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Continental divergence in male reproductive plasticity to thermal and resource stress in a widespread dung fly

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Introduction: Phenotypic plasticity in critical life-history traits can buffer populations against environmental change, but its magnitude and direction may vary across geographic lineages.

Methods: Here, we investigated how two major developmental stressors, rising temperatures and food limitation, affect life-history traits. We quantified variation in juvenile survival, development time, adult body size and male reproductive investment (testis size; sperm length) in *Sepsis punctum* (Fabricius, 1794), a widespread dung fly with different mating strategies and contrasting sexual size dimorphism (SSD) between North America (females larger) and Europe (males larger).

Results and Discussion: Elevated temperatures reduced larval survival and accelerated development, generating smaller adults, particularly in European populations, with no changes in SSD. Food limitation also reduced adult size but had little effect on juvenile survival. Instead, resource stress differentially impacted SSD; female-biased SSD persisted in North American populations, but male-biased SSD did not persist in European populations. Male reproductive investment showed partial resilience, where absolute trait sizes declined under both stressors, but size-independent investment remained largely stable. Testis size decreased with increasing temperature, while sperm length did not, suggesting potential canalization of sperm quality and a strategic resource allocation that trades off quantity under stress. We revealed continental divergence in phenotypic plasticity in *S. punctum*, highlighting the importance of geography and stressor type in shaping life-history trajectories. These findings indicate that thermal stress may have stronger evolutionary consequences than resource limitations, with implications for population resilience and reproduction under global change.

Introduction

Rapid environmental changes are intensifying the frequency and severity of developmental stressors experienced by organisms (Chevin and Hoffmann, 2017; IPCC, 2023; Peters and Lovejoy, 1994). Rising temperatures and fluctuations in resource availability directly influence an organism's early development, altering key fitness-related traits such as survival, growth rate, and reproduction (Crean and Immler, 2021; Johnson et al., 2016). Resilience to stressful conditions partly depends on phenotypic plasticity, which is a flexible response that allows individuals to alter their phenotypes in response to environmental variation (Bonamour et al., 2019; Pigliucci et al., 2006; Stearns, 1989; West-Eberhard, 2003). However, plasticity is not universally beneficial and can involve critical trade-offs between survival (e.g., thermal tolerance) and reproduction (e.g., fecundity), which vary among sexes and populations (Bogan et al., 2024; Jarrold et al., 2019; Sisodia and Singh, 2002). Thus, adaptive plasticity in resource allocation strategies is fundamental in influencing the evolution of life-history traits, and understanding developmental stress responses in different populations is relevant for predicting resilience to global change, particularly for widespread species (Chevin and Hoffmann, 2017; Kelly, 2019; Levins, 2012; Nylin and Gotthard, 1998).

Insects are powerful models for studying developmental plasticity, given their short generation times and sensitivity to environmental stress (Gillott, 1980; Moczek, 2010; Yoon et al., 2023). In holometabolous species that undergo complete metamorphosis, juveniles often experience starkly different environmental selection pressures than adults (Truman, 2019; Weaving et al., 2022). Larval developmental conditions tend to impact overall fitness more critically, as they influence both survival to the adult stage and fitness parameters such as reproduction and longevity (Blanckenhorn et al., 2014; Boggs, 2009; English and Barreaux, 2020; Nylin and Gotthard, 1998). Abiotic factors, such as temperature, influence the overall development, reproduction, and population dynamics of various ectotherms (Adamo and Lovett, 2011; Atkinson, 1994; Barton and Ives, 2014; Gourgoulianni et al., 2023), whereas biotic stressors, such as early resource or food limitation, can influence adult dispersal and reproductive capacities (Daniel et al., 2022; Chafino et al., 2019; Dillon et al., 2009; Zhang and Puniamoorthy, 2025). Studies investigating reproductive plasticity often focus on indicators vital for mate acquisition, that is, precopulatory traits such as overall body size and/or secondary sexual structures (Emlen, 1994; Harrison et al., 2023; Yap et al., 2024). The effects on traits that mediate fertilization success or post-copulatory reproductive success, such as sperm competition, mate guarding, or increased refractory periods, are less explored (Devost and Turgeon, 2016; Hagg et al., 2024; Himuro et al., 2022; Parker, 1970). Even sperm traits can be influenced by resource investments and affect reproductive fitness via gamete quantity and quality (Boggs, 1981, 2009; Macartney et al., 2018; Mehlis et al., 2015).

Here, we focus on *Sepsis punctum* (Diptera: Sepsidae), a widespread dung fly with natural populations distributed across North America and Europe (Pont and Meier, 2002). Adults are commonly found on ephemeral dung pats and exhibit sexual dimorphism. Prior studies have documented continental divergence in mating system dynamics and associated changes in mating behavior and sexual size dimorphism, with European populations expressing male-biased SSD and North American populations expressing female-biased SSD (Dmitriew and Blanckenhorn, 2012; Puniamoorthy et al., 2012a, b). These populations have likely experienced distinct evolutionary pressures, including varying climate regimes and intensities of sexual selection, making them ideal for testing whether plastic responses to stress are consistent or lineage-specific, although existing studies have mainly focused on single populations or within-continent variation (Berger et al., 2013, 2014; Lee et al., 2024). We subjected larvae from different populations to two common forms of developmental stress (elevated temperatures and reduced food availability) and quantified their effects on juvenile survival, development time, adult body size, and male postcopulatory traits: Testis size, a proxy for sperm quantity, and sperm length, a proxy for sperm quality (Simmons et al., 1999, 2003; Snook, 2005). By comparing responses across continents, sexes, and stress types, we aim to uncover the extent to which environmental stress alters fitness-related traits and whether reproductive investment is buffered against developmental perturbations.

We predicted that developmental stress would negatively impact fitness-related traits, but the magnitude would vary between continents, with larger-bodied European populations exhibiting greater sensitivity to stress-induced size reductions. Specifically, we expect increasing temperatures to reduce larval survival, shorten developmental time and reduce overall body size, consistent with the temperature-size rule, which predicts that ectotherms mature smaller at warmer temperatures owing to the differential thermal responses of growth and development rates (Atkinson, 1994; Sheridan and Bickford, 2011; Verberk et al., 2021). Food limitation may impose similar effects on survival and growth, but may lead to prolonged development owing to potential trade-offs in reproductive allocation (Gittleman and Thompson, 1988; Martin, 1987).

Methods

Fly cultures and maintenance

Here, we studied both European and North American populations of *S. punctum*. Laboratory stocks were established from wild-caught mixtures collected in 2018. These flies were allowed to mate freely within each container, expanding the population size to 200–400 adults per generation, and were kept for multiple (>15) generations before the experiments were conducted in 2019. All stock cultures were maintained under

standardized laboratory conditions of 26°C, 60% humidity, and 14:10 L:D photoperiod with fresh untreated cow dung, sugar, and water provided *ad libitum*.

Experimental design

We conducted two independent experiments to evaluate the effects of (1) developmental temperature and (2) resource availability on survival, development, adult body size, and male reproductive traits of the wasps. Each experiment included four populations (two per continent) selected to span latitudinal and ecological gradients, with one overlapping European population (Italy) to facilitate cross-stressor comparisons (Figure 1). To obtain test individuals, stock cultures were provided with cattle dung *ad libitum* for 2 h to 3 h for females to oviposit. First-instar larvae from each population were split into replicates of 10 larvae, which were randomly distributed into different environmental treatments within 30 h of oviposition.

Experiment 1: Thermal stress

Two European (Sweden, SW: 58.52°N, 16.84°E; Italy, IT: 42.56°N, 12.64°E) and two North American (USA, GA: 32.17°N, −82.90°W; USA, NY: 43.711°N, −76.977°E) populations were reared at four temperatures within an ecologically relevant range: 16°C, 20°C, 24°C, and 28°C (Berger et al., 2013; Dallo et al., 2024; Rohner and Berger,

2025). Temperatures above 30°C were excluded from this study because of their known detrimental effects on reproductive success (Berger et al., 2013). For each temperature × population combination, 100 larvae (10 replicates of 10 larvae each) were reared in *ad libitum* dung under controlled humidity and photoperiod (60% humidity, 14:10 L:D).

Experiment 2: Resource stress

Two European (Germany, DE: 49.48°N, 8.44°E; Italy, IT: 42.56°N, 12.64°E) and two North American (Canada, ON: 45.42°N, 75.67°W; USA, ND: 46.90°N, −96.80°W) populations were reared on five amounts of dung around previously defined thresholds: 1.5 g, 5.5 g, 9.5 g, 13.5 g, and 17.5 g, with 0.3 g per larvae previously recorded to cause substantially smaller adults (Berger et al., 2013; Blanckenhorn, 1998; Rohner et al., 2016). For each resource × population combination, 50 larvae (five replicates of 10 larvae each) were reared at a constant temperature, humidity, and photoperiod (26°C, 60% humidity, 14:10 L:D).

Life history traits

Larval survival and development time

Larval survival to adulthood, development time, and adult morphological traits were recorded for both experiments. For each replicate, the survival rate was calculated as the proportion
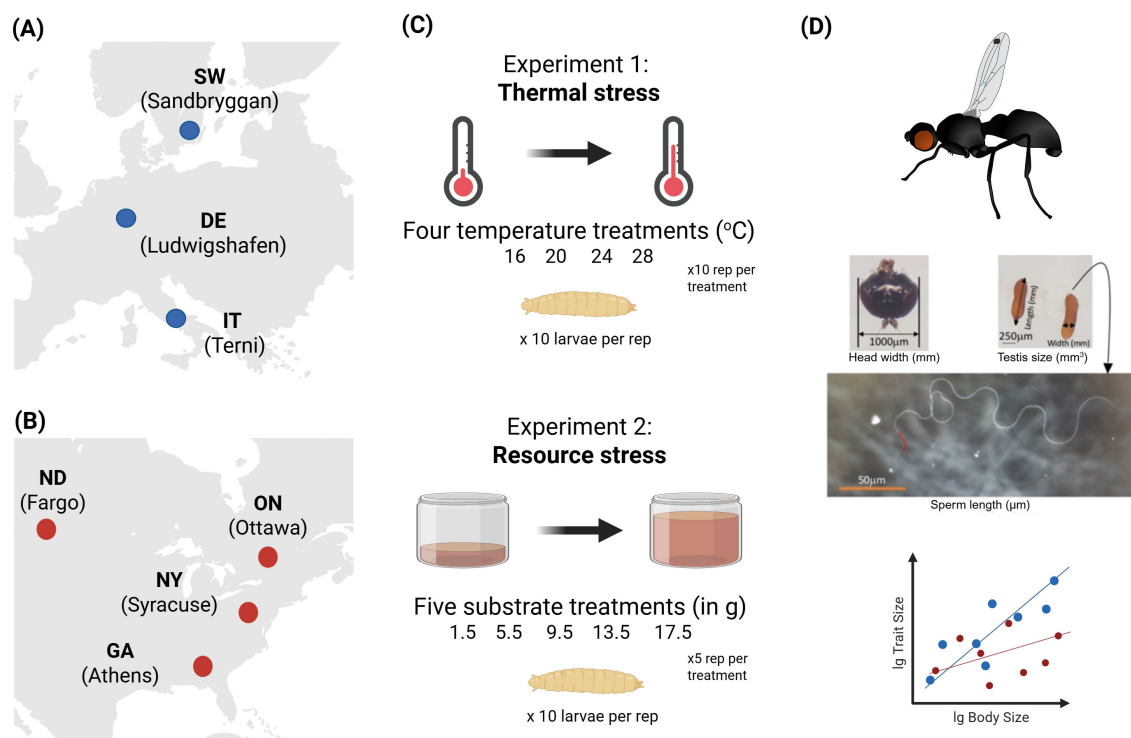
FIGURE 1
Experimental design. Locations of *S. punctum* populations in **(A)** Europe (blue) and **(B)** North America (red). Schematic summary of the two experiments conducted to assess the effects of environmental stress during larval development: higher temperatures and resource limitations **(C)**. Head width measurements were taken as an indicator of body size, and male postcopulatory traits (testes size and sperm length) were scaled against body size **(D)**. Source: Created in BioRender. https://BioRender.com/l95vat2.

of individuals that successfully emerged as adults. Development time was recorded as the number of days from oviposition to eclosion for each adult insect. Adult flies were morphologically sexed upon emergence and freeze-killed at −40°C for two to three days after eclosion before being preserved in 70% ethanol.

Adult morphological traits (body size and male postcopulatory traits)

We assessed whether adult body size and male post-copulatory traits (i.e., testis volume and sperm length) changed in response to environmental stress during larval development. We measured head width as a standard proxy for adult body size (Blanckenhorn et al., 2004; Puniamoorthy et al., 2012a, b) for both sexes using an Olympus SZX10 microscope. The testes of each male fly were dissected and transferred onto a glass slide with phosphate-buffered saline (PBS). The length (L) and width (W) of each testis were recorded to calculate the ellipsoid volume (V = 1/6 × L × W^2 × π) (Blanckenhorn and Henseler, 2005). Subsequently, sperm were extracted from one testis on a fresh glass slide and fixed with a methanol: acetic acid (3:1) solution for 1 min before staining with 4',6'-diamidino-2-phenylindole (DAPI) solution in the dark for 5 min. DAPI binds to nucleic acids, allowing the visualization of sperm cell DNA under an Olympus BX50 fluorescent microscope (Tanious et al., 1992). The stained sperm were mounted with one drop of glycerol before sealing with a cover slip and clear nail polish and dried in the dark. Ten sperm per male were measured (from head to tail) to obtain the average sperm length of each male using ImageJ (Abrámoff et al., 2004) and Micro-manager software (Edelstein et al., 2014).

Statistical analysis

We analyzed all data in R (version 4.0.5) (R Core Team, 2021), checking for normality with Shapiro–Wilk tests and Q–Q plots. Given the deviations from normality and the hierarchical structure of our experimental design, we used generalized linear mixed models (GLMMs) with the *lmerTest* package (Kuznetsova et al., 2017) or linear mixed-effects models (LMEs) with *lme4* (Bates et al., 2015), depending on the response variable. For all models, we used the Akaike Information Criterion with an additional term to correct for small samples (AICc) (Burnham and Anderson, 2004) using the *model.sel* function from the *MuMIn* R package (Barton, 2013) (ΔAICc <2). We checked the diagnostic plots to ensure that the model assumptions were met and removed outliers based on Cook's distance. Figures were constructed using the *ggplot2* package (Wickham, 2016). We assessed significance using the Kruskal–Wallis test (*kruskal.test* function in base R), followed by Dunn's test using the *dunn_test* function from the *rstatix* package (Kassambara, 2023). We calculated the size dimorphism index (SDI) of Lovich and Gibbons (1992) using the means of each sex per treatment (Supplementary Table S1.1) and Dunn's test to statistically test for the presence or absence of sexual size dimorphism.

Larval survival and development

We constructed candidate GLMMs with larval development time (days) and survival rate as separate response variables for each treatment. For larval development time, we used sex, population, and either temperature or resources as fixed effects and replicates as random effects. For survival rate, we used only population and either temperature or resource as fixed effects and replicates as random effects. All GLMMs were checked for any overdispersion.

Adult body size and male reproductive traits

For adult body size, we constructed a set of candidate LMEs with sex, population, and either temperature or resources as fixed effects and replicates as a random effect. For male reproductive traits, we constructed candidate LMEs with testis volume and sperm length as separate response variables and included population and either temperature or resources as fixed effects, replicates as a random effect, and body size as a covariate. We also analyzed residual reproductive investment by regressing reproductive traits on body size and using the residuals as response variables in the treatment models.

Allometric analyses

To evaluate changes in the reproductive allocation of male post-copulatory traits across populations under environmental stress (temperature/resource), we performed a log–log regression of linearized testis size and sperm length against body size and fitted global regression models across all treatment levels. We compared linear, quadratic, and cubic models using AICs and conducted separate regressions to assess geographic differences in allometric scaling.

Results

Thermal stress reduces survival and accelerates larval development across populations

Thermal stress significantly impacted survival and development time across populations, with the number of eclosed adults declining as temperature increased (Figure 2A; $x^2_{3,160}$ = 63.091, p <0.001). With a total of 1,348 out of 1,616 larvae surviving to adulthood, the highest mortality was observed at 28°C, where survival dropped by up to 36% relative to 16°C (Table 1; GLMM, p <0.001). Populations from higher latitudes (NY and SW) also showed a sharp decrease in survivability (Figure 2A). Development time decreased with increasing temperature (Figure 2B; $x^2_{3,1348}$ = 1,045.3, p <0.01), reflecting the expected acceleration of development under warmer conditions (Table 1; GLMM, p <0.001). Overall, at 28°C, flies emerged 37%–38% faster than at 16°C. We also observed an expected cross-continental reversal in development time between sexes in accordance with the differing SSD trends between continents (Figures 2B, C; NA, $x^2_{1,661}$ = 8.4107, p <0.01; EU, $x^2_{1,687}$ = 10.461, p <0.01).
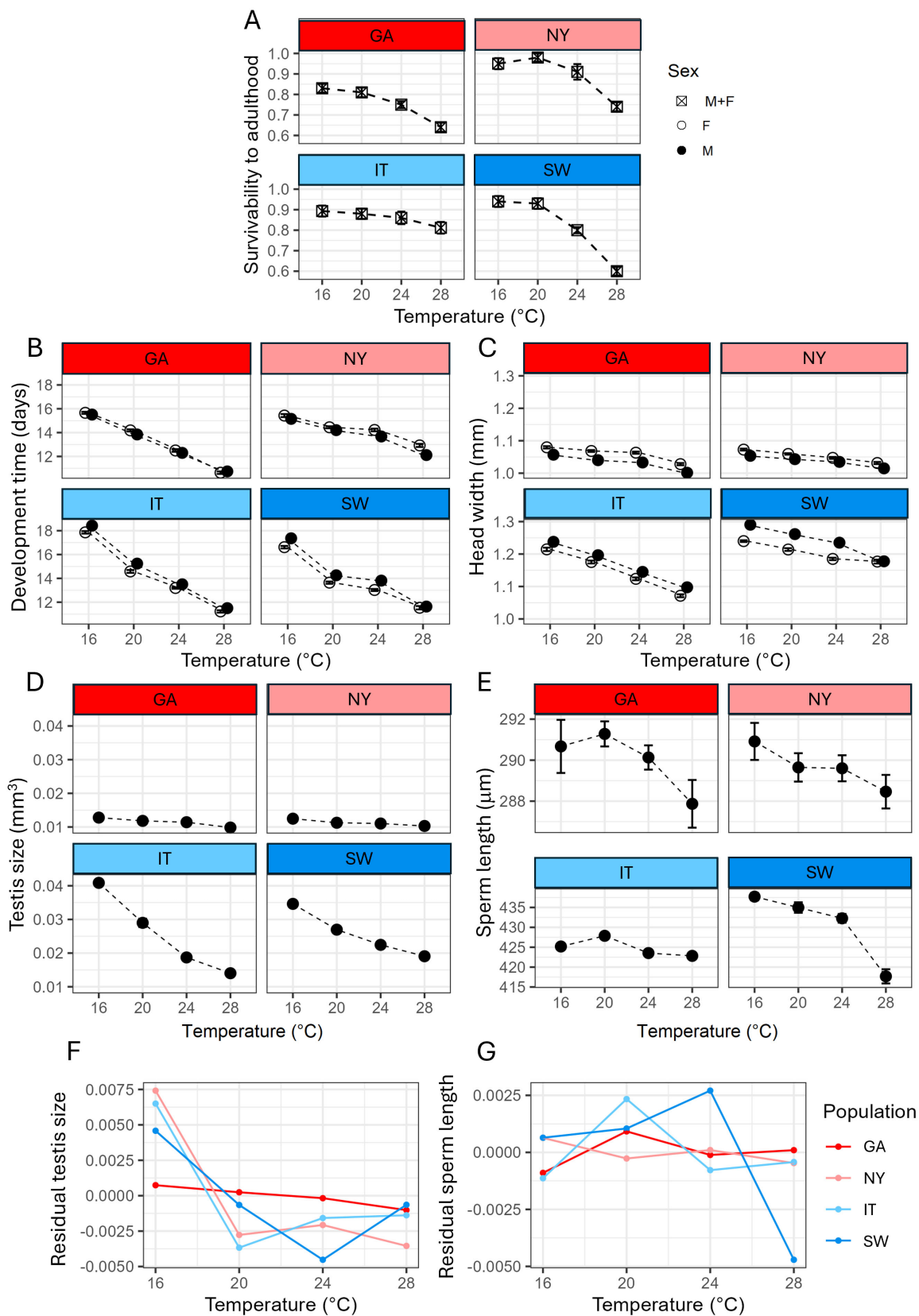
FIGURE 2
Mean ± SE trends of thermal stress on *S. punctum* **(A)** larval survivability rate, **(B)** larval development time, **(C)** head width as a proxy for body size, **(D)** testis size, and **(E)** sperm length, along with the respective size-independent residuals **(F, G)** across four populations from two continents.

TABLE 1 Summary of best fit LME and GLMM models for *S. punctum* development and morphological trait measurements under thermal stress.

Fixed effects	Estimate	Std. Error	T value/Z value	Pr(>|t|)
Survival Rate (model: GLMM)				
(Intercept)	1.586	0.266	5.956	2.58E−09***
Population (SW)	1.166	0.498	2.340	0.019*
Population (NY)	1.359	0.531	2.561	0.010*
28°C	−0.892	0.343	−2.601	0.009**
Population (SW): 28°C	−1.454	0.580	−2.505	0.012*
Development Time (model: averaged GLMM) ***Conditional results. Variables in bold are included in all competing models**				
(Intercept)	2.746	0.028	97.620	<2.00E−16***
20°C	−0.106	0.041	2.601	0.009**
24°C	−0.229	0.043	5.322	1.00E−07***
28°C	−0.378	0.047	7.981	<2.00E−16***
Population (SW)	0.086	0.037	2.294	0.022*
Population (IT)	0.150	0.037	4.062	4.86E−05***
Population (NY): 24°C	0.137	0.058	2.367	0.018*
Population (NY): 28°C	0.179	0.063	2.829	0.005**
Body Size (model: LME)				
(Intercept)	1.080	0.004	288.864	<2.00E−16***
20°C	−0.011	0.005	−2.195	0.028*
24°C	−0.017	0.005	−3.158	0.002**
28°C	−0.052	0.006	−9.442	<2.00E−16***
Population (SW)	0.160	0.005	31.983	<2.00E−16***
Population (IT)	0.135	0.005	27.233	<2.00E−16***
Sex (M)	−0.023	0.005	−4.331	1.60E−05***
20°C: Population (SW)	−0.015	0.007	−2.115	0.035*
24°C: Population (SW)	−0.038	0.007	−5.256	1.72E−07***
20°C: Population (IT)	−0.028	0.007	−4.020	6.16E−05***
24°C: Population (IT)	−0.075	0.007	−10.234	<2.00E−16***
28°C: Population (IT)	−0.091	0.007	−12.477	<2.00E−16***
Population (SW): Sex (M)	0.073	0.007	10.137	<2.00E−16***
Population (IT): Sex (M)	0.046	0.007	6.325	3.46E−10***
28°C: Population (SW): Sex (M)	−0.046	0.011	−4.092	4.54E−05***
Testis Size (model: LME)				
(Intercept)	−0.042	0.020	−2.100	0.036*
Body Size	0.052	0.019	2.744	0.006**
Population (SW)	−0.263	0.030	−8.876	<2.00E−16***
Population (IT)	−0.129	0.024	−5.309	1.55E−07***
20°C: Population (SW)	0.199	0.041	4.828	1.74E−06***
24°C: Population (SW)	0.200	0.044	4.503	8.04E−06***

(Continued)

TABLE 1 Continued

Fixed effects	Estimate	Std. Error	T value/Z value	Pr(>|t|)
Testis Size (model: LME)				
28°C: Population (SW)	0.281	0.039	7.156	2.41E−12***
20°C: Population (IT)	−0.107	0.036	−2.943	0.003**
28°C: Population (IT)	0.106	0.034	3.119	0.002**
Body Size: Population (SW)	0.212	0.025	8.335	5.17E−16***
Body Size: Population (IT)	0.120	0.022	5.445	7.54E−08***
20°C: Body Size: Population (SW)	−0.157	0.036	−4.345	1.63E−05***
24°C: Body Size: Population (SW)	−0.160	0.038	−4.161	3.62E−05***
28°C: Body Size: Population (SW)	−0.226	0.035	−6.419	2.76E−10***
20°C: Body Size: Population (IT)	0.085	0.033	2.564	0.011*
28°C: Body Size: Population (IT)	−0.099	0.032	−3.092	0.002**
Sperm Length (model: LME)				
(Intercept)	150.630	70.370	2.141	0.033*
Body Size	131.620	66.130	1.990	0.047*
Population (SW)	227.830	90.040	2.530	0.012*
Population (IT)	273.080	83.310	3.278	0.001**

Only significant predictors are shown. The full model summaries are presented in Supplementary Table S1.11. Significance codes: 0 "***" 0.001 "**" 0.01 "*" 0.05.

Thermal stress reduces adult body size and even sexual dimorphism at high latitudes

Adult body size, measured as head width, declined with increasing thermal stress across populations, consistent with the temperature-size rule (Figure 2C) ($x^2_{3,1348}$ = 180.59, p <0.001) (Table 1, Supplementary Table S1.5). Consistent with previous studies, we observed a male-biased SSD in European populations and a female-biased SSD in North American populations (Figure 2C, Supplementary Tables S1.1, S1.7). European flies were larger than North American flies across temperatures, and their size declined more steeply with heat stress regardless of latitude (Table 1: EU populations × treatment, p <0.01). Three-way interactions between temperature, population, and sex were only significant at 28°C for the population at the highest latitude (SW), which even records a minimization of SSD from 24°C to 28°C (Figure 2C), suggesting a stronger effect of thermal selection than sexual selection on body size at high temperatures.

Thermal stress reduces reproductive investment with partial resilience in sperm quality but not quantity

Both absolute testis size ($x^2_{3,643}$ = 112.42, p <0.001) and sperm length ($x^2_{3,398}$ = 18.099, p <0.001) decreased with increasing temperatures across populations (Figures 2D, E). However, this was also attributed to the effect of body size rather than temperature alone (Table 1: LME, p <0.05). Most interestingly, when controlling for body

size, only residual testis size showed a significant decline at high temperatures ($x^2_{3,16}$ = 8.625, p <0.05; Table 1: temperature × population, p <0.01), while residual sperm length remained stable across temperatures in all but the highest latitude population (SW), although this was not significant ($x^2_{3,16}$ = 4.9191, p >0.05) (Figures 2F, G). This phenotypic difference suggests that under thermal stress, males reduce sperm quantity but maintain their quality.

Resource stress has limited effects on larval survival but influences sex- and continent-specific development across populations

Of the 1,010 larvae, 704 survived to adulthood. Larval survival generally improved with increasing dung availability (Table 2), but there were no significant differences ($x^2_{4,101}$ = 8.4082, p >0.05). Instead, there were population-level differences, with DE consistently showing the highest survival and ND the lowest (Figure 3A; $x^2_{3,101}$ = 19.332, p <0.001). Hence, unlike thermal stress, food limitation did not significantly alter overall survival (Figure 3A, Table 2: GLMM, p >0.05) or systematically affect overall development time across continents (Table 2: LME, p >0.05). Instead, we documented sex- ($x^2_{1,684}$ = 15.435, p <0.001) and continent-specific ($x^2_{1,684}$ = 233.03, p <0.001) effects on larval development (Table 2). While North American flies show similar development rates in both sexes, European males exhibited significantly prolonged development under high-resource conditions, a trend that disappeared under severe food limitation

TABLE 2 Summary of best fit LME and GLMM models for *S. punctum* development and morphological trait measurements under resource stress.

Fixed effects	Estimate	Std. Error	T value/Z value	Pr(>|t|)
Survival Rate (model: GLMM)				
(Intercept)	−0.198	0.185	−1.065	0.287
Population (DE)	1.003	0.198	5.063	4.13E−07***
Population (ON)	0.687	0.190	3.618	2.97E−04***
Population (IT)	0.943	0.194	4.855	1.20E−06***
9.5 g	0.449	0.214	2.095	0.036*
13.5 g	0.704	0.223	3.151	0.002**
17.5 g	0.622	0.221	2.813	0.005**
Development Time (model: averaged GLMM) ***Conditional results. Variables in bold are included in all competing models**				
(Intercept)	2.50324	0.02733	91.588	<2.00E−16***
Population (DE)	0.07968	0.03192	2.496	0.0126*
Population (IT)	0.0635	0.03172	2.002	0.0453*
Body Size (model: LME)				
(Intercept)	0.816	0.010	85.087	<2.00E−16***
Population (ON)	−0.031	0.012	−2.501	0.013*
Population (IT)	−0.037	0.013	−2.838	0.005**
5.5 g	0.159	0.013	12.030	<2.00E−16***
9.5 g	0.179	0.013	13.891	<2.00E−16***
13.5 g	0.156	0.012	12.879	<2.00E−16***
17.5 g	0.168	0.012	13.753	<2.00E−16***
Population (DE): 5.5 g	0.103	0.017	6.009	0.000***
Population (IT): 5.5 g	0.083	0.018	4.634	0.000***
Population (DE): 9.5 g	0.163	0.017	9.600	<2.00E−16***
Population (ON): 9.5 g	−0.039	0.017	−2.260	0.024*
Population (IT): 9.5 g	0.125	0.017	7.183	0.000***
Population (DE): 13.5 g	0.158	0.016	9.610	<2.00E−16***
Population (ON): 13.5 g	−0.034	0.017	−2.042	0.042*
Population (IT): 13.5 g	0.131	0.017	7.781	0.000***
Population (DE): 17.5 g	0.146	0.016	8.898	<2.00E−16***
Population (ON): 17.5 g	−0.036	0.017	−2.145	0.032*
Population (IT): 17.5 g	0.139	0.017	8.205	0.000***
Testis Size (model: LME)				
(Intercept)	−0.023	0.010	−2.278	0.023*
Population (DE)	−0.064	0.012	−5.363	0.000***
Population (IT)	−0.042	0.012	−3.406	0.001***
Body Size	0.035	0.011	3.197	0.002**
Population (DE): Body Size	0.083	0.012	6.715	0.000***
Population (IT): Body Size	0.053	0.013	4.179	0.000***

(Continued)

TABLE 2 Continued

Fixed effects	Estimate	Std. Error	T value/Z value	Pr(>|t|)
Sperm Length (model: LME)				
(Intercept)	247.830	117.870	2.103	0.037*
Population (IT): 5.5 g	1,070.650	439.780	2.435	0.016*
Population (IT): 5.5 g: Body Size	−1,118.460	468.090	−2.389	0.018*

Only significant predictors are shown. The full model summaries are presented in Supplementary Table S1.12. Significance codes: 0 "***" 0.001 "**" 0.01 "*" 0.05.

due to sharper reductions in male size compared to females (Figure 3B, Supplementary Table S1.10).

Resource stress reduces body size and affects size dimorphism across continents, but has limited effects on male postcopulatory traits

Similar to thermal stress, resource limitation also reduced adult body size across populations (Figure 3C) (Table 2, Supplementary Table S1.8; $x^2_{4,684}$ = 274.83, p <0.001). However, the magnitude and direction of size reduction varied according to sex and continent. European males were generally larger than females ($x^2_{1,684}$ = 236.42, p <0.001), although this difference disappeared under extreme food limitation (Figure 3C, Supplementary Tables S1.2, S1.10). In contrast, North American populations consistently exhibited female-biased SSD, regardless of food level (Figure 3C, Supplementary Tables S1.2, S1.10).

Similar to the temperature experiment, European populations maintained a larger testis size ($x^2_{1,329}$ = 176.64, p <0.001) and sperm length ($x^2_{1,208}$ = 154.89, p <0.001) than North American populations (Supplementary Table S1.9). Dunn's tests indicated significant differences in testis size, mainly between the lowest (1.5 g) and higher dung availabilities (Supplementary Table S1.8, Figure 3D), whereas sperm length variation fluctuated. However, the best-fit models suggest that neither post-copulatory trait was significantly influenced by resource availability alone. Testis size was positively associated with body size (Table 2: LME, p <0.01), and sperm length was only significantly influenced by body size and resources for the IT population (Table 2). Unlike thermal stress, resource stress had no significant effect on size-independent investment in male postcopulatory traits, as evidenced by the residual variation in testis size ($x^2_{4,20}$ = 2.5713, p >0.05) and sperm length ($x^2_{4,20}$ = 2.2571, p >0.05) across treatments (Figures 3F, G).

Continental divergence in allometric scaling of reproductive traits under environmental stress

Under thermal stress, testis size and sperm length remained positively correlated with male body size, but allometric relationships diverged between continents, possibly reflecting the differing mating systems (Figure 4). For instance, best-fit models suggest that testis size scales non-linearly with body size in Europe, while North American populations maintained a linear trend (Figure 4A, Table 3). Even after accounting for body size, temperature still showed an independent negative influence on residual (i.e., size-controlled) testis size (Figure 2F; $x^2_{3,16}$ = 8.625, p <0.05). In contrast, sperm length relationships did not differ between continents (Figure 4B, Table 3), although low R2 values suggest that body size did not fully explain the variability in sperm length. Indeed, residual sperm length showed similar trends to absolute sperm length across temperatures (Figures 2E, G; $x^2_{3,16}$ = 4.919, p >0.05).

In contrast, resource stress induced population-specific scaling in testis size instead of a clear continental effect, indicating local adaptations. Resource availability showed no independent effect on residual testis size (Figure 3F, $x^2_{4,20}$ = 2.257, p >0.05). Sperm length showed a positive hypo-allometric relationship with body size, similar to the temperature treatments (Figure 4D, Table 3), but the residual variation indicated no clear trend with resource availability (Figures 2E, G, $x^2_{3,20}$ = 0.703, p >0.05). Overall, North American flies displayed more variable scaling relationships without a clear pattern across treatments, unlike the significantly stronger allometries observed in European flies, suggesting that European populations exhibit greater plasticity and potentially greater constraints on reproductive investment under environmental stress.

Reproductive investments may be less dependent on body size under increased stress to maintain reproductive advantages, and cross-stressor comparisons suggest that increased temperatures are more stressful, particularly for sperm length (Figures 4E, F). *S. punctum* sperm length scales hypo-allometrically with body size under optimal circumstances (Lee et al., 2024); however, *b* was much shallower under warmer temperatures than under dung restriction (Table 3).

Discussion

Here, we examined the effects of environmental stress, specifically high larval developmental temperature and food limitation, on *S. punctum* populations from North America and Europe. We show that developmental stress during the juvenile stage can have profound consequences on adult reproductive traits and highlight geographic and stress-specific patterns of plasticity.
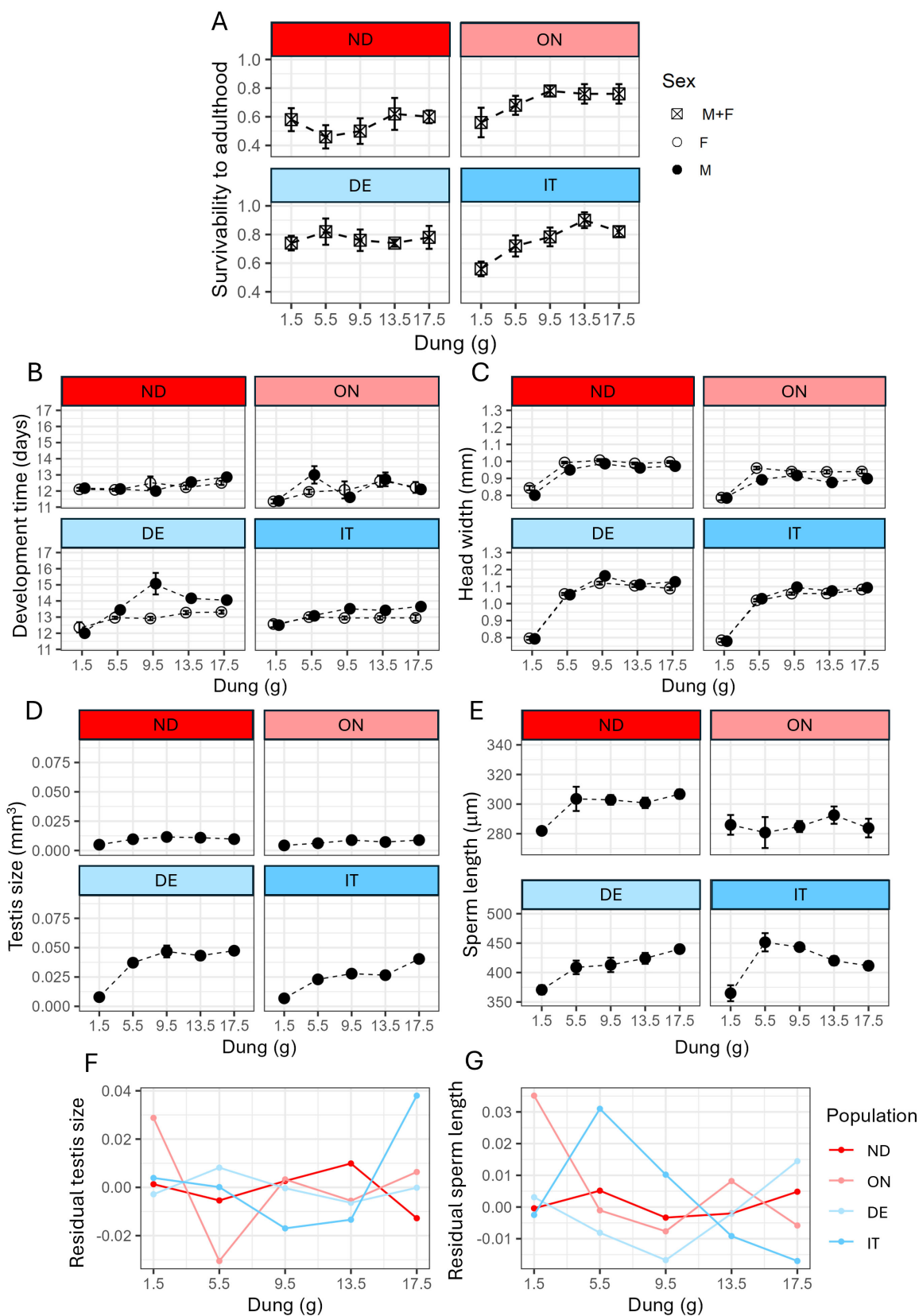
FIGURE 3
Mean ± SE trends of resource availability on *S. punctum* **(A)** larval survivability rate, **(B)** larval development time, **(C)** head width as a proxy for body size, **(D)** testis size, and **(E)** sperm length, along with the respective size-independent residuals **(F, G)** across four populations from two continents.
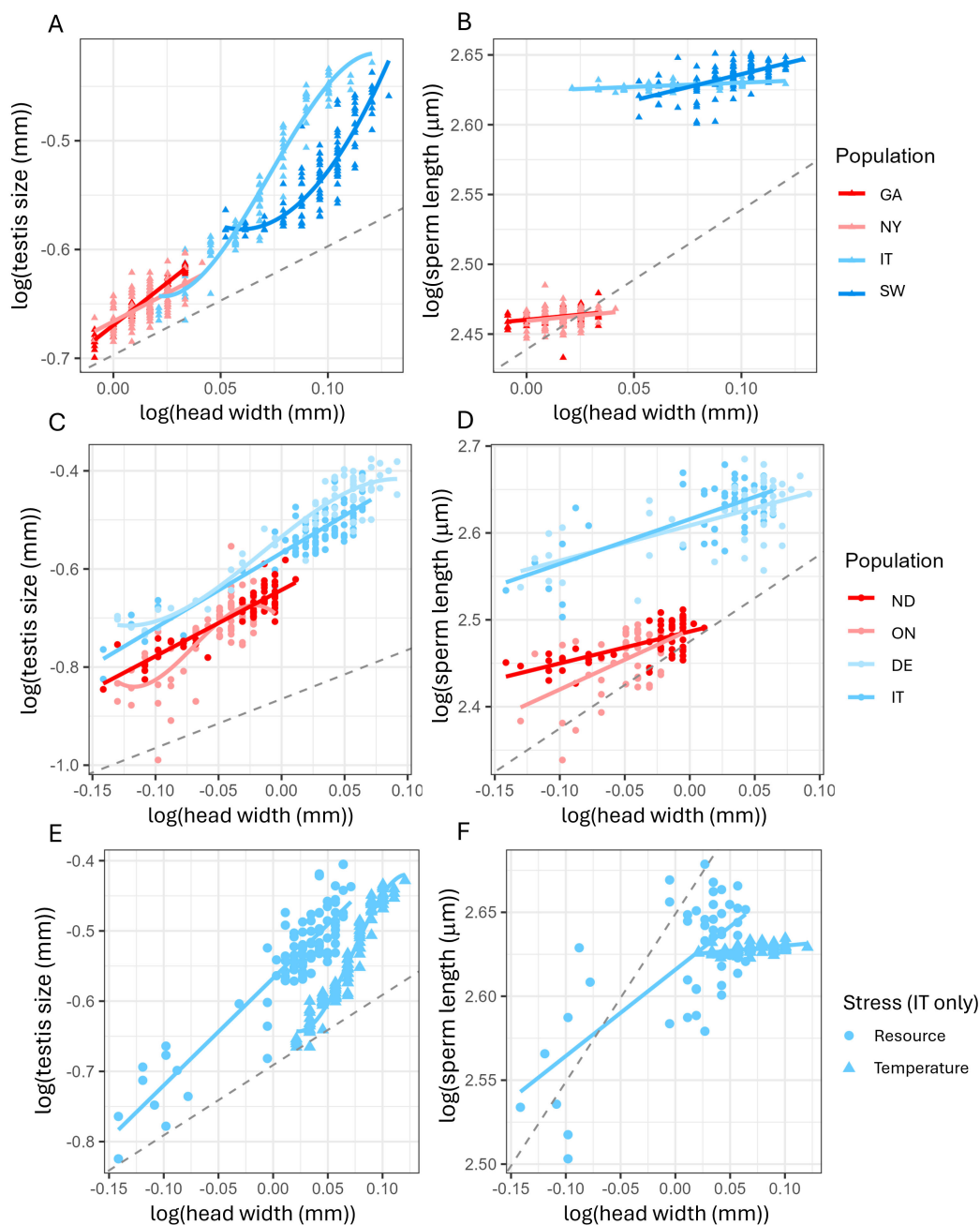
FIGURE 4
Allometric relationships between *S. punctum* body size and testis size **(A, C)** or sperm length **(B, D)** when exposed to thermal **(A, B)** or resource stress **(C, D)**. Cross-stressor comparisons using Italy as the focal population showed diverging allometric trajectories in the testes **(E)**, and particularly in sperm **(F)**. The dotted gray lines indicate an isometric slope of 1.

Developmental stress alters fitness-related traits, with temperature exerting stronger effects than resource

We demonstrated that developmental stress from elevated temperatures and resource limitation significantly alters *S. punctum* life-history traits, but with divergent effects depending on the type of stress. Thermal stress imposed the strongest fitness costs, reducing larval survival, accelerating development, and leading to smaller adult body sizes. This aligns with the predictions of thermal reaction norms and the temperature-size rule (Atkinson, 1994), mirroring the stress responses observed in other holometabolous insects (Sheridan and Bickford, 2011; Verberk et al., 2021). In contrast, food limitation had minimal

TABLE 3 Allometric equations for male postcopulatory traits under environmental stress.

Env. stress	Population	Trend	a	bx	cx^2	dx^3	Adj. R2	RSE	F value	p
					Testis Size					
	GA	Linear	−0.670	1.553	–	–	0.931	0.005	1953.000	***
	NY	Linear	−0.666	1.033	–	–	0.349	0.013	92.210	***
	IT	Cubic	−0.600	−3.947	100.713	−461.161	0.938	0.016	852.700	***
Thermal	SW	Quadratic	−0.465	−3.916	32.821	–	0.784	0.018	48.960	***
	ND	Linear	−0.643	1.350	–	–	0.809	0.036	285.500	***
	ON	Cubic	−0.704	−2.912	−79.629	−382.607	0.629	0.044	48.960	***
	DE	Cubic	−0.534	2.061	−2.806	−61.666	0.906	0.031	242.900	***
Resource	IT	Linear	−0.567	1.527	–	–	0.808	0.036	395.900	***
					Sperm Length					
	GA	Linear	2.460	0.147	–	–	0.079	0.006	7.695	**
	NY	Linear	2.459	0.153	–	–	0.038	0.006	5.715	*
	IT	Linear	2.624	0.060	–	–	0.231	0.002	24.720	***
Thermal	SW	Linear	2.599	0.374	–	–	0.360	0.008	66.730	***
	ND	Linear	2.487	0.367	–	–	0.451	0.016	48.670	***
	ON	Linear	2.488	0.685	–	–	0.252	0.031	16.840	***
	DE	Linear	2.609	0.404	–	–	0.403	0.031	34.790	***
Resource	IT	Linear	2.616	0.514	–	–	0.488	0.028	47.650	***

Significance codes: 0 "***" 0.001 "**" 0.01 "*" 0.05.

impact on larval survival, suggesting that *S. punctum* larvae may be buffered against short-term nutrient deficits, which is a possible adaptation to ephemeral and patchy dung substrates in their natural habitat (Blanckenhorn, 1998).

Interestingly, while thermal stress consistently shortened the development time, food limitation did not produce uniform effects. In contrast, larger European males exhibited stark reductions in development time under severe resource scarcity compared to their smaller female counterparts, suggesting sex-specific trade-offs in growth and reproduction. Emerging earlier and smaller with increasing resource limitation is a generally rare life-history response (Stearns and Koella, 1986), but is common for species inhabiting heterogeneous environments, such as dung pats, where quantity and quality vary unpredictably in time and space (Blanckenhorn, 1998). While prolonged development increases body size and reproductive fitness, the benefits of reducing time in stressful larval environments and early breeding may outweigh the disadvantages of being small (Reim et al., 2006; Roff, 1992).

Geographic divergence in plasticity may influence climate resilience

Plastic responses to environmental stressors appear to be mediated by population background. Across the latitudinal gradient, we did not find resource-induced patterns in life history traits, similar to Berger et al. (2014), although lower latitude populations showed higher thermal tolerance, particularly with regard to survivability and sperm length. However, previous studies have also highlighted that these patterns in thermal tolerance may be broadly explained by the extreme temperatures experienced within different localities (Berger et al., 2014; Sunday et al., 2019).

Instead of a longitudinal gradient, we observed a sharp divergence in size-based sexual selection between North American and European populations, consistent with previous studies. Differential investment in pre- *vs.* postcopulatory sexual selection led to male-biased SSD in Europe, with stronger sexual selection on male size and sperm production, as reflected here, and female-biased SSD in North America, with males investing more in precopulatory mate acquisition (Dmitriew and Blanckenhorn, 2012; Puniamoorthy et al., 2012a, b; Rohner et al., 2016). European flies are also generally larger than North American flies (Puniamoorthy et al., 2012a, b), which in turn influenced the strength of plasticity responses between continents, consistent with previous invertebrate studies (Tseng et al., 2018; Wonglersak et al., 2021), with larger European flies showing sharper decreases in body and testis sizes in response to environmental stress.

Interestingly, while continent-specific SSD trends were maintained across extreme temperatures, this was not the case under resource limitations. The loss of male-biased SSD in European populations under low food conditions indicates that developmental constraints can override sexually selected growth advantages when resources are limited (Miller et al., 2016) and that the larger sex tends to be more sensitive to resource stress (Teder and Kaasik, 2023).

Partial resilience in male post-copulatory trait investment against environmental stress

Sperm quantity and quality are vital for male reproductive fitness (Crean and Immler, 2021; Pattarini et al., 2006; Pitnick, 1996). These traits play crucial roles in post-copulatory courtship, where either intrasexual sperm competition or cryptic female choice occurs (Macartney et al., 2019; Snook, 2005). After accounting for the effects of body size, only the effect of temperature on testis size (a proxy for sperm quantity) remained significant, suggesting that these dung flies are better adapted to compensate for resource constraints over extreme temperatures (Gourgoulianni et al., 2025; Hellriegel and Blanckenhorn, 2002). Investment in male post-copulatory traits may be buffered or canalized against resource variation to maintain reproductive advantages (Lee et al., 2024; Macartney et al., 2019; Waddington, 1942; Wagner et al., 1997).

Testis size investment was reduced with increasing temperatures in accordance with Bergmann's rule extended to ectotherms (Atkinson and Sibly, 1997; Blanckenhorn and Henseler, 2005; Hellriegel and Blanckenhorn, 2002). While both sperm quantity and quality contribute to fertilization success, it is theorized trade-offs may occur under physical or energetic constraints (Parker, 1970; Pattarini et al., 2006; Pitnick, 1996). Testis size, a common proxy for sperm quantity, may be more condition-dependent owing to body size constraints under temperature stress. Although classic sperm competition theory focuses on sperm quantity advantages, sperm quality also plays an important role in post-copulatory sexual selection (Crean and Immler, 2021; Macartney et al., 2019; Pattarini et al., 2006). Our results suggest the potential canalization of sperm quality and strategic resource allocation trading off quantity under stress.

Conclusion: implications for climate change resilience

In widespread species exposed to varying environments, responses to environmental stress vary owing to local adaptations (Berger et al., 2013, 2014; Chakraborty et al., 2023), with implications for species persistence with global change. Here, we documented phenotypic plasticity in *S. punctum* life-history traits in response to temperature and resource stress, the strength of which varied between the type of stress and geographic lineage. Reproductive investment in males may be less sensitive to environmental stress than developmental investment, as body size-independent investment in male post-copulatory traits was not affected by environmental stress, except for testis size at higher temperatures. This suggests that

warmer temperatures could be more stressful than resource limitations for dung flies accustomed to ephemeral dung pats. While we tested development under constant environmental parameters, this may not reflect field conditions, as wild populations experience frequent temperature variations across their lifetimes. Future studies should explore fluctuating stress regimes that are more ecologically relevant, with possible consequences for trait expression (Hoffmann et al., 2003; Kjærsgaard et al., 2013). We also acknowledge that we only studied reproductive post-copulatory investments in males, and analyzing the impacts of environmental stress on females is crucial for the persistence of the species as a whole because of sex-specific ecological repertoires (Kjærsgaard et al., 2013; Lande, 1980). Additionally, investigating transgenerational variation in a quantitative genetic framework would improve our understanding of how natural populations respond to changing environments.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

NLYL: Methodology, Data curation, Formal analysis, Project administration, Visualization, Writing – original draft. JWT: Investigation, Data curation, Formal analysis, Writing – original draft. YW: Investigation, Formal analysis, Writing – original draft. TRHT: Formal analysis, Project administration, Validation, Visualization, Writing – review & editing. NP: Conceptualization, Funding acquisition, Resources, Methodology, Supervision, Visualization, Writing – review & editing.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

Abrámoff, M. D., Magalhães, P., and Ram, S. J. (2004). Image Processing with Image J. *Biophotonics International* 11, 36–41.

Adamo, S. A., and Lovett, M. M. E. (2011). Some like it hot: The effects of climate change on reproduction, immune function and disease resistance in the cricket Gryllus texensis. *J. Exp. Biol.* 214, 1997–2004. doi: 10.1242/jeb.056531

Atkinson, D. (1994). Temperature and organism size—A biological law for ectotherms? *Adv. Ecol. Res.* 25, 1–58. doi: 10.1016/S0065-2504(08)60212-3

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

Barton, K. (2013). *MuMIn: Multi-model inference. R package version 1.9.5.* Available online at: http://CRAN.R-project.org/package=MuMIn (Accessed October 04, 2025).

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Barton, B. T., and Ives, A. R. (2014). Direct and indirect effects of warming on aphids, their predators, and ant mutualists. *Ecology* 95, 1479–1484. doi: 10.1890/13-1977.1

Berger, D., Postma, E., Blanckenhorn, W. U., and Walters, R. J. (2013). Quantitative genetic divergence and standing genetic (co)variance in thermal reaction norms along latitude. *Evolution* 67, 2385–2399. doi: 10.1111/EVO.12138

Berger, D., Walters, R. J., and Blanckenhorn, W. U. (2014). Experimental evolution for generalists and specialists reveals multivariate genetic constraints on thermal reaction norms. *J. Evolutionary Biol.* 27, 1975–1989. doi: 10.1111/jeb.12452

Blanckenhorn, W. U. (1998). Adaptive phenotypic plasticity in growth, development, and body size in the yellow dung fly. *Evolution* 52, 1394–1407. doi: 10.1111/j.1558-5646.1998.tb02021.x

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

Blanckenhorn, W. U., and Henseler, C. (2005). Temperature-dependent ovariole and testis maturation in the yellow dung fly. *Entomologia Experimentalis Applicata* 116, 159–165. doi: 10.1111/j.1570-7458.2005.00316.x

Blanckenhorn, W. U., Kraushaar, U. R. S., Teuschl, Y., and Reim, C. (2004). Sexual selection on morphological and physiological traits and fluctuating asymmetry in the black scavenger fly Sepsis cynipsea. *J. Evolutionary Biol.* 17, 629–641. doi: 10.1111/j.1420-9101.2004.00693.x

Bogan, S. N., Porat, O. I., Meneses, M. J., and Hofmann, G. E. (2024). Thermal plasticity has higher fitness costs among thermally tolerant genotypes of Tigriopus californicus. *Funct. Ecol.* 38, 1562–1577. doi: 10.1111/1365-2435.14568

Boggs, C. L. (1981). Nutritional and life-history determinants of resource allocation in holometabolous insects. *Am. Nat.* 117, 692–709. doi: 10.1086/283753

Boggs, C. L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Funct. Ecol.* 23, 27–37. doi: 10.1111/j.1365-2435.2009.01527.x

Bonamour, S., Chevin, L.-M., Charmantier, A., and Teplitsky, C. (2019). Phenotypic plasticity in response to climate change: The importance of cue variation. *Philos. Trans. R. Soc. B: Biol. Sci.* 374, 20180178. doi: 10.1098/rstb.2018.0178

Burnham, K. P., and Anderson, D. R. (2004). Multimodel Inference: Understanding AIC and BIC in Model Selection. *Sociological Methods & Research* 33, 261–304. doi: 10.1177/0049124104268644

Chafino, S., Ureña, E., Casanova, J., Casacuberta, E., Franch-Marro, X., and Martín, D. (2019). Upregulation of E93 gene expression acts as the trigger for metamorphosis independently of the threshold size in the beetle tribolium castaneum. *Cell Rep.* 27, 1039–1049.e2. doi: 10.1016/j.celrep.2019.03.094

Chakraborty, A., Walter, G. M., Monro, K., Alves, A. N., Mirth, C. K., and Sgrò, C. M. (2023). Within-population variation in body size plasticity in response to combined nutritional and thermal stress is partially independent from variation in development time. *J. Evolutionary Biol.* 36, 264–279. doi: 10.1111/jeb.14099

Chevin, L.-M., and Hoffmann, A. A. (2017). Evolution of phenotypic plasticity in extreme environments. *Philos. Trans. R. Soc. B: Biol. Sci.* 372, 20160138. doi: 10.1098/rstb.2016.0138

Crean, A. J., and Immler, S. (2021). Evolutionary consequences of environmental effects on gamete performance. *Philos. Trans. R. Soc. B: Biol. Sci.* 376, 20200122. doi: 10.1098/rstb.2020.0122

Dallo, R., Kapun, M., and Blanckenhorn, W. (2024). Lacking sex-specific temperature preferences of 9 coexisting temperate sepsid dung fly species (Diptera: Sepsidae). *Research Square.* doi: 10.21203/rs.3.rs-4252799/v1

Daniel, G. M., Noriega, J. A., da Silva, P. G., Deschodt, C. M., Sole, C. L., Scholtz, C. H., et al. (2022). Soil type, vegetation cover and temperature determinants of the diversity and structure of dung beetle assemblages in a South African open woodland and closed canopy mosaic. *Austral Ecology* 47, 79–91. doi: 10.1111/aec.13138

Devost, E., and Turgeon, J. (2016). The combined effects of pre- and post-copulatory processes are masking sexual conflict over mating rate in Gerris buenoi. *J. Evolutionary Biol.* 29, 167–177. doi: 10.1111/jeb.12772

Dillon, M. E., Wang, G., Garrity, P. A., and Huey, R. B. (2009). Thermal preference in drosophila. *J. Thermal Biol.* 34, 109–119. doi: 10.1016/j.jtherbio.2008.11.007

Dmitriew, C., and Blanckenhorn, W. U. (2012). The role of sexual selection and conflict in mediating among-population variation in mating strategies and sexually dimorphic traits in sepsis punctum. *PloS One* 7, e49511. doi: 10.1371/journal.pone.0049511

Edelstein, A. D., Tsuchida, M. A., Amodaj, N., Pinkard, H., Vale, R. D., and Stuurman, N. (2014). Advanced methods of microscope control using µManager software. *J. Biol. Methods* 1, e10. doi: 10.14440/jbm.2014.36

Emlen, D. J. (1994). Environmental control of horn length dimorphism in the beetle Onthophagus acuminatus (Coleoptera: Scarabaeidae). *Proc. R. Soc. London. Ser. B: Biol. Sci.* 256, 131–136. doi: 10.1098/rspb.1994.0060

English, S., and Barreaux, A. M. (2020). The evolution of sensitive periods in development: Insights from insects. *Curr. Opin. Behav. Sci.* 36, 71–78. doi: 10.1016/j.cobeha.2020.07.009

Gillott, C. (1980). "The Abiotic Environment," in *Entomology* (Boston, MA: Springer US), 595–625. doi: 10.1007/978-1-4615-6918-3_22

Gittleman, J. L., and Thompson, S. D. (1988). Energy allocation in mammalian reproduction. *Am. Zoologist* 28, 863–875. doi: 10.1093/icb/28.3.863

Gourgoulianni, N., Kümmerli, R., and Blanckenhorn, W. U. (2025). Nutritional effects on growth and development of sepsid flies. *Entomologia Experimentalis Applicata* 173, 105–117. doi: 10.1111/eea.13524

Gourgoulianni, N., Schäfer, M. A., Kapun, M., Busso, J. P., and Blanckenhorn, W. U. (2023). Temperature-dependent melanism and phenoloxidase activity in the dimorphic

sepsid fly Sepsis thoracica. *J. Thermal Biol.* 112, 103473. doi: 10.1016/j.jtherbio.2023.103473

Hagg, L., LaMere, C. J., and Zuk, M. (2024). Postcopulatory song as a mate-guarding tactic in the Pacific field cricket, Teleogryllus oceanicus. *Anim. Behav.* 207, 201–207. doi: 10.1016/j.anbehav.2023.11.001

Harrison, L. M., Vega-Trejo, R., and Jennions, M. D. (2023). The effect of brief or prolonged bouts of winning or losing male-male contests on plasticity in sexually selected traits. *Am. Nat.* 201, 442–459. doi: 10.1086/722829

Hellriegel, B., and Blanckenhorn, W. U. (2002). Environmental influences on the gametic investment of yellow dung fly males. *Evolutionary Ecol.* 16, 505–522. doi: 10.1023/A:1020875021823

Himuro, C., Honma, A., Ikegawa, Y., and Kumano, N. (2022). The female Euscepes postfasciatus refractory period is induced by the male but length is determined by the female. *J. Insect Physiol.* 142, 104427. doi: 10.1016/j.jinsphys.2022.104427

Hoffmann, A. A., Sørensen, J. G., and Loeschcke, V. (2003). Adaptation of Drosophila to temperature extremes: Bringing together quantitative and molecular approaches. *J. Thermal Biol.* 28, 175–216. doi: 10.1016/S0306-4565(02)00057-8

IPCC. (2023). *Climate Change 2022 – Impacts, Adaptation and Vulnerability: Working Group II Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge: Cambridge University Press). doi: 10.1017/9781009325844

Jarrold, M. D., Chakravarti, L. J., Gibbin, E. M., Christen, F., Massamba-N'Siala, G., Blier, P. U., et al. (2019). Life-history trade-offs and limitations associated with phenotypic adaptation under future ocean warming and elevated salinity. *Philos. Trans. R. Soc. B: Biol. Sci.* 374, 20180428. doi: 10.1098/rstb.2018.0428

Johnson, C. A., Coutinho, R. M., Berlin, E., Dolphin, K. E., Heyer, J., Kim, B., et al. (2016). Effects of temperature and resource variation on insect population dynamics: The bordered plant bug as a case study. *Funct. Ecol.* 30, 1122–1131. doi: 10.1111/1365-2435.12583

Kassambara, A. (2023). rstatix: Pipe-Friendly Framework for Basic Statistical Tests. *R package version 0.7.2.* Available online at: https://rpkgs.datanovia.com/rstatix/ (Accessed October 20, 2025).

Kelly, M. (2019). Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philos. Trans. R. Soc. B: Biol. Sci.* 374, 20180176. doi: 10.1098/rstb.2018.0176

Kjærsgaard, A., Pertoldi, C., Loeschcke, V., and Blanckenhorn, W. U. (2013). The effect of fluctuating temperatures during development on fitness-related traits of scatophaga stercoraria (Diptera: scathophagidae). *Environ. Entomology* 42, 1069–1078. doi: 10.1603/EN13074

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* 82, 1–26. doi: 10.18637/jss.v082.i13

Lande, R. (1980). Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34, 292–305. doi: 10.1111/j.1558-5646.1980.tb04817.x

Lee, N. L. Y., Kuan, P. S. Y., Hua, Q. Q. H., and Puniamoorthy, N. (2024). Experimental evolution under predation reduces body size in dung flies but courtship displays persist in males (Diptera: Sepsidae). *Behav. Processes* 220, 105073. doi: 10.1016/j.beproc.2024.105073

Levins, R. (2012). *Evolution in changing environments : some theoretical explorations.* Princeton, NJ: Princeton University Press.

Lovich, J. E., and Gibbons, J. W. (1992). A review of techniques for quantifying sexual size dimorphism. *Growth Dev. Aging* 56, 269–281.

Macartney, E. L., Crean, A. J., Nakagawa, S., and Bonduriansky, R. (2019). Effects of nutrient limitation on sperm and seminal fluid: A systematic review and meta-analysis. *Biol. Rev.* 94, 1722–1739. doi: 10.1111/brv.12524

Macartney, E. L., Nicovich, P. R., Bonduriansky, R., and Crean, A. J. (2018). Developmental diet irreversibly shapes male post-copulatory traits in the neriid fly Telostylinus angusticollis. *J. Evolutionary Biol.* 31, 1894–1902. doi: 10.1111/jeb.13384

Martin, T. E. (1987). Food as a limit on breeding birds: A life-history perspective. *Annu. Rev. Ecol. Systematics* 18, 453–487. doi: 10.1146/annurev.es.18.110187.002321

Mehlis, M., Rick, I. P., and Bakker, T. C. M. (2015). Dynamic resource allocation between pre- and postcopulatory episodes of sexual selection determines competitive fertilization success. *Proc. R. Soc. B: Biol. Sci.* 282, 20151279. doi: 10.1098/rspb.2015.1279

Miller, C. W., McDonald, G. C., and Moore, A. J. (2016). The tale of the shrinking weapon: Seasonal changes in nutrition affect weapon size and sexual dimorphism, but not contemporary evolution. *J. Evolutionary Biol.* 29, 2266–2275. doi: 10.1111/jeb.12954

Moczek, A. P. (2010). Phenotypic plasticity and diversity in insects. *Philos. Trans. R. Soc. B: Biol. Sci.* 365, 593–603. doi: 10.1098/rstb.2009.0263

Nylin, S., and Gotthard, K. (1998). Plasticity in life-history traits. *Annu. Rev. Entomology* 43, 63–83. doi: 10.1146/annurev.ento.43.1.63

Parker, G. A. (1970). Sperm competition and its evolutionary consequences in insects. *Biol. Rev.* 45, 525–567. doi: 10.1111/j.1469-185X.1970.tb01176.x

Pattarini, J. M., Starmer, W. T., Bjork, A., and Pitnick, S. (2006). Mechanisms underlying the sperm quality advantage in Drosophila melanogaster. *Evolution* 60, 2064–2080. doi: 10.1554/06-142.1

Peters, R. L., and Lovejoy, T. E. (1994). *Global Warming and Biological Diversity* (New Haven, CT: Yale University Press).

Pigliucci, M., Murren, C. J., and Schlichting, C. D. (2006). Phenotypic plasticity and evolution by genetic assimilation. *J. Exp. Biol.* 209, 2362–2367. doi: 10.1242/jeb.02070

Pitnick, S. (1996). Investment in testes and the cost of making long sperm in Drosophila. *Am. Nat.* 148, 57–80. doi: 10.1086/285911

Pont, A., and Meier, R. (2002). The Sepsidae (Diptera) of Europe. *Fauna Entomologica Scandinavica* 37, 1–221.

Puniamoorthy, N., Blanckenhorn, W. U., and Schäfer, M. A. (2012a). Differential investment in pre- vs. Post-copulatory sexual selection reinforces a cross-continental reversal of sexual size dimorphism in Sepsis punctum (Diptera: Sepsidae). *J. Evolutionary Biol.* 25, 2253–2263. doi: 10.1111/j.1420-9101.2012.02605.x

Puniamoorthy, N., Schäfer, M. A., and Blanckenhorn, W. U. (2012b). Sexual selection accounts for the geographic reversal of sexual size dimorphism in the dung fly, Sepsis punctum (Diptera: Sepsidae). *Evolution* 66, 2117–2126. doi: 10.1111/j.1558-5646.2012.01599.x

R Core Team(2021). *R: A Language and Environment for Statistical Computing* (Vienna, Austria: R Foundation for Statistical Computing).

Reim, C., Teuschl, Y., and Blanckenhorn, W. U. (2006). Size-dependent effects of larval and adult food availability on reproductive energy allocation in the Yellow Dung Fly. *Funct. Ecol.* 20, 1012–1021. doi: 10.1111/j.1365-2435.2006.01173.x

Roff, D. A. (1992). *The Evolution of life histories: Theory and analysis* (London: Chapman & Hall).

Rohner, P. T., and Berger, D. (2025). Macroevolution along developmental lines of least resistance in fly wings. *Nat. Ecol. Evol.* 9, 639–651. doi: 10.1038/s41559-025-02639-1

Rohner, P. T., Blanckenhorn, W. U., and Puniamoorthy, N. (2016). Sexual selection on male size drives the evolution of male-biased sexual size dimorphism via the prolongation of male development. *Evolution* 70, 1189–1199. doi: 10.1111/evo.12944

Sheridan, J. A., and Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nat. Climate Change* 1, 401–406. doi: 10.1038/nclimate1259

Simmons, L. W., Tomkins, J. L., and Hunt, J. (1999). Sperm competition games played by dimorphic male beetles. *Proc. R. Soc. London. Ser. B: Biol. Sci.* 266, 145–150. doi: 10.1098/rspb.1999.0614

Simmons, L. W., Wernham, J., García-González, F., and Kamien, D. (2003). Variation in paternity in the field cricket Teleogryllus oceanicus: No detectable influence of sperm numbers or sperm length. *Behav. Ecol.* 14, 539–545. doi: 10.1093/beheco/arg038

Sisodia, S., and Singh, B. N. (2002). Effect of temperature on longevity and productivity in drosophila ananassae: evidence for adaptive plasticity and trade-off between longevity and productivity. *Genetica* 114, 95–102. doi: 10.1023/A:1014640604740

Snook, R. (2005). Sperm in competition: Not playing by the numbers. *Trends Ecol. Evol.* 20, 46–53. doi: 10.1016/j.tree.2004.10.011

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Funct. Ecol.* 3, 259. doi: 10.2307/2389364

Stearns, S. C., and Koella, J. C. (1986). The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution* 40, 893–913. doi: 10.1111/j.1558-5646.1986.tb00560.x

Sunday, J., Bennett, J. M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A. L., et al. (2019). Thermal tolerance patterns across latitude and elevation. *Philos. Trans. R. Soc. B.* 374, 20190036. doi: 10.1098/rstb.2019.0036

Tanious, F. A., Veal, J. M., Buczak, H., Ratemeyer, L. S., and Wilson, W. D. (1992). DAPI (4',6-diamidino-2-phenylindole) binds differently to DNA and RNA: minor-groove binding at AT sites and intercalation at AU sites. *Biochemistry* 31, 3103–3112. doi: 10.1021/bi00127a010

Teder, T., and Kaasik, A. (2023). Early-life food stress hits females harder than males in insects: A meta-analysis of sex differences in environmental sensitivity. *Ecol. Lett.* 26, 1419–1431. doi: 10.1111/ELE.14241

Truman, J. W. (2019). The evolution of insect metamorphosis. *Curr. Biol.* 29, R1252–R1268. doi: 10.1016/j.cub.2019.10.009

Tseng, M., Kaur, K. M., Soleimani Pari, S., Sarai, K., Chan, D., Yao, C. H., et al. (2018). Decreases in beetle body size linked to climate change and warming temperatures. *J. Anim. Ecol.* 87, 647–659. doi: 10.1111/1365-2656.12789

Verberk, W. C. E. P., Atkinson, D., Hoefnagel, K. N., Hirst, A. G., Horne, C. R., and Siepel, H. (2021). Shrinking body sizes in response to warming: Explanations for the temperature–size rule with special emphasis on the role of oxygen. *Biol. Rev.* 96, 247–268. doi: 10.1111/BRV.12653

Waddington, C. H. (1942). Canalization of development and genetic assimilation of acquired characters. *Nature* 150, 563–565. doi: 10.1038/150563a0

Wagner, G. P., Booth, G., and Bagheri-Chaichian, H. (1997). A population genetic theory of canalization. *Evolution* 51, 329–347. doi: 10.1111/j.1558-5646.1997.tb02420.x

Weaving, H., Terblanche, J. S., Pottier, P., and English, S. (2022). Meta-analysis reveals weak but pervasive plasticity in insect thermal limits. *Nat. Commun.* 13, 5292. doi: 10.1038/s41467-022-32953-2

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution* (Oxford, U.K.: Oxford University Press).

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. (New York: Springer-Verlag). Available online at: https://ggplot2.tidyverse.org (Accessed October 27, 2025).

Wonglersak, R., Fenberg, P. B., Langdon, P. G., Brooks, S. J., and Price, B. W. (2021). Insect body size changes under future warming projections: A case study of Chironomidae (Insecta: Diptera). *Hydrobiologia* 848, 2785–2796. doi: 10.1007/s10750-021-04597-8

Yap, S., Toh, K. X., and Puniamoorthy, N. (2024). Male reproductive traits display increased phenotypic variation in response to resource quality and parental provisioning in a tropical rainforest dung beetle, Onthophagus c.f. babirussa. *Ecol. Evol.* 14, e70421. doi: 10.1002/ece3.70421

Yoon, K. J., Cunningham, C. B., Bretman, A., and Duncan, E. J. (2023). One genome, multiple phenotypes: Decoding the evolution and mechanisms of environmentally induced developmental plasticity in insects. *Biochem. Soc. Trans.* 51, 675–689. doi: 10.1042/BST20210995

Zhang, Q.-H., and Puniamoorthy, N. (2025). Impact of rearing substrates on black soldier fly growth and fertility: A semi-industrial scale study to optimize egg collection. *Insects* 16, 142. doi: 10.3390/insects16020142