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

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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 plasticity of behavioural thermoregulation strategies using the invasive fly *Drosophila suzukii*, including *Wolbachia*-infected individuals, and long-term exposure to contrasting thermal regimes across multiple generations. 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.

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

48 to behavioural thermoregulation (Gvoždík, 2012; Llewelyn et al., 2017). These levels of
49 plasticity can also vary across ontogeny and may be linked to shifts in thermoregulation
50 strategies, as reported in *D. melanogaster* (Deconninck et al., 2025). For example,
51 thermoregulation precision may vary with temperature depending on the costs associated
52 with sublethal temperature avoidance and the benefits of reaching the optimal temperature.
53 This recent conceptual development of behavioural thermoregulation plastic responses
54 (Deconninck et al., 2025) remains to be applied more broadly to infer the extent to which
55 behavioural plasticity may help ectotherms to adapt rapidly to varying temperature
56 conditions, including resistance or tolerance to extreme heat.
57 In this study, we explore the developmental plasticity of thermoregulation strategies in
58 the invasive fly *Drosophila suzukii* (Mastumura, 1931). This pest species of soft fruit shows
59 various seasonal adaptations (Tait et al., 2020, 2021), exploits different habitats (e.g., crops,
60 woodland, urban areas; Ulmer et al., 2024), has invaded temperate and tropical areas (Walsh
61 et al., 2011), has plastic thermal limits (Raynaud-Berton et al., 2024) and is an important
62 example of a successful invader that is benefiting from climate change (Ward and Masters,
63 2007; Diez et al., 2012; Cornelissen et al., 2019; Little et al., 2020; Robinson et al., 2020;
64 Skendžić et al., 2021; Deconninck et al., 2026). We used thermal preference (Tp) mean and
65 variance as proxies of behavioural thermoregulation strategies using the framework
66 developed by Deconninck et al. (2025). In this framework, the mean Tp reflects the thermal
67 need (higher Tp likely reflecting a shift of optimum temperature of physiological processes to
68 warmer temperature; Martin and Huey, 2015), the variance of Tp reflects the precision (the
69 lower the variance, the higher the precision) and their relationship allows deciphering of
70 behavioural thermoregulation strategies (Deconninck et al., 2025). We measured the Tp of *D.*
71 *suzukii* across ontogeny (larvae, young and old adults), including males, virgin and fertile
72 females, infected or not 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 also assessed the stability of thermal responses across successive generations and expected Tp and behavioural strategy to be consistent among the four generations tested (Paranjpe et al., 2013; Castañeda et al., 2019).

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 four generations. 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

Thermal preference (Tp) was measured in a linear thermal gradient (13–28 °C), using an apparatus similar to those described byTakeuchi et al. (2009), Goda et al. (2014) and Deconninck et al. (2025). The system consisted of an aluminium slab (500 × 250 × 20 mm)

heated at one end and cooled at the other using 45W Peltier devices (Quick-cool®, Germany) controlled by Arduino units (UNO R3). Water cooling prevented overheating of the Peltier elements and the apparatus was maintained in a climate-controlled laboratory at 22°C.

Ten lanes (400 × 10 × 5 mm) were machined into the slab and covered with transparent Makrolon® strips to prevent escape while allowing position tracking. Adults were introduced through small openings that were sealed during trials, whereas larval lanes were filled with a homogeneous gel (distilled water, sucrose 50 $g.L^{-1}$, agar $7g.L^{-1}$) to permit movement. Insects' positions were recorded every 5 min using a camera (Kodak PixPro AZ422) mounted above the apparatus.

Before experiments, temperatures were calibrated in each lane using T-type thermocouples connected to HOBO data loggers (UX120-014M, ONSET). Temperatures were recorded every 30 sec for 60 min at 4-cm intervals, and linear regression were used to predict temperature from position. Gradients were highly linear (all $R^2 > 0.99$) and stable over time and between assays.

Thermal preference measurement

We use two units of the gradient apparatus described above, with all electrical parameters kept constant throughout the study, to measure the Tp of wandering third-instar larvae (L3), young adults (3 day-old) and old adults (13 day-old), including males, virgin and fertile females and W– and W+ individuals. We focused on L3 as earlier stages were delicate to manipulate in our design, and whether they exhibit similar thermoregulation behaviour remains unknown. The life stage, sex and reproductive status of adults were grouped under 'Life stage' (LifeS) for analyses and included seven levels: larvae (L3), 3- and 13-day-old virgin females (Fem_V_3d; Fem_V_13d), 3- and 13-day-old fertile females (Fem_F_3d;

Fem_F_13d), and 3- and 13-day-old males (Male_3d; Male_13d) (Table 1; Figure 1B). Measurements were made on individuals from generations 1 to 4 (G1–G4; Figure 1A).

To assess the range of Tp at the population level, 50 individuals with a unique combination of variables were introduced into five lanes (10 × 5 replicates; Figure 1C). In total, about 14,000 individuals were measured (50 × 7 life stages × 5 temperature regimes × 2 *Wolbachia* infection status × 4 generations). The lanes were considered as independent replicates because no communication or contact was possible among individuals from different lanes. Before starting the assays, the gradient was switched on 15 min to allow stabilisation. Adult flies or larvae were placed in the centre of the lane at a temperature of ~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 avoid confounding effects of CO_2 anaesthesia (Nilson et al., 2006; Milton and Partridge, 2008).

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–G4), *Wolbachia* infection status, life stage, and all pairwise interactions among these factors as

fixed effects. Gradient and lane nested within gradient system were included as random effects to account for the experimental design. Higher-order interactions were evaluated but were not retained because they substantially increased model complexity without improving model support (ΔAIC = 89.4), produced non-estimable marginal means for sparse factor combinations, and did not alter the biological interpretation of the results.

Model-estimated contrasts were obtained from estimated marginal means (EMMs) using the *emmeans* package (Lenth and Piaskowski 2026). Pairwise treatment-versus-control contrasts were calculated using 20 °C (temperature treatment), L3 (life stage), or *Wolbachia*-uninfected (W–) individuals as the reference level, depending on the factor analysed. Estimated contrasts (ΔTp relative to the reference level) and their standard errors were extracted and visualised as point estimates with 95% confidence intervals.

To identify thermoregulation strategies, we quantified the mean Tp, used as an index of thermal need, and the variance in Tp among individuals within each experimental lane, used as an index of thermoregulatory precision (following Deconninck et al., 2025). Each lane contained a group of 10 individuals sharing the same combination of developmental temperature, life stage and *Wolbachia* infection status from the same generation. For each lane, we calculated the mean Tp and variance of Tp. We then averaged these lane-level means and within-lane variances across the five replicate lanes corresponding to each developmental temperature treatment. This approach preserves the biological interpretation of within-lane behavioural variation while allowing lane to be treated as the experimental replicate in subsequent analyses (see below). The relationship between mean Tp and within-lane Tp variance was first explored using a LOESS smoothing procedure (span = 10) within each life stage × *Wolbachia* combination, following Deconninck et al. (2025). This non-parametric approach allowed visual exploration of potential thermoregulation strategies within each life stage, without imposing a predefined functional form while accounting for

197 the broad variation in Tp induced by developmental temperature treatments. We then
198 formally tested these relationships using second-order polynomial linear models including
199 mean Tp, *Wolbachia* infectious status, life stage, and their interactions as fixed effects. The
200 significance of the Linear (β_1) and quadratic (β_2) components was used to classify the
201 relationship for each life stage × *Wolbachia* combination as linear, U-shaped, inverted U-
202 shaped, or non-significant. These analyses on thermoregulation strategies were performed on
203 pooled data from generations G1–G4 to increase the power of the test, and because
204 generation had only a weak effect. We were mostly interested in the variation induced by
205 temperature treatments to assess thermoregulation strategies of each life stage, but the
206 thermoregulation strategies can also be assessed within each temperature treatment following
207 the same procedure to explore the variation induced by ontogeny (Figure S3).
208
209 **RESULTS**
210 **Important Tp plasticity**
211 All variables and most of their interactions had significant effects on Tp, with temperature
212 treatment, life stage and *Wolbachia* having the strongest effects (all $p < 0.0001$; Table 2;
213 Figure S1). Temperature treatment effect depended on life stage with larval Tp being much
214 less influenced (Figure 2A) but most adults selecting lower temperatures when developed at
215 16 °C and higher temperatures when developed at 24 °C (mean ± se: 19.0 ± 0.1 and 20.5 ±
216 0.1 °C, respectively; Figure 2A). Individuals that developed under the low fluctuating regime
217 (18–22 °C) had Tp similar to those developed at constant 20 °C (both 19.9 ± 0.1 °C) but
218 selected lower Tp under the high fluctuating regime (16–24 °C), similar to the constant 16 °C
219 regime (19.1 ± 0.1 °C; Figure 2A). Regarding life stage, males had the lowest Tp (18.8 ± 0.1
220 °C both 3 d and 13 d), virgin females had intermediate Tp (3 d: 20.2 ± 0.1; 13 d: 19.2 ± 0.1
221 °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 change before ectotherms reach their thermal limits. However, understanding its adaptive significance requires integrating multiple sources of phenotypic plasticity rather than considering Tp alone (Gvoždík, 2012). Here, we show that Tp in *D. suzukii* is highly plastic, responding to developmental temperature, life stage and *Wolbachia* infection, with strong interactions among these factors. Despite this plasticity, thermoregulatory strategies were relatively conserved across ontogeny and were generally characterised by "precision at extreme" (low Tp variance near deleterious

247 temperatures). A notable exception was the contrasting effect of *Wolbachia* on larval and
248 adult thermoregulation, suggesting different underlying mechanisms. Overall, our results
249 indicate that predicting thermoregulatory behaviour in the field requires knowledge of an
250 individual's thermal history, life stage, reproductive and infection status.
251
252 **Multiple drivers of thermal preference plasticity**
253 The Tp of *D. suzukii* was highly plastic, responding to all variables tested, with temperature,
254 life stage (including ontogeny and sex) and *Wolbachia* having the strongest effects.
255 Increasing developmental temperature generally increased Tp, a pattern already reported
256 in other ectotherms, including seahorses, shrimps, killifish and spiders (Podrabsky et al.,
257 2008; Alfaro et al., 2013; Reiser et al., 2014; Mascaró et al., 2019). Studies in *Drosophila*
258 have likewise found positive effects of temperature on Tp (Yamamoto and Ohba, 1984;
259 McDaniel et al., 1995), although others reported no effect (MacLean et al., 2019) or reduced
260 Tp at temperatures above the thermal optimum (Krstevska and Hoffmann, 1994). Our results
261 suggest that the inconsistencies between studies may reflect differences in sex, age and
262 reproductive status, all of which modulated the magnitude, and sometimes the direction, of
263 the temperature effect. Exposure to stressful temperatures beyond the thermal optimum, as
264 well as thermal hardening, is also expected to reduce Tp (McDaniel et al., 1995; Dillon et al.,
265 2009).
266 The Tp of *D. suzukii* responded differently to constant and fluctuating temperature
267 regimes. Low amplitude fluctuations (18–22°C) produced the same Tp as a constant
268 temperature with the same mean (20 °C), consistent with findings in *D. melanogaster*
269 (Deconninck et al., 2025). In contrast, high-amplitude fluctuations (16–24 °C) reduced Tp to
270 levels similar to those observed after development at a constant 16 °C. One possible
271 explanation is a lower Tp protects against potentially harmful high temperatures, as suggested

for killifish (Podrabsky et al., 2008), although *D. suzukii* tolerates temperatures well above 30 °C (Enriquez and Colinet, 2017). Alternatively, because *D. suzukii* that developed at a constant 24 °C selected a higher Tp than those reared under high fluctuations, the lower end of the fluctuating regime may drive the reduction in Tp. This is consistent with recent findings reporting that the thermal optimum for survival shifts from ~25 to 18 °C under constant versus similarly fluctuating temperatures (Raynaud-Berton et al., 2024), suggesting that the lower Tp is an adaptive response to improved performance at lower mean temperature.

In *Drosophila*, both Tp and its plasticity generally differ between larvae and adults (MacLean et al., 2019). In *D. suzukii*, larvae had higher Tp than adults overall, although similar to fertile females. Selecting warmer temperatures allows faster larval development, providing a competitive advantage and reducing predation risk before emergence (Nunney 1990; Grainger and Levine, 2022; but see also 'grow now, pay later' hypothesis of Metcalfe and Monaghan (2001)). Although larval sex could not be determined, the low variance in Tp suggests that males and females display similar thermoregulatory behaviour at this stage. Adult females had higher Tp than males, particularly after females had mated. Fertile females may select higher temperatures to accelerate oogenesis (Cohet and David, 1978; Gandara and Drummond-Barbosa, 2022), and to optimise larval fitness as shown in Destierdt et al. (2026). Conversely, virgin females and older adults may prefer cooler temperatures to reduce metabolic costs and increase longevity (Marshall and Sinclair, 2010). Males had the lowest Tp, consistent with evidence that male fertility is more sensitive to elevated temperatures than female fertility (Jørgensen et al., 2006; van Heerwaarden and Sgrò, 2021; Ørsted et al., 2024).

Wolbachia-infected adults exhibited a lower Tp, consistent with findings in *D. melanogaster*, where multiple *Wolbachia* strains reduced adult Tp by 2–8 °C (Truitt et al., 2019), arguing against the hypothesis that *Wolbachia* effects on Tp reflect confounding

297 factors (Strunov et al., 2023). A lower Tp could be a self-medicating behaviour to reduce
298 bacterial proliferation ("behavioural chill"; Fedorka et al., 2016) and thereby limit fitness
299 costs associated with *Wolbachia* over-proliferation (Strunov et al., 2013a,b; 2024). In
300 contrast, infected larvae exhibited a slightly higher Tp (up to 0.8 °C), a pattern not previously
301 reported that could reflect *Wolbachia*-mediated manipulation of host behaviour (see
302 thermoregulation strategy section below).
303 Finally, the generation had only a minor influence on *D. suzukii* Tp. In ectotherms, Tp
304 often displays low to moderate heritability (see Bestion et al. (2023) for an example in
305 lizards), whereas maternal effects may contribute more strongly to variation in Tp (Paranjpe
306 et al., 2013). In D. melanogaster, bet-hedging limits the heritability of Tp, supposedly
307 reflecting adaptation to seasonal environments (Kain et al., 2015). We propose that a similar
308 strategy applies to *D. suzukii* whose life cycle is particularly adapted to seasonal variations
309 (De Ro et al., 2025). This prediction could be evaluated by analysing inter-individual
310 variation in Tp across generations and under different selection regimes (see also Deconninck
311 et al., 2025).
312
313 **Thermoregulation strategy: Be precise when approaching thermal extremes**
314 The increase in mean Tp associated with warmer developmental temperatures was generally
315 accompanied by more precise temperature selection (lower Tp variance), consistent with the
316 "precision at extremes" and "precision at high temperature" strategies described by
317 Deconninck et al. (2025). This pattern is consistent with studies showing that behavioural
318 thermoregulation in herbivorous insects primarily aims to avoid overheating rather than
319 maximise reproductive performance (Leith et al., 2024). In contrast, spider mites appear to
320 rely more on physiological than behavioural mechanisms to tolerate heat because their leaf
321 surface habitat provides limited thermal heterogeneity (Caillon et al., 2014; Ma et al., 2021).

Fruit flies, by comparison, can readily move or fly to cooler microhabitats and are expected to benefit from behavioural avoidance of sublethal heat stress, particularly because fertility declines at temperatures below those affecting survival (Jørgensen et al., 2006; Parratt et al., 2021; van Heerwaarden and Sgrò, 2021; Ørsted et al., 2024). More generally, Tp variance was lower in *D. suzukii* than reported for *D. melanogaster* (Deconninck et al., 2025), even in life stages that showed little evidence of plastic thermoregulatory strategies. This consistently high precision may contribute to the invasion success of *D. suzukii* by allowing exploitation of fine scale thermal refuges, although direct evidence is still lacking. Alternatively, the weak relationship between Tp mean and Tp variance could reflect limited plasticity in *D. suzukii* or to the relatively permissive thermal regimes used here.

Regardless of *Wolbachia* infection, larvae preferred warmer temperatures than any other life stage. This is consistent with their ecology, as larvae develop within fruit that can reach temperatures well above ambient air temperature under solar radiation (Woolf and Ferguson, 2000; Saudreau et al., 2009), and their developmental optimum ((~28 °C) exceeds that of adults (~24–27 °C) (Sánchez-Ramos et al., 2019; Winkler et al., 2021; Baser et al., 2025; Raynaud-Berton et al., 2024). Although larvae are confined to their host fruit, they may exploit within-fruit thermal heterogeneity to avoid overheating or approach their thermal optimum (Kührt et al., 2005; Saudreau et al., 2007, 2009, 2011). Notably, *Wolbachia* not only increased larval Tp, opposite to its effect in adults, but also shifted thermoregulation towards a "precision at optimum" strategy (Deconninck et al., 2025), with infected larvae selecting intermediate temperatures more precisely than uninfected larvae. Assuming that Tp reflects the thermal optimum for performance (Martin and Huey, 2008), this behaviour could enhance larval foraging and development, which is the main function of thermoregulation behaviour reported in reptiles (Ladyman et al., 2003; Taylor et al., 2021). Alternatively, *Wolbachia* may manipulate larval thermoregulation to favour its own proliferation by promoting the selection

of warmer microhabitats (Fedorka et al., 2016; Strunov et al., 2013a,b). Although speculative, this contrasts with the proposed "behavioural chill" response in adults and suggests that Wolbachia-host interactions may differ fundamentally between larval and adult stages.

CONCLUSIONS

Drosophila suzukii exhibited marked plasticity in Tp, with all tested variables influencing its expression. Although we could not directly assess the fitness consequences of this plasticity, modulation of Tp may help individuals to cope with extreme or novel environments (Gvoždík, 2012; Ma et al., 2021). In *D. suzukii* and other drosophilids, behavioural thermoregulation plasticity may therefore have contributed to range expansion and invasive success (Deconninck et al., 2026). Whether Tp measured in artificial thermal gradients reflects thermoregulatory behaviour in more complex, natural conditions remains uncertain. Nevertheless, our approach of inferring thermoregulatory strategies from both the mean and variance of Tp provides a useful conceptual framework. Future studies should determine whether Tp plasticity is adaptive under natural conditions, facilitates colonisation and persistence during range expansion, and itself evolves during range expansion, as predicted by theoretical models (Eriksson and Rafajlović, 2022; Usui et al., 2023; Swaegers et al., 2024).

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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.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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665 **TABLES**

666 **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+)
Generation	
Generation	G1, G2, G3, G4

667

668

669 **Table 2** Effects of temperature treatment, generation, *Wolbachia* infection status, life stage
670 and their pairwise interactions on thermal preference (Tp) in *Drosophila suzukii*, estimated
671 using linear mixed-effects models with gradient and lane nested within gradient included as
672 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	***

673

FIGURES

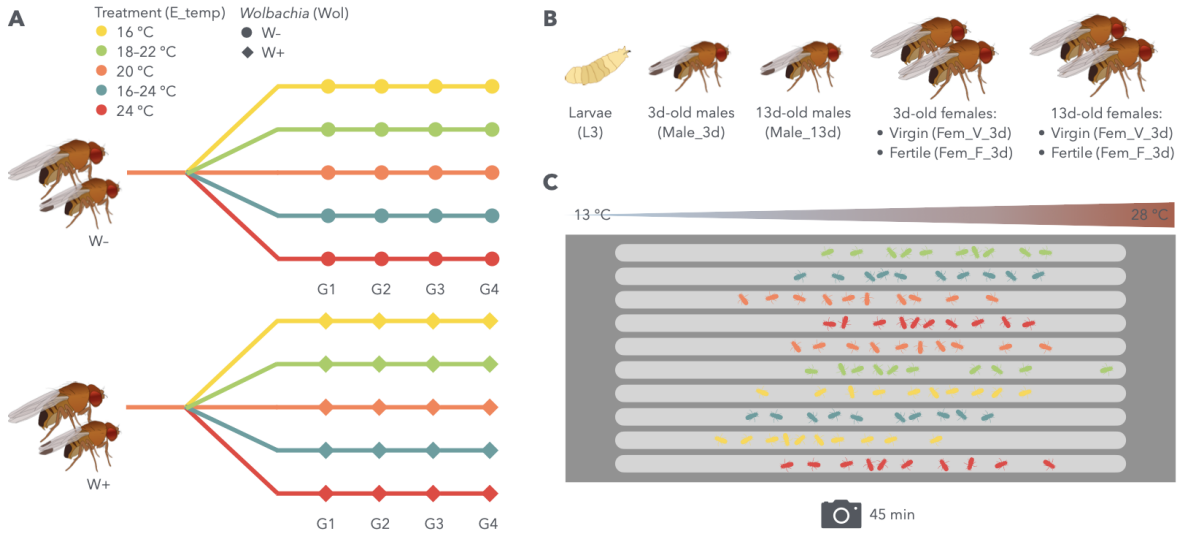

Figure 1 Experimental design. (A) From two source lines of *Drosophila suzukii*, *Wolbachia*-uninfected (W–; circle) and infected (W+; diamond), initially raised at 20 °C, five subpopulations of each were allocated to different temperature regimes (E_temp: 16, 18–22, 20, 16–24 and 24 °C; yellow, green,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

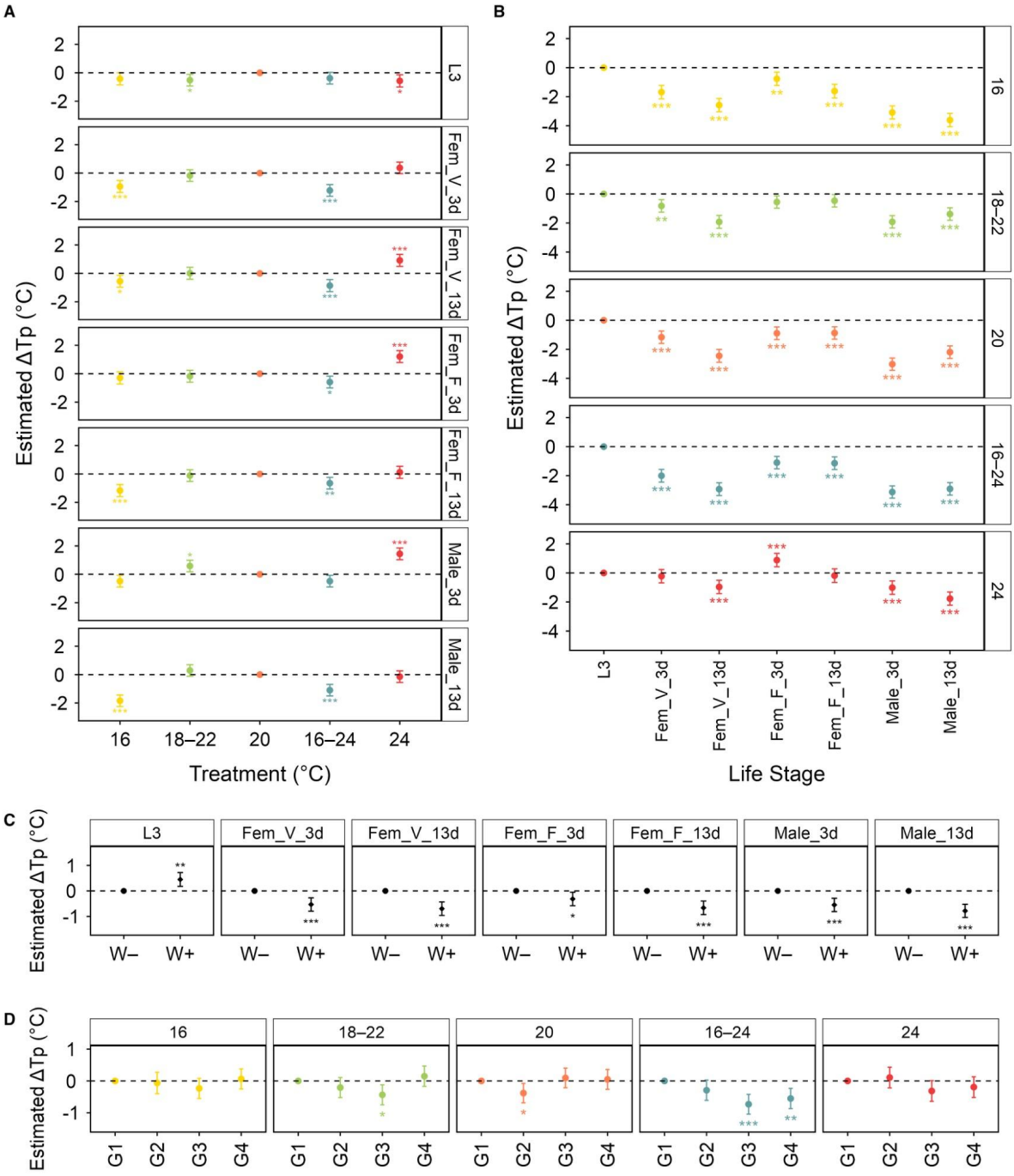

Figure 2 Context-dependent effects of temperature treatment, life stage, generation and *Wolbachia* infection on mean thermal preference (Tp) in *Drosophila suzukii*. Estimated contrasts were obtained from linear mixed-effects models using estimated marginal means (EMMs). Panels show the estimated effects 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. Contrasts are expressed relative to the

appropriate reference level for each panel (20 °C for temperature, L3 for life stage, W– for *Wolbachia* infection status, and G1 for generation). Points indicate estimated contrasts (ΔTp, °C), error bars indicate 95% confidence intervals, and asterisks denote significant differences from the corresponding reference level (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

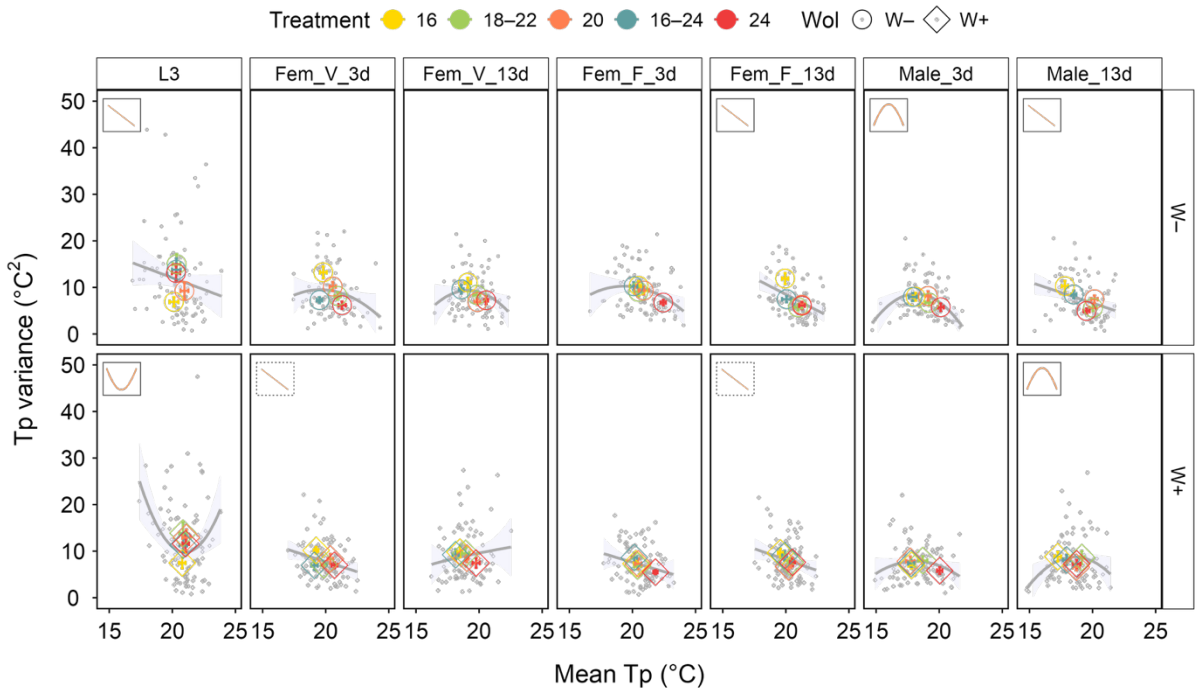

Figure 3 Thermal preference (Tp) mean and variance patterns, with plastic responses of Tp across life stages and *Wolbachia* infection status in *Drosophila suzukii*. Relationship between Tp mean and Tp variance of Tp across temperature treatments (constant regimes: 16, 20 and 24°C, in yellow, orange 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).

SUPPORTING INFORMATION

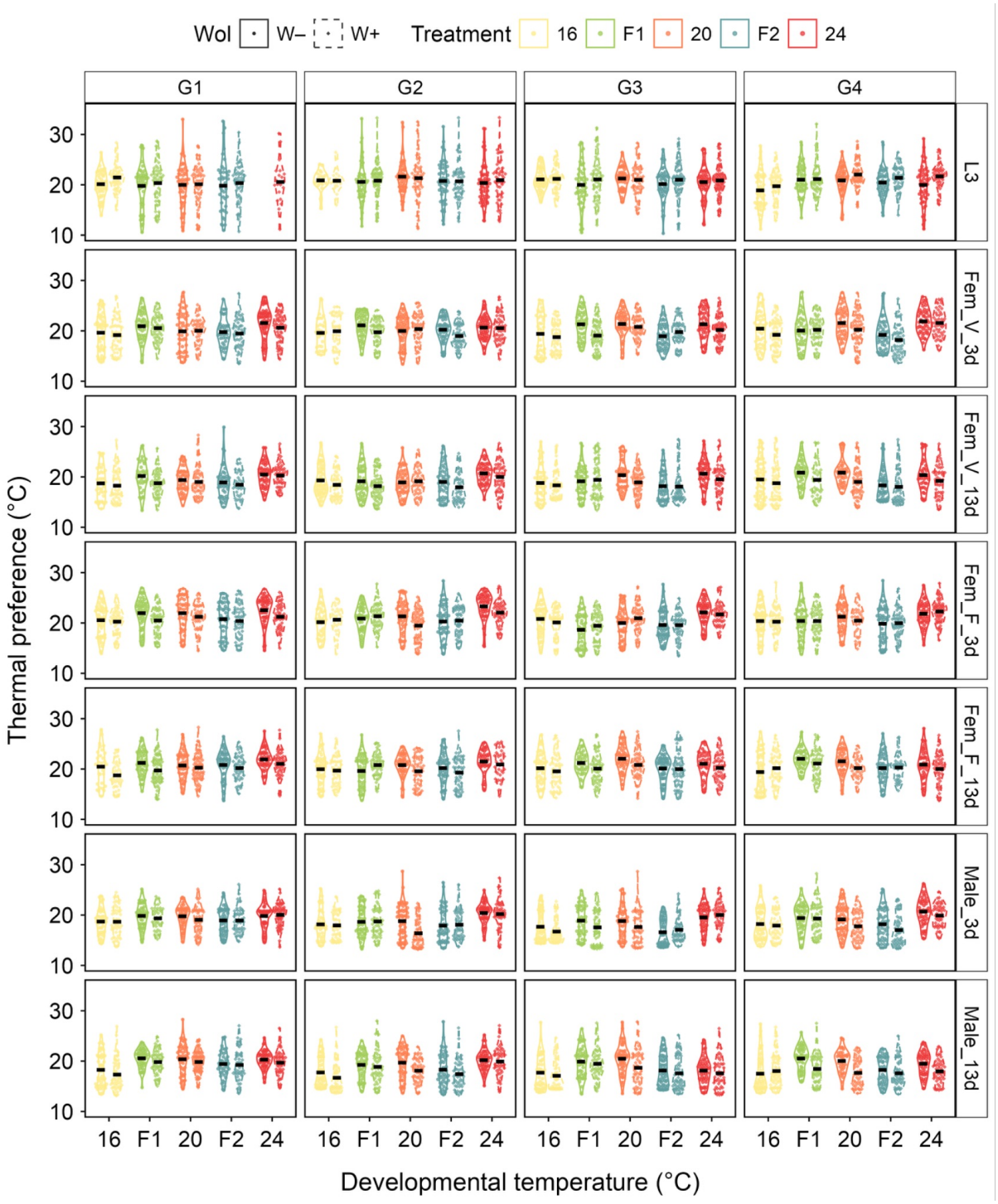

Figure S1 Thermal preference (Tp) across temperature treatments, generations, life stages and *Wolbachia* infection status in *Drosophila suzukii*. Points represent mean Tp (± SE) for each combination of variables. Colours indicate temperature treatment (constant: 16, 20 or 24°C, in yellow, orange and 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.

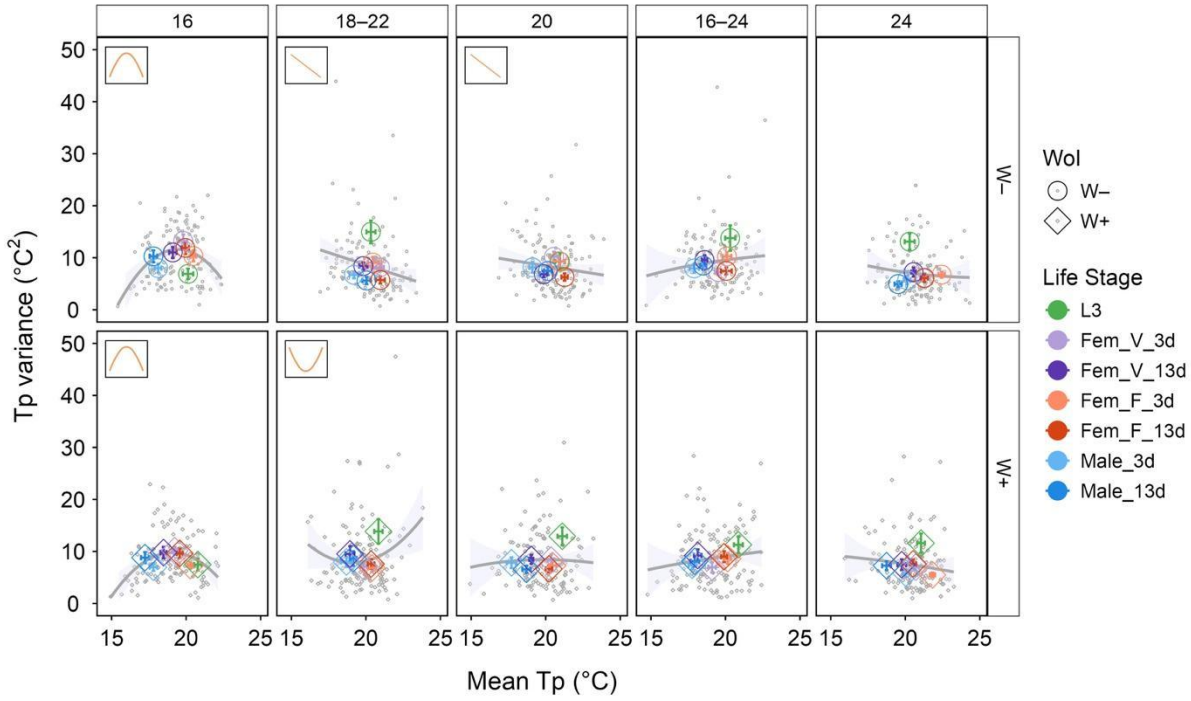

Figure S2 Thermal preference (Tp) mean and variance patterns, with plastic responses of Tp across temperature treatments and *Wolbachia* infection status in *Drosophila suzukii*. Relationship between Tp mean and Tp variance of Tp across temperature treatments (constant regimes: 16, 20 and 24°C, in yellow, 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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