bonnet X, Lourdais O, Bradshaw D, Naulleau G. 2003 Gestation,
519 thermoregulation, and metabolism in a viviparous snake, *Vipera aspis*: Evidence for
520 fecundity-independent costs. *Physiol. Biochem. Zool.* **76**, 497–510. (doi:10.1086/376420)
521 Leith NT, Miller EA, Fowler-Finn KD. 2024 Thermoregulation enhances survival but not
522 reproduction in a plant-feeding insect. *Funct. Ecol.* (doi:10.1111/1365-2435.14546)

523 Little CM, Chapman TW, Hillier NK. 2020 Plasticity is key to success of *Drosophila suzukii*
524 (Diptera: Drosophilidae) invasion. *J. Insect Sci.* **20**, 5. (doi:10.1093/jisesa/ieaa034)
525 Llewelyn J, Macdonald S, Hatcher A, Moritz C, Phillips BL. 2017 Thermoregulatory
526 behaviour explains countergradient variation in the upper thermal limit of a rainforest
527 skink. Oikos 126, 748–757. (doi:10.1111/oik.03933)
528 Ma C-S, Ma G, Pincebourde S. 2021 Survive a warming climate: Insect responses to extreme
529 high temperatures. *Annu. Rev. Entomol.* **66**, 163–184. (doi:10.1146/annurev-ento-041520-
530 074454)
531 Ma G, Ma C Sen. 2022 Potential distribution of invasive crop pests under climate change:
532 incorporating mitigation responses of insects into prediction models. *Curr. Opin. Insect*
533 *Sci.* **49**, 15–21. (doi:10.1016/j.cois.2021.10.006)
534 MacLean HJ, Sørensen JG, Kristensen TN, Loeschcke V, Beedholm K, Kellermann V,
535 Overgaard J. 2019 Evolution and plasticity of thermal performance: An analysis of
536 variation in thermal tolerance and fitness in 22 *Drosophila* species. *Philos. Trans. R. Soc.*
537 *B Biol. Sci.* **374**, 20180548. (doi:10.1098/rstb.2018.0548)
538 Marshall KE, Sinclair BJ. 2010 Repeated stress exposure results in a survival-reproduction
539 trade-off in *Drosophila melanogaster*. *Proc. R. Soc. B Biol. Sci.* **277**, 963–969.
540 (doi:10.1098/rspb.2009.1807)
541 Martin TL, Huey RB. 2008 Why 'suboptimal' is optimal: Jensen's inequality and ectotherm
542 thermal preferences. *Am. Nat.* **171**, 102–118. (doi:10.1086/527502)
543 Mascaró M, Horta JL, Diaz F, Paschke K, Rosas C, Simões N. 2019 Effect of a gradually
544 increasing temperature on the behavioural and physiological response of juvenile
545 *Hippocampus erectus*: Thermal preference, tolerance, energy balance and growth. *J.*
546 *Therm. Biol.* **85**, 102406. (doi:10.1016/j.jtherbio.2019.102406)

May ML. 1979 Insect thermoregulation. *Annu. Rev. Entomol.* **24**, 313–349. (doi:10.1146/annurev.en.24.010179.001525)

McDaniel R, Hostert EE, Seager RD. 1995 Acclimation and adaptive behavior of *Drosophila robusta* and *D. tripunctata* adults in response to combined temperature and desiccation stress. *Am. Midl. Nat.* **133**, 52. (doi:10.2307/2426347)

Metcalfe NB, Monaghan P. 2001 Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**, 254–260. (doi:10.1016/S0169-5347(01)02124-3)

Morley SA, Peck LS, Sunday JM, Heiser S, Bates AE. 2019 Physiological acclimation and persistence of ectothermic species under extreme heat events. *Glob. Ecol. Biogeogr*. 28, 1018–1037. (doi:10.1111/geb.12911)

Nunney L. 1990 *Drosophila* on oranges: colonization, competition, and coexistence. *Ecology* **71**, 1904–1915. (doi:10.2307/1937598)

Ørsted M, Willot Q, Olsen AK, Kongsgaard V, Overgaard J. 2024 Thermal limits of survival and reproduction depend on stress duration: A case study of *Drosophila suzukii*. *Ecol. Lett.* **27**, 1–13. (doi:10.1111/ele.14421)

Paranjpe DA, Bastiaans E, Patten A, Cooper RD, Sinervo B. 2013 Evidence of maternal effects on temperature preference in side-blotched lizards: implications for evolutionary response to climate change. *Ecol. Evol.* **3**, 1977–1991. (doi:10.1002/ece3.614)

Parratt SR, Walsh BS, Metelmann S, White N, Manser A, Bretman AJ, Hoffmann AA, Snook RR, Price TAR. 2021 Temperatures that sterilize males better match global species distributions than lethal temperatures. *Nat. Clim. Chang.* **11**, 481–484. (doi:10.1038/s41558-021-01047-0)

Pecl GT *et al.* 2017 Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science.* **355**, 1389. (doi:10.1126/science.aai9214)

Pincebourde S, Casas J. 2015 Warming tolerance across insect ontogeny: influence of joint shifts in microclimates and thermal limits. *Ecology* **96**, 986–997. (doi:10.1890/14-0744.1)

Pincebourde S, Woods HA. 2020 There is plenty of room at the bottom: microclimates drive insect vulnerability to climate change. *Curr. Opin. Insect Sci.* **41**, 63–70. (doi:10.1016/j.cois.2020.07.001)

Podrabsky JE, Clelen D, Crawshaw LI. 2008 Temperature preference and reproductive fitness of the annual killifish *Austrofundulus limnaeus* exposed to constant and fluctuating temperatures. *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.* **194**, 385–393. (doi:10.1007/s00359-008-0313-7)

R Core Team. 2021 R: A language and environment for statistical computing. Austria, Vienna.

Rajpurohit S, Schmidt PS. 2016 Measuring thermal behavior in smaller insects: A case study in *Drosophila melanogaster* demonstrates effects of sex, geographic origin, and rearing temperature on adult behavior. *Fly (Austin).* **10**, 149–161. (doi:10.1080/19336934.2016.1194145)

Raynaud-Berton B, Gibert P, Suppo C, Pincebourde S, Colinet H. 2024 Modelling thermal reaction norms for development and viability in *Drosophila suzukii* under constant, fluctuating and field conditions. *J. Therm. Biol.* **123**, 103891. (doi:10.1016/j.jtherbio.2024.103891)

Reiser S, Herrmann JP, Temming A. 2014 Thermal preference of the common brown shrimp (*Crangon crangon*, L.) determined by the acute and gravitational method. *J. Exp. Mar. Bio. Ecol.* **461**, 250–256. (doi:10.1016/j.jembe.2014.08.018)

Renner SS, Zohner CM. 2018 Climate change and phenological mismatch in trophic interactions among plants, insects, and vertebrates. *Annu. Rev. Ecol. Evol. Syst.* **49**, 165–182. (doi:10.1146/annurev-ecolsys-110617-062535)

596 Robinson TB, Martin N, Loureiro TG, Matikinca P, Robertson MP. 2020 Double trouble:
597 the implications of climate change for biological invasions. *NeoBiota* **62**, 463–487.
598 (doi:10.3897/neobiota.62.55729)
599 Sánchez-Ramos I, Fernández CE, González-Núñez M. 2019 Comparative analysis of
600 thermal performance models describing the effect of temperature on the preimaginal
601 development of *Drosophila suzukii*. *J. Pest Sci. (2004).* **92**, 523–541.
602 (doi:10.1007/s10340-018-1030-9)
603 Saudreau M, Marquier A, Adam B, Monney P, Sinoquet H. 2009 Experimental study of fruit
604 temperature dynamics within apple tree crowns. *Agric. For. Meteorol.* **149**, 362–372.
605 (doi:10.1016/j.agrformet.2008.09.001)
606 Saudreau M, Marquier A, Adam B, Sinoquet H. 2011 Modelling fruit-temperature dynamics
607 within apple tree crowns using virtual plants. *Ann. Bot.* **108**, 1111–1120.
608 (doi:10.1093/aob/mcr054)
609 Saudreau M, Sinoquet H, Santin O, Marquier A, Adam B, Longuenesse JJ, Guilioni L, Chelle
610 M. 2007 A 3D model for simulating the spatial and temporal distribution of temperature
611 within ellipsoidal fruit. *Agric. For. Meteorol.***147**, 1–15.
612 (doi:10.1016/j.agrformet.2007.06.006)
613 Sinclair BJ *et al.* 2016 Can we predict ectotherm responses to climate change using thermal
614 performance curves and body temperatures? *Ecol. Lett.* **19**, 1372–1385.
615 (doi:10.1111/ele.12686)
616 Skendžić S, Zovko M, Živković IP, Lešić V, Lemić D. 2021 Effect of climate change on
617 introduced and native agricultural invasive insect pests in Europe. *Insects* **12**, 1–21.
618 (doi:10.3390/insects12110985)

619 Strunov AA, Ilinskii YY, Zakharov IK, Kiseleva E V. 2013a Effect of high temperature on
620 survival of *Drosophila melanogaster* infected with pathogenic strain of *Wolbachia*
621 bacteria. *Russ. J. Genet. Appl. Res.* **3**, 435–443. (doi:10.1134/S2079059713060099)
622 Strunov A, Kiseleva E, Gottlieb Y. 2013b Spatial and temporal distribution of pathogenic
623 *Wolbachia* strain *w*MelPop in *Drosophila melanogaster* central nervous system under
624 different temperature conditions. *J. Invertebr. Pathol.* **114**, 22–30.
625 (doi:10.1016/j.jip.2013.05.001)
626 Strunov A, Lerch S, Blanckenhorn WU, Miller WJ, Kapun M. 2022 Complex effects of
627 environment and *Wolbachia*infections on the life history of Drosophila melanogaster
628 hosts. *J. Evol. Biol.* **35**, 788–802. (doi:10.1111/jeb.14016)
629 Strunov A, Schoenherr C, Kapun M. 2023 *Wolbachia* has subtle effects on thermal
630 preference in highly inbred *Drosophila melanogaster* which vary with life stage and
631 environmental conditions. *Sci. Rep.* **13**, 1–10. (doi:10.1038/s41598-023-40781-7)
632 Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB. 2014
633 Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and
634 elevation. *Proc. Natl. Acad. Sci. U. S. A.* 111, 5610–5615. (doi:10.1073/pnas.1316145111)
635 Swaegers J, De Cupere S, Gaens N, Lancaster LT, Carbonell JA, Sánchez Guillén RA, Stoks
636 R. 2024 Plasticity and associated epigenetic mechanisms play a role in thermal evolution
637 during range expansion. *Evol. Lett.* **8**, 76–88. (doi:10.1093/evlett/qrac007)
638 Tait G, Cabianca A, Grassi A, Pfab F, Oppedisano T, Puppato S, Mazzoni V, Anfora G,
639 Walton VM. 2020 *Drosophila suzukii* daily dispersal between distinctly different habitats.
640 *Entomol. Gen.* **40**, 25–37. (doi:10.1127/entomologia/2019/0876)
641 Tait G *et al.* 2021 *Drosophila suzukii* (Diptera: Drosophilidae): A decade of research towards
642 a sustainable integrated pest management program. *J. Econ. Entomol.* **114**, 1950–1974.
643 (doi:10.1093/jee/toab158)

644 Takeuchi KI *et al.* 2009 Changes in temperature preferences and energy homeostasis in
645 dystroglycan mutants. *Science* **323**, 1740–1743. (doi:10.1126/science.1165712)
646 Taylor EN *et al.* 2021 The thermal ecology and physiology of reptiles and amphibians: A
647 user's guide. *J. Exp. Zool. Part A Ecol. Integr. Physiol.* **335**, 13–44. (doi:10.1002/jez.2396)
648 Truitt AM, Kapun M, Kaur R, Miller WJ. 2019 *Wolbachia* modifies thermal preference in
649 *Drosophila melanogaster*. *Environ. Microbiol.* **21**, 3259–3268. (doi:10.1111/1462-
650 2920.14347)
651 Ulmer R *et al.* 2024 Urban ecology of *Drosophila suzukii*. *Urban Ecosyst.* **in press**.
652 (doi:https://doi.org/10.1007/s11252-024-01554-w)
653 Usui T *et al.* 2023 The evolution of plasticity at geographic range edges. *Trends Ecol. Evol.*
654 **38**, 831–842. (doi:10.1016/j.tree.2023.04.004)
655 van Heerwaarden B, Sgrò CM. 2021 Male fertility thermal limits predict vulnerability to
656 climate warming. *Nat. Commun.* **12**, 2214. (doi:10.1038/s41467-021-22546-w)
657 Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ, Walton VM, O'Neal SD,
658 Zalom FG. 2011 *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening
659 soft fruit expanding its geographic range and damage potential. *J. Integr. Pest Manag.* **2**,
660 3–9. (doi:10.1603/IPM10010)
661 Ward NL, Masters GJ. 2007 Linking climate change and species invasion: An illustration
662 using insect herbivores. *Glob. Chang. Biol.* **13**, 1605–1615. (doi:10.1111/j.1365-
663 2486.2007.01399.x)
664 Winkler A, Jung J, Kleinhenz B, Racca P. 2021 Estimating temperature effects on
665 *Drosophila suzukii* life cycle parameters. *Agric. For. Entomol.* **23**, 361–377.
666 (doi:10.1111/afe.12438)
667 Woolf AB, Ferguson IB. 2000 Postharvest responses to high fruit temperatures in the field.
668 *Postharvest Biol. Technol.* **21**, 7–20. (doi:10.1016/S0925-5214(00)00161-7)

669 Yamamoto A, Obha S. 1984 Temperature preferences of eleven *Drosophila* species from
670 Japan: the relationship between preferred temperature and some ecological characteristics
671 in their natural habitats. *Zoolog. Sci.* **1**, 631–640.
672

673 **FIGURES AND TABLES**

674 **Table 1** Variables considered to induce plasticity in *D. suzukii* thermal preference

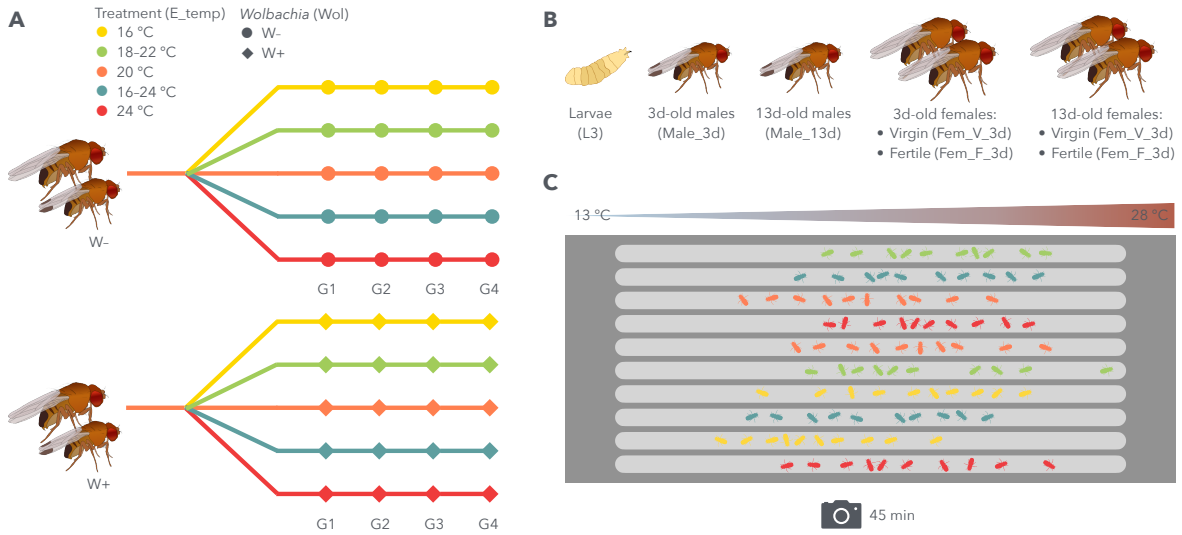
Environmental	
Temperature treatment	Constant (16, 20, 24°C) Fluctuating (18-22, 16-24°C)
Life stage	
Age	Larvae (L3) 3-day-old adult (D3) 13-day-old adult (D13)
Sex	Larvae (None) Male (M) Female (F)
Reproductive status	Larvae (L3) Virgin female (Female_V) Fertile female (Female_F) Male (Male)
Life stage (LifeS; integrative variable of Sex, Age and Reproductive status)	Larvae (L3) 3-day-old virgin females (Fem_V_3d) 3-day-old fertile females (Fem_F_3d) 13-day-old virgin females (Fem_V_13d) 13-day-old fertile females (Fem_F_13d) 3-day-old males (Male_3d) 13-day-old males (Male_13d)
Symbionts	
Wolbachia infection	Uninfected (W–) Infected (W+)
Transgenerational	
Generation	G1, G2, G3, G4

675

676

Table 2 Effects of temperature treatment, generation, *Wolbachia* infection status, life stage and their pairwise interactions on thermal preference (Tp) in *Drosophila suzukii*, estimated using linear mixed-effects models with gradient and lane nested within gradient included as random intercepts.

	Chisq	**Df**	***p*-value**	
Temperature treatment (E_temp)	392.27	4	<2.2e-16	***
Generation (Gen)	25.52	3	1.2e-05	***
Wolbachia (Wol)	78.97	1	<2.2e-16	***
Life stage (LifeS)	1046.12	6	<2.2e-16	***
E_temp × Gen	40.76	12	5.4e-05	***
E_temp × Wol	11.09	4	0.02561	*
E_temp × LifeS	179.25	24	<2.2e-16	***
Gen × Wol	0.49	3	0.9207	
Gen × LifeS	86.07	18	7.3e-11	***
Wol × LifeS	57.01	6	1.8e-10	***



682

683 **Figure 1** Experimental design. (A) From two source lines of *Drosophila suzukii*, *Wolbachia*-
684 uninfected (W–; circle) and infected (W+; diamond), initially raised at 20 °C, five
685 subpopulations of each were allocated to different temperature regimes (E_temp: 16, 18–22,
686 20, 16–24 and 24 °C; yellow, green, orange, blue and red, respectively). The populations were
687 maintained for 15 generations (expect for 16 °C, 10 generation; and 18–22 °C, 4 generations).
688 (B) We measured thermal preference (Tp) among different life stages: larvae (L3), young and
689 old males (Male_3d, Male_13d) and young and old females, virgin and fertile (Fem_V_3d,
690 Fem_V_13d, Fem_F_3d, Fem_F_13d), for both W– and W+ populations. (C) Tp measure
691 consisted in placing 10 individuals with a unique combination of variables within a lane of a
692 thermal gradient made of aluminium and with temperature ranging from 13 to 28 °C. A total
693 of 50 individuals was measured for each variable combination. Photographs were taken every
694 5 min for 60 min and the position at 45 min was recorded to assess Tp (see Deconninck et al.
695 (2025) for review on Tp measurement in *Drosophila*).

696

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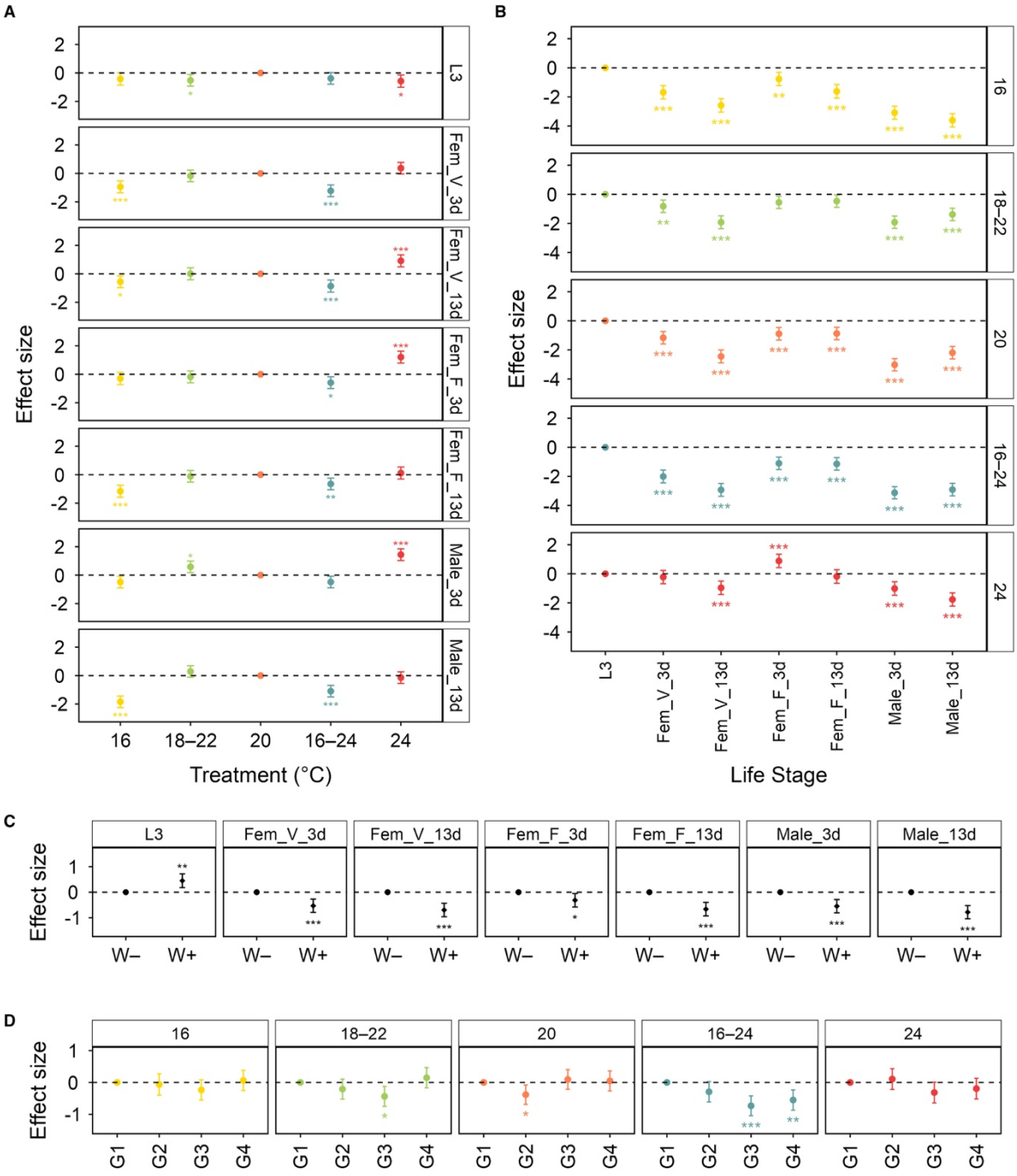

Figure 2 Context-dependent effects of temperature treatment, life stage, generation and *Wolbachia* infection on mean thermal preference (Tp) in *Drosophila suzukii*. Effect sizes were estimated from linear mixed-effects models. Panels show the effects of (A) temperature treatment within life stages, (B) life stage within temperature treatments, (C) *Wolbachia* infection within life stages, and (D) generation within temperature treatments. Effect sizes represent contrasts relative to reference conditions (20 °C, L3 stage and W– infection status).

706 Points indicate estimated contrasts and error bars represent 95% confidence intervals. Asterisks
707 denote significant differences from reference levels (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

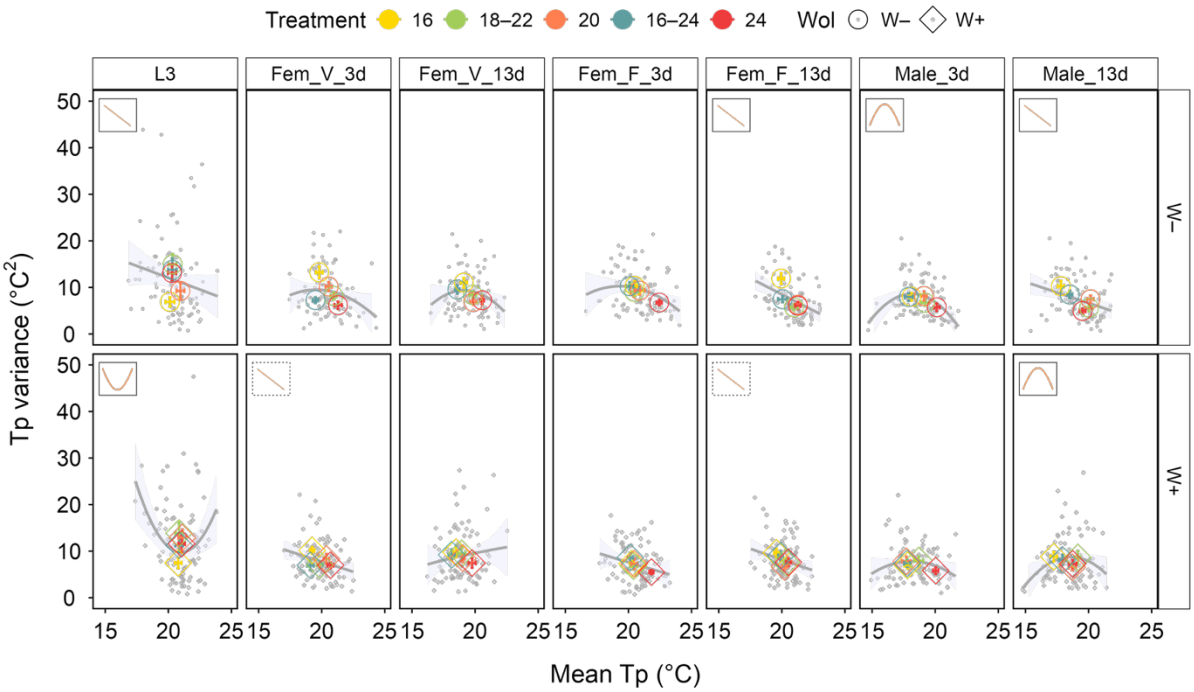

Figure 3 Thermal preference (Tp) mean and variance patterns, with plastic responses of Tp across life stages and *Wolbachia* infection status in *Drosophila suzukii*. Relationship between Tp mean and Tp variance of Tp across temperature treatments (constant regimes: 16, 20 and 24°C, in yellow, orange and red; fluctuating regimes: 18–22 and 16–24°C, in green and blue), life stages (L3: third larval stage; Fem_V_3d, Fem_V_13d, Fem_F_3d, Fem_F_13d: 3d and 13d-old virgin and fertile females; Male_3d, Male_13d: 3d and 13d-old males) and *Wolbachia* infection status (W–: uninfected, circle; W+: infected, diamond). Grey points represent averaged lane-level observations (n = 5 lanes for each temperature treatment and generation). Coloured symbols indicate treatment-level mean values (± SE) of Tp mean and Tp variance. Analyses were conducted using pooled data from generations G1 to G4. Fitted linear, quadratic or loess (span 10) regressions are shown as solid lines with 95% confidence bands. Symbols indicate inferred behavioural thermoregulation strategies following Deconninck et al. (2025) when the relationship between Tp variance and Tp mean was statistically significant (or marginally significant, shown as dashed symbols; see Table S1 for details).

Supplementary Materials

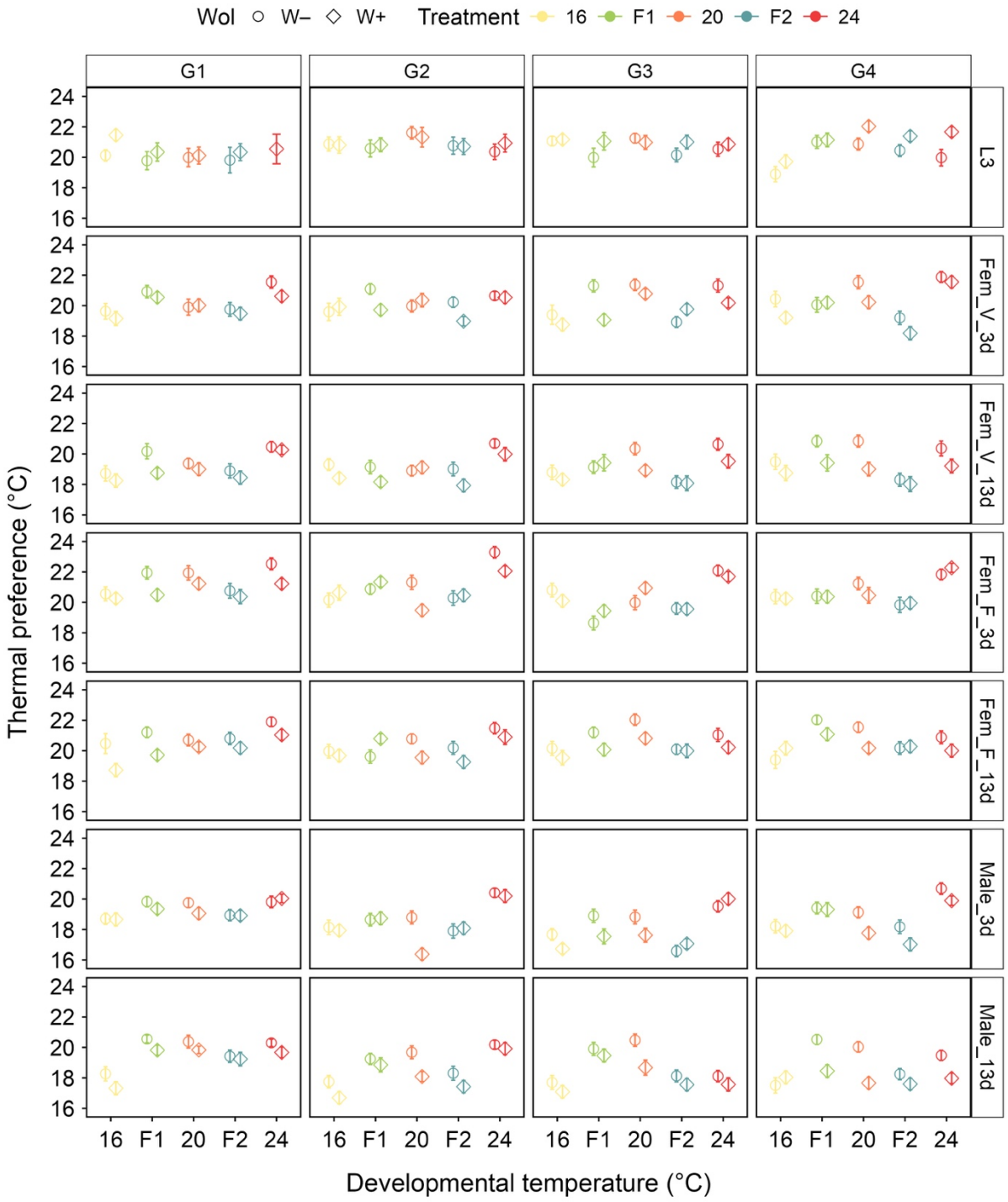

Figure S1 Thermal preference (Tp) across temperature treatments, generations, life stages and *Wolbachia* infection status in *Drosophila suzukii*. Points represent mean Tp (± SE) for each combination of variables. Colours indicate temperature treatment (constant: 16, 20 or 24°C, in yellow, orange and red; fluctuating regimes: F1 = 18-22 and F2 = 16-24°C, in green and blue)

and symbols indicate infection status (W–: uninfected, circle; W+: *Wolbachia*-infected, diamond). Panels are arranged by life stage (rows) and generation (columns). Tp measurements at G1, L3, W+, 24 °C could not be obtained due to technical constraints.

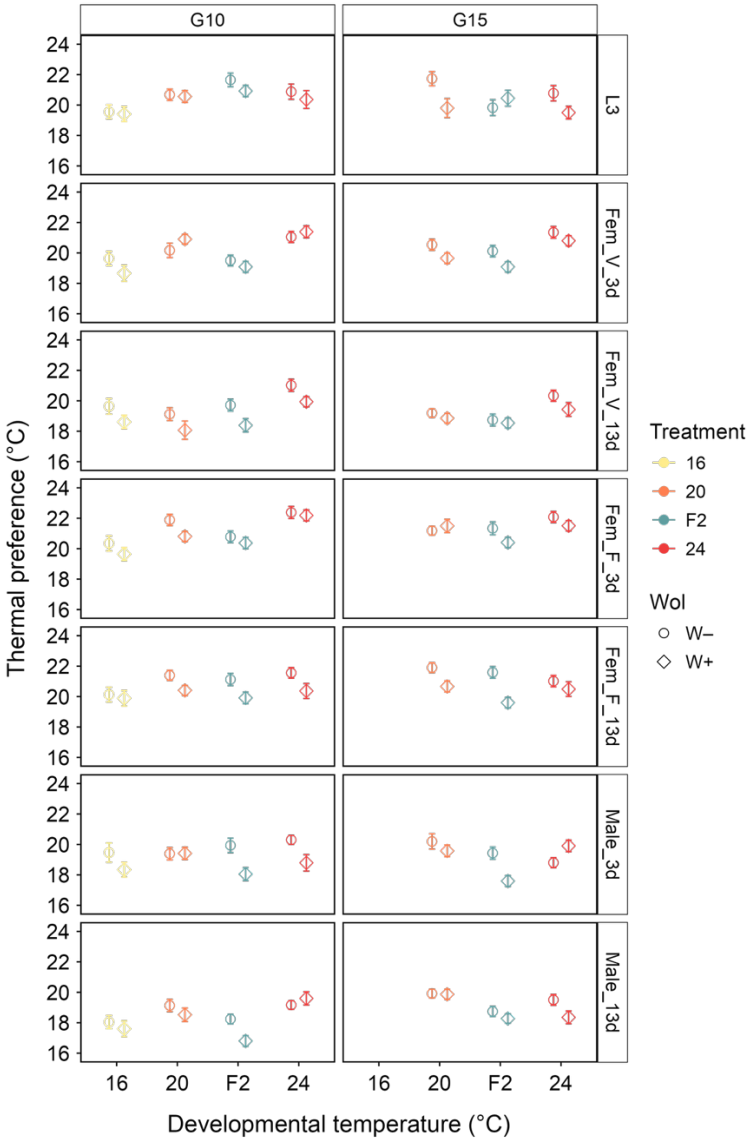

Figure S2 Thermal preference (Tp) across temperature treatments, generations, life stages and *Wolbachia* infection status in *Drosophila suzukii*. Points represent mean Tp (± SE) for each combination of variables. Colours indicate temperature treatment (constant: 16, 20 or 24°C, in yellow, orange and red; fluctuating regimes: 18-22 and 16-24°C, in green and blue) and symbols indicate infection status (W–: uninfected, circle; W+: *Wolbachia*-infected, diamond). Panels are arranged by life stage (rows) and generation (columns).

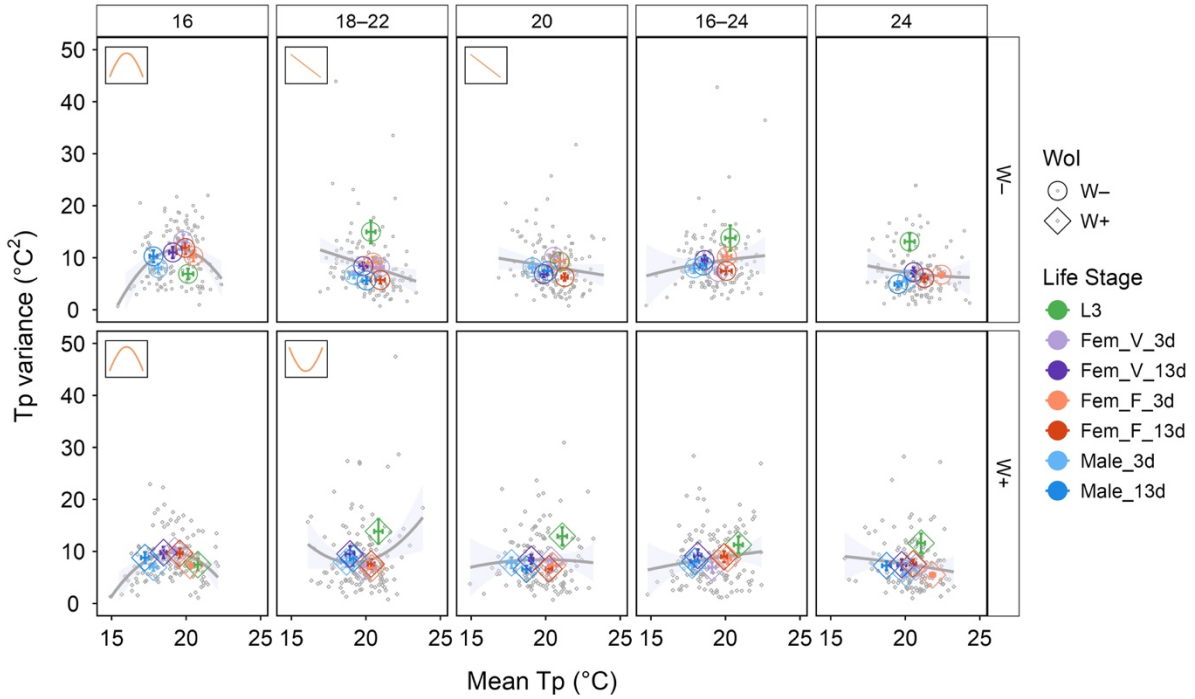

Figure S3 Thermal preference (Tp) mean and variance patterns, with plastic responses of Tp across temperature treatments and *Wolbachia* infection status in *Drosophila suzukii*. Relationship between Tp mean and Tp variance of Tp across temperature treatments (constant regimes: 16, 20 and 24°C, in yellow, orange and red; fluctuating regimes: 18–22 and 16–24°C, in green and blue), life stages (L3: third larval stage; Fem_V_3d, Fem_V_13d, Fem_F_3d, Fem_F_13d: 3d and 13d-old virgin and fertile females; Male_3d, Male_13d: 3d and 13d-old males) and *Wolbachia* infection status (W–: uninfected, circle; W+: infected, diamond). Grey points represent averaged lane-level observations (n = 5 lanes for each temperature treatment and generation). Coloured symbols indicate treatment-level mean values (± SE) of Tp mean and Tp variance. Analyses were conducted using pooled data from generations G1 to G4. Fitted linear, quadratic or loess (span 10) regressions are shown as solid lines with 95% confidence bands. Symbols indicate inferred behavioural thermoregulation strategies following Deconninck et al. (2025) when the relationship between Tp variance and Tp mean was statistically significant (see Table S2 for details). Interpretation: Life stage is used to induce plasticity. For a given temperature treatment, the mean and variance of Tp co-vary during

ontogeny. The inverted U-shape relationship between mean and variance of Tp indicates that the life stage with the highest mean Tp is also the one with highest precision (e.g., W+ larvae at 16 °C).

Table S1 Group-specific linear (β_1) and quadratic (β_2) effects of mean Tp on Tp variance for each life stage, with inferred relationship shape (when $p < 0.05$).

Wol	LifeS	β_1	β_1 *p*-value	β_2	β_2 *p*-value	Inferred shape
W–	L3	-75.84	0.0019	32.12	0.1953	Linear decreasing
	Fem_V_3d	-1.35	0.9679	-34.01	0.1803	ns
	Fem_V_13d	-25.75	0.2888	-58.42	0.1219	ns
	Fem_F_3d	-7.48	0.8057	-32.02	0.1340	ns
	Fem_F_13d	-101.63	0.0244	20.04	0.6112	Linear decreasing
	Male_3d	-89.76	0.0012	-68.20	0.0043	Inverted U (∩)
	Male_13d	-65.26	0.0105	-21.65	0.4390	Linear decreasing
W+	L3	-194.52	0.0000	152.74	0.0000	U-shape
	Fem_V_3d	-46.73	0.0739	-20.75	0.5870	Marginal - Linear decreasing
	Fem_V_13d	33.14	0.2408	-3.06	0.9232	ns
	Fem_F_3d	-47.72	0.2277	-0.25	0.9946	ns
	Fem_F_13d	-63.11	0.0748	18.27	0.7011	Marginal - Linear decreasing
	Male_3d	-50.00	0.1178	-38.40	0.0801	ns
	Male_13d	-55.92	0.1373	-60.48	0.0221	Inverted U (∩)

Table S2 Group-specific linear (β_1) and quadratic (β_2) effects of mean Tp on Tp variance for each temperature treatment, with inferred relationship shape (when $p < 0.05$).

Wol	E_temp	β_1	β_1 *p*-value	β_2	β_2 *p*-value	Inferred shape
W–	16	-12.59	0.5359	-85.74	0.0003	Inverted U (∩)
	18–22	-58.46	0.0075	6.13	0.8018	Linear decreasing
	20	-55.50	0.0258	38.84	0.0895	Linear decreasing
	16–24	23.58	0.2019	-4.16	0.8170	ns
	24	-25.40	0.3971	5.13	0.8018	ns

W+	16	-32.20	0.1387	-65.77	0.0013	Inverted U (∩)
	18–22	23.15	0.1874	51.22	0.0073	U-shape
	20	3.83	0.8132	-13.72	0.3471	ns
	16–24	23.93	0.1785	-2.02	0.9132	ns
	24	-24.26	0.2089	-2.32	0.9044	ns

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