Microbiome-mediated resilience and cross-generational consequences in male *Drosophila* exposed to combined environmental stressors

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Abstract

Environmental stressors like heat extremes and pesticide exposure can significantly threaten insect reproduction, yet the interplay of these stressors and the potential mitigating role of the gut microbiome remain poorly understood, particularly across generations. This study investigated the interactive effects of acute heat stress and sublethal imidacloprid exposure on male *Drosophila melanogaster* reproductive success and the subsequent fitness of their offspring. We manipulated the gut microbiome of male flies (germ-free or colonized with one or five bacterial species) and subjected them to individual or combined stress. Our findings reveal that combined stress synergistically impairs male fitness traits, an effect partially buffered by higher microbiome diversity. We further demonstrate cross-generational consequences, with paternal stress exposure also impacting offspring fitness. Our results highlight the crucial role of the gut microbiome in mediating resilience to environmental stress and underscore the importance of considering multi-stressor and intergenerational effects in ecological risk assessments.

Introduction

The increasing prevalence of human-induced stressors poses a significant challenge to global biodiversity (Navarro-Ortega *et al.* 2015; Piggott *et al.* 2015b, a; Przeslawski *et al.* 2015; Segner *et al.* 2014), with climate change and pesticide being key drivers of biodiversity loss and their interaction presenting unique ecological risks (Beketov *et al.* 2013; Heino *et al.* 2009; Stehle & Schulz 2015). Rising temperatures are projected to intensify pesticide use due to intensified pest outbreaks (Deutsch *et al.* 2018), while simultaneously altering the response of ectotherms to chemical exposure (Fournier-Level *et al.* 2016; Hooper *et al.* 2013; Noyes *et al.* 2009). For ectotherms, which lack internal temperature regulation (Deutsch *et al.* 2008;

43 Paaijmans *et al.* 2013), warming elevates metabolic rates and feeding activity (potentially
44 increasing pesticide uptake), leading to energetic trade-offs between detoxification and
45 essential functions like growth or reproduction (Jutfelt *et al.* 2021; Pörtner *et al.* 2017).

46 While the individual effects of temperature and pesticides are relatively well-documented
47 (Dougherty *et al.* 2024; Wan *et al.* 2025), their combined impacts remain poorly resolved but
48 have shown variable outcomes. Studies report additive (Arambourou & Stoks 2015),
49 antagonistic (Meng *et al.* 2022), or synergistic (Wang et al., 2019) interactions, with synergism
50 being most concerning for non-target species as their combined harm exceeds the sum of
51 individual effects (Folt *et al.* 1999; Siviter *et al.* 2021; Verheyen & Stoks 2019, 2023). Such
52 synergisms may arise from physiological trade-offs, such as heat stress diverting resources
53 from detoxification (Dai *et al.* 2023) combined with a pesticide-induced antioxidant depletion,
54 crucial for thermal resilience (Silva *et al.* 2025). This dual depletion could disproportionately
55 harm gonads and gametes that are particularly prone to oxidative stress due to their high cell
56 proliferation, metabolic rate and generation of reactive oxygen species (Simmons *et al.* 2018).
57 By eroding reproductive resilience, synergisms threaten to accelerate biodiversity loss in ways
58 that single stressors would not (Shahid *et al.* 2024).

59 Beyond direct physiological impacts, host-associated microbial communities (microbiomes)
60 are increasingly recognized as mediators for environmental stress tolerance (Feldhaar 2011;
61 Osborne *et al.* 2023; Sepulveda & Moeller 2020; Shokal *et al.* 2016). Commensal microbes
62 can enhance host thermal performance (Renoz *et al.* 2019; Sepulveda & Moeller 2020), either
63 as obligate or facultative symbionts (Dunbar *et al.* 2007; Gruntenko *et al.* 2017; Montllor *et al.*
64 2002; Russell & Moran 2006; Zhang *et al.* 2008), and a healthy microbiome can bolster insect
65 resistance to insecticides through direct pesticide metabolism or by inducing the expression of
66 host detoxification genes (Cheng *et al.* 2017; Jones *et al.* 2015; Kikuchi *et al.* 2012; Pang *et al.*

2018). Despite progress in studying individual stressors, how microbiomes modulate *combined* effects remains largely unknown, but microbiome disruption by one stressor could amplify susceptibility to another, potentially explaining observed synergisms (Karimzadeh *et al.* 2020; Mao *et al.* 2019; Zhang *et al.* 2008; Zhang, Cai, *et al.* 2021).

Another critical knowledge gap concerns stress effects on male reproduction. While much research focuses on acute toxicity or sterility thresholds (De França *et al.* 2017; Desneux *et al.* 2007; Walsh *et al.* 2014; Zwoinska *et al.* 2020), sublethal exposures can have significant fitness consequences. Sublethal effects on female reproductive function are relatively well-characterized, the studies on males are lagging behind but have already revealed that male fertility can be more vulnerable than female fecundity, at least to heat stress (Iossa 2019; Sales *et al.* 2018; Zwoinska *et al.* 2020). Heat and pesticides can impair spermatogenesis, sperm DNA integrity, and overall ejaculate quality (Aulsebrook *et al.* 2020; Dougherty *et al.* 2024; Walsh *et al.* 2019), key determinants fertlization success, particularly under competitive conditions in promiscuous species (Fitzpatrick & Lüpold 2014; Parker & Pizzari 2010; Pizzari & Parker 2009; Simmons & Fitzpatrick 2012).

Sperm damage can also extend the environmental stress effects across generations through epigenetic modifications or sperm-borne small RNAs (Bonduriansky *et al.* 2012; Morgan & Watkins 2019), affecting population stability and evolution (Aulsebrook *et al.* 2020; Crean & Immler 2021). While parental exposure to combined thermal and chemical stress has been shown to reduce offspring performance in some species (DeCourten & Brander 2017; Fathy 2024), other studies find no clear effects (Massot *et al.* 2021; Meng *et al.* 2022) or even reduced offspring vulnerability (Tran *et al.* 2018), indicating interspecific variation due to stressor intensity (including the thermal sensitivity of pesticides themselves), species-specific acclimation, or compensatory maternal provisioning. Understanding these cross-generational

91 impacts is crucial for predicting long-term ecological consequences and evolutionary
92 adaptation potential.

93 To address these knowledge gaps, we investigated the interactive effects of acute heat stress
94 and sublethal pesticide exposure on the reproductive fitness of male *D. melanogaster*, focusing
95 on microbiome mediation and cross-generational consequences. We combined sublethal acute
96 heat stress (4 hours at 36°C), previously shown to negatively affect testicular function and the
97 fertilizing capacity of sperm (Meena *et al.* 2024a, b), with exposure to imidacloprid, a widely
98 used systemic insecticide targeting nicotinic acetylcholine receptors (nAChRs) in the central
99 nervous system (Ihara *et al.* 2017). Sublethal imidacloprid exposure is known to affect various
100 aspects of insect physiology and fitness (De França *et al.* 2017; He *et al.* 2011; Kang *et al.*
101 2018; Li *et al.* 2018; Xiao *et al.* 2016; Zhang *et al.* 2021b; Zhou *et al.* 2017). To isolate the role
102 of the microbiome, we compared germ-free (axenic) males with males associated with defined
103 bacterial communities: mono-association of *Acetobacter tropicalis* or *Lactobacillus brevis*, or
104 a five-species consortium of *Acetobacter* and *Lactobacillus* species that are common in
105 *Drosophila* (Broderick & Lemaitre 2012; Gould *et al.* 2018; Newell & Douglas 2014; Ridley
106 *et al.* 2013). This gnotobiotic approach permitted precise assessment of microbial contributions
107 to stress tolerance. Furthermore, we assessed the cross-generational consequences of paternal
108 stress exposure on offspring fitness, testing if the father's microbiome status influenced these
109 effects while minimizing bacterial transmission to offspring to focus on direct paternal effects
110 (Marshall *et al.* 2010; Moore *et al.* 2019; Mousseau *et al.* 2009).

111 Based on the evidence outlined above, we tested the following five predictions: (1) Combined
112 sublethal heat and imidacloprid exposure will synergistically reduce male reproductive fitness.
113 (2) Gut microbes will partially mitigate the negative impacts of individual and combined
114 stressors on male reproductive fitness compared to germ-free males. (3) Different bacterial

115 compositions will vary in their protective capacity, with the complex community offering
116 greater resilience than either mono-association. (4) Paternal exposure to individual and
117 combined stressors will negatively impact offspring fitness (cross-generational effects), with
118 potential synergistic amplification. (5) The paternal microbiome composition will influence the
119 magnitude of these cross-generational effects.

120

121 **Materials and Methods**

122 ***Experimental populations***

123 Throughout this study, we used an outbred *D. melanogaster* population (LH_m wild-type strain),
124 maintained under constant conditions of 25°C, 60% humidity, and a 12h:12h light:dark cycle.
125 First, we generated axenic (germ-free) focal flies by collecting eggs (within six hours of
126 oviposition) from four large population containers supplied with Petri dishes containing grape-
127 juice agar medium (300 mL water, 670 mL grape juice, 22 g agar, 20 g sugar, 19 g yeast, 20
128 mL nipagin per litre) supplemented with a smear of yeast paste. Eggs were washed off the
129 plates into a strainer, pooled, and dechorionated by immersion in 3.5% bleach for 2 minutes,
130 followed by a rinse in 70% ethanol and three rinses in sterile water.

131 Next, eggs were aseptically transferred to sterile glass vials containing autoclaved standard fly
132 food (75 g glucose, 100 g fresh yeast, 55 g corn, 10 g flour, and 15 mL nipagin antimicrobial
133 agent per liter food media) supplemented with antibiotics [a cocktail of following antibiotics
134 with their respective final concentrations: Ampicillin (50 μg/mL), Kanamycin (50 μg/mL),
135 Erythromycin (10 μg/mL), Tetracycline (10 μg/mL)]. Vials were then sealed with sterile plugs
136 and incubated under standard laboratory conditions until adult eclosion. All steps of vial
137 preparation were conducted in a laminar flow chamber to avoid contamination.

138 Upon eclosion, axenic status was confirmed using two methods on a subsample of flies. First,
139 homogenated flies were plated on Lysogeny Broth (LB) and de Man, Rogosa and Sharpe
140 (MRS) agar plates, incubated, and checked for bacterial colony growth. Second, genomic DNA
141 was extracted from pools of 3-5 putatively germ-free and conventional flies (positive control),
142 respectively, using Quick-DNA midiprep plus kit following the instructions provided by the
143 manufacturer. Universal primers (27F and 1492R) were used as forward and reverse primer,
144 respectively. To prepare the PCR master mix, 10 µL of nuclease-free water, 0.5 µL of forward
145 and reverse primer, 1 µL of template DNA and 13 µL Taq green master mix were mixed in a
146 sterile eppendorf tube. PCR conditions were: initial denaturation at 95°C for 90s, followed by
147 35 cycles of denaturation at 95°C for 30s, annealing at 55°C for 30s and extension at 72°C for
148 90s, and final extension at 72°C for 5 minutes). PCR products were visualized on a 1% agarose
149 gel under ultraviolet light to confirm the absence of bacterial DNA amplification in axenic
150 samples and its presence in controls.

151 ***Bacterial cultures and gnotobiotic associations***

152 Axenic males were associated with defined compositions of microbial species, all originally
153 isolated from the gut of wild-type flies (Obadia *et al.* 2017): *Acetobacter tropicalis* (*At*),
154 and *Lactobacillus brevis* (*Lb*) for mono-associations based on their contrasting effects on male
155 reproductive investments in our previous study (Maggu *et al.* 2025). For comparison, we also
156 included axenic males and males inoculated with a defined five-species community consisting
157 of common and abundant *Drosophila* gut bacteria (*A. tropicalis*, *A. orientalis, A. pasteurianus,*
158 *L. plantarum, L. brevis*), resulting in four distinct microbial treatments.

159 Bacterial strains were cultured overnight in an incubator at 30°C: *Acetobacter* in MRS +
160 mannitol medium with shaking, and *Lactobacillus* in MRS broth under stationary conditions.
161 The following morning, cultures were checked for growth before measuring their optical

162 densities (OD) at 600 nm and centrifuging at 7000 rpm for 5 minutes. Bacterial pellets were
163 then resuspended in sterile phosphate-buffered saline (PBS) to a final concentration of 10^8
164 cells/mL, following established protocols (Koyle *et al.* 2016; Newell & Douglas 2014).

165 To inoculate vials, 50µL of bacterial suspension (approx. 5×10^6 CFUs) was added to sterile
166 food vials, with equal representation of bacterial strains in the 5-species consortia. This
167 bacterial density is comparable to previous reports for conventionally reared flies (Gould *et al.*
168 2018; Newell & Douglas 2014). Inoculated food vials were then allowed to air-dry in a laminar
169 flow cell before introducing ten 1-day-old axenic males to each to generate gnotobiotic flies.
170 Males were transferred to freshly inoculated vials with the same bacterial treatment every 48h
171 for a total of four days to ensure stable colonization. Successful colonization with the intended
172 bacteria was confirmed by plating homogenates of males from each treatment and checking for
173 characteristic colony morphologies (see *Bacterial abundance of males*).

174 ***Stress treatments***

175 Following the 4-day colonization period, males were transferred to vials containing sterile food
176 and exposed to pesticide and heat stress in a fully factorial design. Specifically, half of the
177 males from each microbial group were subjected to food supplemented with imidacloprid,
178 whilst the remaining half were maintained on uncontaminated standard food. We used a
179 concentration of 1 ppm (parts per million) of imidacloprid as concentrations of 0.04 to 20 ppm
180 have been used effectively in studies on larval or adult *D. melanogaster*, depending on the
181 exposure regime (Charpentier *et al.* 2014; Frantzios *et al.* 2008; Martelli *et al.* 2020). These
182 concentrations are low enough to avoid immediate lethality but sufficient to induce sublethal
183 effects on behavior, reproduction, and physiology. Males were exposed to imidacloprid for an
184 acute window of 18 h, following an additional thermal treatment of 4-hour in a hot water bath.
185 To this end, half of the males from each microbiome and pesticide treatment were subjected to

186 acute heat stress by transferring them to vials containing only sterile 1% agar (to prevent
187 dehydration) and placing these vials in a water at 36°C for 4 hours. The remaining males
188 (thermal controls) were treated identically but at benign 24.5°C. All males were then allowed
189 to recover overnight in standard food vials under standard laboratory conditions.

190 ***Hatching success and fertility***

191 To quantify the fitness consequences of heat and pesticide exposure and their potential
192 mitigation by the microbiome on the morning after the stress treatments, experimental males
193 were individually mated to standard females of the same population. All females had been
194 collected as virgins (within 7 hours of eclosion) and reared under axenic conditions along with
195 the experimental males to minimize confounding contributions of female microbes to the
196 measured outcomes. After mating ended, females were allowed to lay eggs for 24 h, and
197 another 30 h later (allowing time for embryonic development), oviposition vials were frozen
198 for later quantification of the proportion of eggs hatching as a proxy of fertilization success,
199 and the total number of larvae estimating male-mediated fecundity. This assay was performed
200 in two blocks, each with 25 males per treatment combination, totaling 800 experimental males
201 (4 bacterial treatments × 2 pesticide treatments × 2 temperature treatments × 2 blocks × 25
202 males).

203 ***Sperm competitiveness***

204 To assess male sperm competitiveness, the above experimental design was repeated, but this
205 time using standard females that had mated once with standard competitor males two days
206 before the focal mating. These standard competitors were of the same strain but genetically
207 modified to ubiquitously express green fluorescent protein (GFP), permitting paternity
208 assignment against the focal wild-type males (Lüpold *et al.* 2012, 2020). Pairs were monitored
209 for remating for up to 8 h. Remated females were then isolated in fresh vials for oviposition,

210 while pairs not mating were separated and given another 8-hour mating opportunity on the
211 following day. Females still not remating were excluded. Offspring paternity (focal vs.
212 competitor) was assessed via GFP fluorescence to calculate P_2 as the proportion of offspring
213 sired by the second (focal) male. The treatment design of this assay was identical to the non-
214 competitive experiment above, but this time with 30 trials per treatment combination in each
215 of the two experimental blocks (i.e., 960 trials in total).

216 ***Bacterial abundance in males***

217 To investigate the impact of stress treatments on the bacterial load, colony forming units
218 (CFUs) were compared between ten random males from each experimental treatment, surface-
219 sterilized with 70% ethanol, and individually homogenized in 1.5 mL microcentrifuge tubes
220 containing sterile PBS using a pestle. Homogenates were serially diluted to different dilutions
221 at a final volume of 100 µL, then plated on MRS media for quantification of CFUs at
222 appropriate dilutions (approx. 100 CFUs/plate). For the control groups with both *Acetobacter*
223 and *Lactobacillus* species, MRS agar was supplemented with 10 µg/mL ampicillin to avoid the
224 growth of *Lactobacillus,* whilst replicate plates were incubated in a CO_2 atmosphere to exclude
225 the growth of *Acetobacter*. The presence of only target bacterial species on plates was
226 confirmed based on colony morphologies, with *A. tropicalis* producing small tan-brown
227 colonies and *L. brevis* producing small yellowish colonies. CFUs per fly were calculated as:
228 CFU = $C \times D/P \times V$, where C = number of colonies counted, D = dilution, P = volume plated,
229 V = volume of fly homogenate (Koyle *et al.* 2016). For control males, with all the five bacterial
230 species, bacterial colonies were identified by their morphology and total CFUs per fly was
231 obtained by adding CFUs from all the five bacterial species.

232

233

234 ***Cross-generational effects***

235 To assess cross-generational impacts of paternal (F_0) stress exposure, we quantified hatching
236 success and fecundity in both male and female offspring (F_1) of our experimental males. Within
237 each experimental block, up to 20 freshly hatched larvae from each of ten randomly selected
238 vials per parental treatment combination (i.e., 16 combinations) were transferred to rearing
239 vials containing sterilized standard fly food. This immediate transfer minimized the potential
240 acquisition of parental microbes via consumption of the egg chorion or contaminated food.
241 These larvae developed under standard laboratory conditions.

242 Upon eclosion, 4-5 virgin F_1 male and female offspring were collected from each rearing vial,
243 separated by sex. At 2-3 days of age, three males and three females from each lineage were
244 individually mated with a virgin standard fly of the opposite sex reared under axenic conditions
245 (to avoid potential confounding effects of their microbiota). Following copulation, hatching
246 success and fecundity were measured for these F_1 flies as in their parents (see above). These
247 offspring themselves were not subjected to any experimental treatment, thus assessing solely
248 the cross-generational effects. This experiment was carried out in two independent blocks of
249 480 offspring per sex from 160 F_0 males (i.e. total of 1,920 mating trials).

250 ***Statistical analysis***

251 All statistical analyses were performed in R version 4.4.3 (R Core Team 2025), using
252 generalized linear models (GLMs) with a Poisson (fecundity) or binomial error distribution
253 (hatching success, paternity shares) in the experimental generation, and the corresponding
254 mixed-effects models (GLMMs) in the offspring generation with sire as a random effect to
255 account for non-independence among siblings. Each model included microbiome composition
256 (4-level factor) along with the binary thermal and pesticide treatments with all two- and three-
257 way interactions as fixed effects, along with experimental block as a nuisance factor accounting

258 for inter-block variation. Given that this factor only had two levels except for the assay of CFUs
259 with three experimental blocks, thereby limiting estimates of random variance, we included it
260 as a fixed effect in all models.

261 The binomial model of the relative abundance of Acetobacter versus Lactobacillus exhibited
262 slight overdispersion (dispersion factor = 1.12), which we addressed by fitting a mixed-effects
263 model with an observation-level random effect. In contrast, the remaining non-linear models
264 showed moderate underdispersion (dispersion factors ≈ 0.9). Alternative model families such
265 as Tweedie, negative binomial, and quasibinomial did not improve model fit, so the original
266 models were retained for clarity and interpretability.

267 Model performance was checked using the performance package (Lüdecke *et al.* 2021), and
268 output plots were generated was plotted using ggplot2 (Wickham 2016) based on means and
269 95% confidence intervals estimated via emmeans (Lenth 2025).

270 The impact of the different experimental treatments was also analyzed for the total bacterial
271 abundances (CFUs) and, separately, for compositional shifts in the five-species consortia. For
272 the former, we used a linear mixed-effects model with block as a random effect. For
273 microbiome community composition in the five-species consortium, we quantified the species-
274 specific abundances per sample, then applied a Hellinger transformation to these count data
275 and computed Bray-Curtis distances using the *vegdist* function in the package vegan (Oksanen
276 *et al.* 2025). We assessed treatment effects (temperature, pesticide, and their interaction) in a
277 permutational multivariate analysis of variance (PERMANOVA) using *adonis2* in the same
278 package. To visualize compositional shifts, we further ran non-metric multidimensional scaling
279 (NMDS) (*metaMDS*) on the Bray-Curtis dissimilarities and fitted species vectors onto the
280 NMDS ordination with *envfit*, which indicates the direction and strength of each species-
281 specific association with the ordination space. We also compared the relative abundances at

282 the genus level, using a binomial GLMM with block as a random effect and accounting for
283 overdispersion by including an additional observation-level random effect.

284

285 **Results**

286 ***Hatching success***

287 To assess the combined effects of heat stress, pesticide exposure, and microbiome composition
288 on male reproductive function, we first investigated fertilization success, estimated as the
289 proportion of eggs hatched out of all eggs laid by females. A binomial GLM revealed a
290 significant three-way interaction between microbiome, temperature, and pesticide ($N = 650$, χ^2
291 $= 15.19$, $df = 3$, $p = 0.002$; Fig. 1A, Supplementary Table S1), highlighting that the combined
292 effects of these stressors depended on the microbial composition of the focal males. Overall,
293 males associated with all five bacterial strains exhibited the highest hatching success, followed
294 by *At*- and *Lb*-associated males, with axenic males being most severely affected. These general
295 patterns were also confirmed by the significant two-way interactions of temperature ´
296 microbiome ($\chi^2 = 22.01$, $df = 3$, $p < 0.0001$) and microbiome ´pesticide ($\chi^2 = 18.29$, $p <$
297 0.0001), as well as by all main effects (all χ^2 ³67.07, $p < 0.001$; Supplementary Table S1).

298 ***Male-mediated fecundity***

299 Next, we investigated male-mediated fecundity, quantified as the total number of larvae
300 produced per female. In contrast to hatching success, the three-way interaction was not
301 significant ($\chi^2 = 0.68$, $df = 3$, $p = 0.878$) for this metric and was removed from the final model.
302 The simplified model revealed significant two-way interactions between microbiome and
303 temperature ($N = 650$, $\chi^2 = 8.06$, df = 3, $p = 0.042$) and between temperature and pesticide (χ^2

304 = 8.06, df = 1, p = 0.005; Fig. 1B, Supplementary Table S2). All main effects were again
305 significant (all χ^2 ³23.92, $p < 0.0001$).

306 ***Sperm competitiveness***

307 Given reports of adverse effects of increased temperatures and pesticide exposure on sperm
308 quality and function (Chirault *et al.* 2015; Meena *et al.* 2024a; Moreira *et al.* 2022; Roy *et al.*
309 2017; Sales *et al.* 2018, 2024), we evaluated the combined effects of our treatments on the
310 proportion of progeny sired by focal males relative to their standard competitors (i.e., P_2).
311 Although none of the interactions were statistically significant and thus removed from the
312 model (N = 331, all χ^2 = ≤5.26, df = 3, $p \geq 0.15$), all experimental treatments exhibited highly
313 significant main effects on P_2 (all χ^2 ³99.60, p £0.0001; Fig. 1C, Supplementary Table S3).

314 ***Bacterial abundance in males in response to heat stress and pesticide exposure***

315 We also analyzed the effect of heat stress, pesticide exposure and their interactions on the
316 growth and viability of commensal bacteria inside their host under the different stress
317 treatments by quantifying their colony forming units in homogenates of focal males
318 immediately after mating. For total CFU counts, we found a significant three-way interaction
319 (N = 120, $F_{2,106}$ = 4.78, p = 0.010; Fig. 1D, Supplementary Table S4), suggesting that the
320 bacterial strains differed in their accumulation patterns in adult flies depending on the
321 combined effects of stress treatments. Additionally, we documented significant two-way
322 interactions and main effects of all three factors (all F ³10.54, p £0.001)

323 A PERMANOVA controlling for block effects ($F_{1,35}$ = 0.20, p = 0.874) revealed that
324 temperature was also the dominant driver of variation in the relative abundances of the five
325 bacterial species constituting the consortium, explaining 26.3% of the variation in community
326 composition ($F_{1,35}$ = 13.88, p = 0.0001; Supplementary Table S5). In contrast, the main effect
327 of pesticide was not statistically significant (2.2% of variation, $F_{1,35}$ = 1.16, p = 0.314), and the

temperature × pesticide interaction showed a marginal effect (4.8% of variation, $F_{1,35} = 2.55$, $p = 0.068$). NMDS based on Bray-Curtis dissimilarities provided a visual representation of community shifts across treatments. The NMDS ordination (Fig. 2A) indicated that the first axis (NMDS1) predominantly tracked the temperature gradient, while the second axis (NMDS2) aligned more, but only weakly, with pesticide exposure. Overlaying species vectors revealed notable differences in species associations (all R^2 ³0.54, p £0.0001), in that both *Lactobacillus* species showed positive (³0.68), and all *Acetobacter* exhibited negative (£-0.38), loadings on NMDS1 (Supplementary Table S6), suggesting an increase in the relative abundance of *Acetobacter* in the high-temperature regimes. By contrast, loadings on NMDS2 were negative for *A. tropicalis* (-0.92) and *L. brevis* (-0.44) but positive for all remaining species (³0.56). This axis tended to be biased toward negative values in the absence of imidacloprid, but more ambiguous in its presence.

Finally, in a binomial GLMM focusing only on the relative abundances of acetic acid versus lactic acid bacteria, where the three *Acetobacter* species should theoretically constitute 60% of the total CFUs among the five species, temperature and pesticide presence interacted significantly ($N = 40$, $z = 2.15$, $p = 0.032$). Specifically, the relative representation of *Acetobacter* was increased to around 66% under heat stress ($z = 2.84$, $p = 0.013$), whereas pesticide exposure reduced its representation ($z = -2.32$, $p = 0.021$), although only at the benign temperature (Fig. 3B).

Cross-generational effects on offspring fitness

Finally, we investigated how paternal stress exposure affects the next generation by measuring the hatching success and fecundity of F_1 offspring produced by F_0 males from each treatment group. We found a significant interactive effect between paternal temperature and pesticide treatments on the hatching success of both male ($N = 725$, $\chi^2 = 9.45$, df = 1, $p = 0.002$;

352 Supplementary Table S7) and female offspring (N = 740, χ^2 = 4.73, df = 1, p = 0.030;
353 Supplementary Table S8, Fig. 3A,B), indicating that the effects of paternal stress exposure
354 were transmitted to the next generation. However, the paternal microbiome contributed only to
355 very weak and non-statistically significant trends for an interaction with temperature in either
356 offspring sex (χ^2 £6.38, df = 3, p = 0.10), suggesting limited cross-generational effects on F_2
357 hatching.

358 The interactive effect of paternal temperature and pesticide exposure was also significant for
359 the fecundity of F_1 daughters (N = 740, χ^2 = 6.11, df = 1, p = 0.013), along with a main effect
360 of the paternal microbiome composition (χ^2 = 8.91, df = 3, p = 0.030; Fig. 3, Supplementary
361 Table S9). By contrast, the paternal effects on larval numbers in sons was limited to main
362 effects of temperature and pesticide exposure ($\chi^2 \geq 26.84$, df = 1, $p < 0.0001$), with no
363 contribution of the paternal microbiome (χ^2 = 0.52, df = 3, p = 0.915; Fig. 3, Supplementary
364 Table S10).

365

366 **Discussion**

367 We investigated the role of the gut microbiome in modulating male reproductive success in *D.*
368 *melanogaster* under individual and combined exposure to acute heat stress and the pesticide
369 imidacloprid. Our results confirm and extend previous work demonstrating the detrimental
370 effects of sublethal temperatures (Canal Domenech & Fricke 2022; David *et al.* 2005; Fasolo
371 & Krebs 2004; Gandara & Drummond-Barbosa 2022, 2023; Meena *et al.* 2024a; Pilakouta &
372 Baillet 2022; Sales *et al.* 2024; Walsh *et al.* 2019) or imidacloprid (Charpentier *et al.* 2014;
373 Xiao *et al.* 2016; Zhang *et al.* 2021b; Zhou *et al.* 2017) on reproductive functions. Importantly,
374 our study revealed both the combined effects of these stressors and the critical role of the

375 microbiome in mediating the response of stressed males, highlighting the importance of the gut
376 microbiome in mitigating fitness losses. *Acetobacter* generally provided stronger protection
377 than *Lactobacillus*, especially in males with a complex microbial community. Furthermore, we
378 demonstrated that paternal stress exposure imposes fitness costs on the next generation,
379 although these cross-generational effects were not significantly mediated by the paternal
380 microbiome under our experimental conditions designed to minimize direct microbial
381 transmission.

382 ***Interactive effects of combined stressors on male reproduction***

383 Our study revealed that initial exposure to imidacloprid followed by heat shock reduced male
384 reproductive functions to a greater extent than either stressor alone, consistent with previous
385 studies indicating complex, often order-dependent interactions between thermal stress and
386 pesticide toxicity (Ahmed *et al.* 2023; Deng *et al.* 2016; Meng *et al.* 2022; Ricupero *et al.* 2020;
387 Wang *et al.* 2021). While heat stress has been shown to increase the tolerance to subsequent
388 insecticide exposure in diverse insect pests (Cao *et al.* 2019; Ge *et al.* 2013; Gu *et al.* 2010),
389 the reverse order of stress exposure can have opposing effects (Delnat *et al.* 2019; Wang *et al.*
390 2021), emphasizing the importance of stress sequence in cross-protection.

391 Pre-exposure to insecticides can also decrease heat stress tolerance, suggesting that warmer
392 temperatures may exacerbate insecticide toxicity depending on the exposure sequence.
393 Increased imidacloprid toxicity at higher temperatures has been documented in mayflies
394 (Macaulay *et al.* 2020), earthworms and collembolans (Bandeira *et al.* 2020), and the terrestrial
395 ectotherm *Hypogastrura viatica* (Silva *et al.* 2023). Our study supports these findings, showing
396 decreased male reproductive success when pesticide exposure preceded heat stress. These
397 multi-stressor studies highlight the complex temperature-toxicity interplay, which can be
398 positive or negative depending on the insecticide mode of action, exposure route, and insect

399 species. Therefore, the sequence of exposure must be carefully considered when evaluating
400 combined stressor effects on insect performance.

401 The mechanisms underlying these sequence-dependent interactions likely involve intricate
402 physiological adjustments. Exposure to one stressor might compromise pathways needed to
403 cope with the subsequent stressor (lack of cross-tolerance) or trigger shared signaling pathways
404 that amplify susceptibility (negative cross-talk). Resource allocation trade-offs are also
405 probable (Sokolova 2013), where coping with pesticide toxicity may deplete energy reserves
406 or specific molecules (e.g., antioxidants, chaperones) needed for an effective heat stress
407 response. Generally, stressful environments can alter resource allocation among essential
408 processes like growth, reproduction or immune defense to optimize fitness outcomes
409 (Kooijman 2000). As such, elevated temperature may increase somatic maintenance costs
410 (Verberk *et al.* 2016), potentially reducing resources for defense and repair (Hallman & Brooks
411 2015).

412 While the precise mechanisms underlying increased toxicity at high temperatures remain
413 unclear, they may overlap with those explaining the increased toxicity of organophosphate
414 pesticides at higher mean temperatures (Noyes *et al.* 2009; Noyes & Lema 2015). Increased
415 temperatures can enhance the activity of detoxification enzymes like cytochrome P450s (Wang
416 *et al.* 2021), accelerating pesticide breakdown (Ahmad 2007). Increased uptake of chemicals
417 at higher temperatures (Holmstrup *et al.* 2010) has been shown to increase membrane
418 permeability (Noyes & Lema 2015) or inhibit growth (Gardner *et al.* 2011), possibly due to
419 higher basal metabolism (Hooper *et al.* 2013). Stressful conditions can promote the
420 accumulation of reactive oxygen species (ROS) due to reduced respiration and increased water
421 loss, thereby negatively affecting development and reproduction (Dampc *et al.* 2021). Low
422 doses of imidacloprid, for example, can cause ROS formation and metabolic impairment in *D.*

423 *melanogaster* (Martelli *et al.* 2020). Both pesticide tolerance (Bagchi *et al.* 1996) and heat
424 stress (Neven 2000) are also associated with overexpression of heat-shock proteins. In
425 honeybees, Hsp70 and Hsp90 are upregulated in response to both thermal stress and
426 imidacloprid (Manzi *et al.* 2020). Such chaperone activation could also protect *D.*
427 *melanogaster* males against combined stressors. If so, the observed fitness loss under both
428 stressors might at least in part be attributable to the costs associated with enhanced Hsp
429 expression (Hoekstra *et al.* 2013; Li *et al.* 2015), which may become overwhelming under
430 combined stress.

431 ***Microbiome as a modulator of stress resilience***

432 A key finding of our study was the significant role of the gut microbiome in mitigating the
433 detrimental reproductive consequences of heat and pesticide stress. Axenic males consistently
434 performed worse than colonized males across all measured fitness components, highlighting
435 the baseline importance of the microbiome for reproduction and its crucial role under stress.
436 The reduced fitness of axenic males under combined stress suggests an impaired ability to
437 metabolize and excrete imidacloprid, potentially leading to higher internal toxin loads and
438 increased vulnerability to subsequent heat stress.

439 Interestingly, the degree of protection depended on microbial composition. While both
440 *Lactobacillus brevis* and *Acetobacter tropicalis* mono-associations offered benefits over the
441 axenic state, the five-species community consistently provided the greatest resilience,
442 underscoring the potential importance of microbial diversity and inter-species interactions for
443 robust host function (Coyte *et al.* 2015; Freilich *et al.* 2011; Levy & Borenstein 2013;
444 Magnúsdóttir *et al.* 2017; Noecker *et al.* 2019; Zelezniak *et al.* 2015). Mono-associations might
445 lack the metabolic versatility or synergistic cross-feeding capabilities present in more complex
446 consortia, limiting their ability to buffer the host against multiple stressors.

447 Our results partially contrast with Daisley *et al.* (2017), who reported that *Lactobacillus*
448 *plantarum* (Lp39) supplementation rescued *D. melanogaster* from harmful imidacloprid
449 effects, accompanied by increased *Acetobacter* and *Lactobacillus* abundance. We did not
450 observe such a strong rescue with *L. brevis*, nor an increased abundance of either bacterial
451 genus. This discrepancy could stem from strain-specific functions, similar to opposing effects
452 on chlorpyrifos (CP) toxicity between *L. plantarum* ISO and *Lactobacillus ATCC* in *D.*
453 *melanogaster* (Daisley *et al.* 2017), or differences in experimental protocols (e.g., our acute
454 18h imidacloprid exposure versus Daisley *et al.*'s (2017) continued exposure).

455 We also observed shifts in the relative abundance of *Acetobacter* and *Lactobacillus* within the
456 host, aligning with studies showing heat-induced microbiome disruptions (Jaramillo &
457 Castañeda 2021; Kokou *et al.* 2018; Moghadam *et al.* 2018). *Lactobacillus* appeared more
458 abundant and less affected by treatments in mono-associations, whereas *Acetobacter* seemed
459 to be overrepresented in the five-species consortium relative to the expected proportion of 60%.
460 Generally, we found a higher proportional representation of *Acetobacter* following acute heat
461 stress, consistent with prior work (Moghadam *et al.* 2018). Beyond Moghadam *et al.*'s (2018)
462 thermal treatments throughout development, however, our findings demonstrate that such
463 compositional shifts can occur within hours.

464 The mechanisms by which the microbiome enhances stress tolerance likely involve multiple
465 pathways. Beyond direct detoxification (Cheng *et al.* 2017; Jones *et al.* 2015; Kikuchi *et al.*
466 2012; Pang *et al.* 2018), gut microbes significantly impact host nutrition and metabolism
467 (Douglas 2009; Ridley *et al.* 2012), which are intrinsically linked to thermal tolerance in
468 ectotherms (Henry *et al.* 2020; Moeller *et al.* 2020; Moghadam *et al.* 2018; Semsar-Kazerouni
469 *et al.* 2020). In *D. melanogaster*, the gut microbiota influences nutrient allocation and the
470 thermo-protectant trehalose (Overgaard *et al.* 2007; Ridley *et al.* 2012; Schwasinger-Schmidt

471 *et al.* 2012). It can also modulate host immune responses and potentially Hsp expression in the
472 gut epithelium (Arnal & Lalles 2016; Liu *et al.* 2014). Given the critical role of Hsps in
473 mitigating thermal stress in *D. melanogaster* (Calabria *et al.* 2012; Sørensen *et al.* 2003), and
474 their rapid induction after heat exposure (Hoekstra *et al.* 2013), the microbiota may enhance
475 heat tolerance by promoting higher Hsp levels. Disrupting the microbiome can alter these
476 processes, as seen with heat-induced reductions in *Buchnera aphidicola* in pea aphids lowering
477 heat resistance (Dunbar *et al.* 2007), and temperature-driven microbiome shifts increasing
478 imidacloprid susceptibility in *Nilaparvata lugens* (Zhang *et al.* 2021a).

479 ***Cross-generational impacts of parental stress***

480 We found clear evidence for cross-generational fitness costs of paternal stress exposure, with
481 reduced hatching success and fecundity in offspring of stressed fathers. Cross-generational
482 effects can either increase or decrease offspring vulnerability to stressors (Brausch & Salice
483 2011; Kim *et al.* 2014; Pölkki *et al.* 2012; Reátegui-Zirena *et al.* 2017; Schultz *et al.* 2016).
484 Our results corroborate studies reporting detrimental cross- or transgenerational effects of
485 paternal heat stress (Ferrer *et al.* 2013; Guillaume *et al.* 2016; Shama *et al.* 2014; Walsh *et al.*
486 2014) or toxicant exposure (Bhandari *et al.* 2015; Kimberly & Salice 2015; Schultz *et al.* 2016;
487 Yu & Liao 2016), possibly through epigenetic processes (Schultz *et al.* 2016). The observed
488 synergism between paternal heat and pesticide exposure in offspring fitness underscores the
489 potential for long-lasting consequences of multi-stressor events.

490 Offspring from stressed fathers exhibited reduced fecundity and hatching success, indicating
491 that paternal exposure does not buffer against these environmental stressors. Our findings
492 support the view that cross-generational effects of stress are often non-adaptive or costly for
493 offspring (Uller *et al.* 2013), highlighting the potential for cumulative fitness declines across
494 generations in populations facing recurrent multi-stressor events.

Intriguingly, the paternal microbiome did not significantly mediate these cross-generational effects in our study. This could be attributable to our experimental design, which deliberately minimized vertical transmission of microbes by using axenic mothers and transferring these and their offspring to sterile food vials immediately after mating or hatching, respectively. This allowed us to isolate direct paternal effects (potentially epigenetic or related to gamete quality) based on the sire's microbial status. However, we cannot rule out a role for the paternal microbiome if transmission occurs naturally (e.g., during copulation) (Guilhot *et al.* 2025), potentially triggering differential maternal effects or microbial deposition on eggs (direct paternal transmission of microbes to their offspring seems rather unlikely as males have little control over female oviposition). Future studies explicitly allowing or manipulating vertical transmission would be needed to explore this.

Conclusions and broader implications

In conclusion, our study demonstrates that acute heat stress and sublethal pesticide exposure interact, often synergistically, to negatively impact male reproductive fitness in *D. melanogaster*, with consequences extending to offspring viability and fecundity. Crucially, we show that the gut microbiome is a key determinant of resilience, offering significant protection against these stressors, with benefits enhanced by microbial complexity. However, the microbiome itself is vulnerable to disruption by these same stressors.

Our findings underscore the critical need to consider multi-stressor interactions and the role of host-associated microbes when assessing the ecological risks posed by climate change and agrochemical use. The observed negative cross-generational effects further highlight the potential vulnerability of insect populations, particularly those with depleted or altered microbiomes. Future research should explore these interactions across diverse species,

environments, and stressor combinations to improve ecological forecasting and develop strategies for mitigating biodiversity loss in the Anthropocene.

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Author contributions

KM and SL conceived the study and acquired funding; KM, AM, ANDN, BE, SHS and RATS collected the data; KM and SL conducted statistical analyses and drafted the manuscript, which was reviewed and approved by all other authors.

References

Ahmad, M. (2007). Insecticide resistance mechanisms and their management in *Helicoverpa armigera* (Hübner)–A review. *J Agric Res*, 45, 319–335.

Ahmed, T., Masood, H.A., Noman, M., Al-Huqail, A.A., Alghanem, S.M., Khan, M.M., *et al.* (2023). Biogenic silicon nanoparticles mitigate cadmium (Cd) toxicity in rapeseed (*Brassica napus* L.) by modulating the cellular oxidative stress metabolism and reducing Cd translocation. *J. Hazard. Mater.*, 459, 132070.

539 Arambourou, H. & Stoks, R. (2015). Combined effects of larval exposure to a heat wave and
540 chlorpyrifos in northern and southern populations of the damselfly *Ischnura elegans*.
541 *Chemosphere*, 128, 148–154.
542 Arnal, M.-E. & Lalles, J.-P. (2016). Gut epithelial inducible heat-shock proteins and their
543 modulation by diet and the microbiota. *Nutr. Rev.*, 74, 181–197.
544 Aulsebrook, L.C., Bertram, M.G., Martin, J.M., Aulsebrook, A.E., Brodin, T., Evans, J.P., *et*
545 *al.* (2020). Reproduction in a polluted world: implications for wildlife. *Reproduction*,
546 160, R13–R23.
547 Bagchi, D., Bhaitacharya, G. & Stohs, S. (1996). Production of reactive oxygen species by
548 gastric cells in association with *Helicobacter pylori*. *Free Radic. Res.*, 24, 439–450.
549 Bandeira, F.O., Alves, P.R.L., Hennig, T.B., Toniolo, T., Natal-da-Luz, T. & Baretta, D.
550 (2020). Effect of temperature on the toxicity of imidacloprid to *Eisenia andrei* and
551 *Folsomia candida* in tropical soils. *Environ. Pollut.*, 267, 115565.
552 Beketov, M.A., Kefford, B.J., Schäfer, R.B. & Liess, M. (2013). Pesticides reduce regional
553 biodiversity of stream invertebrates. *Proc. Natl. Acad. Sci.*, 110, 11039–11043.
554 Bhandari, R.K., Vom Saal, F.S. & Tillitt, D.E. (2015). Transgenerational effects from early
555 developmental exposures to bisphenol A or 17α-ethinylestradiol in medaka, *Oryzias*
556 *latipes*. *Sci. Rep.*, 5, 9303.
557 Bonduriansky, R., Crean, A.J. & Day, T. (2012). The implications of nongenetic inheritance
558 for evolution in changing environments. *Evol. Appl.*, 5, 192–201.
559 Brausch, J.M. & Salice, C.J. (2011). Effects of an environmentally realistic pesticide mixture
560 on *Daphnia magna* exposed for two generations. *Arch. Environ. Contam. Toxicol.*, 61,
561 272–279.
562 Broderick, N.A. & Lemaitre, B. (2012). Gut-associated microbes of *Drosophila melanogaster*.
563 *Gut Microbes*, 3, 307–321.

564 Brooks, M.E., Kristensen, K., Van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., *et*
565 *al.* (2017). glmmTMB balances speed and flexibility among packages for zero-inflated
566 generalized linear mixed modeling.
567 Calabria, G., Dolgova, O., Rego, C., Castañeda, L., Rezende, E., Balanyà, J., *et al.* (2012).
568 Hsp70 protein levels and thermotolerance in *Drosophila subobscura*: a reassessment of
569 the thermal co-adaptation hypothesis. *J. Evol. Biol.*, 25, 691–700.
570 Canal Domenech, B. & Fricke, C. (2022). Recovery from heat-induced infertility—A study of
571 reproductive tissue responses and fitness consequences in male *Drosophila*
572 *melanogaster*. *Ecol. Evol.*, 12, e9563.
573 Cao, Y., Yang, H., Li, J., Wang, C., Li, C. & Gao, Y. (2019). Sublethal effects of imidacloprid
574 on the population development of western flower thrips *Frankliniella occidentalis*
575 (Thysanoptera: Thripidae). *Insects*, 10, 3.
576 Charpentier, G., Louat, F., Bonmatin, J.-M., Marchand, P.A., Vanier, F., Locker, D., *et al.*
577 (2014). Lethal and sublethal effects of imidacloprid, after chronic exposure, on the
578 insect model *Drosophila melanogaster*. *Environ. Sci. Technol.*, 48, 4096–4102.
579 Cheng, D., Guo, Z., Riegler, M., Xi, Z., Liang, G. & Xu, Y. (2017). Gut symbiont enhances
580 insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis*
581 (Hendel). *Microbiome*, 5, 1–12.
582 Chirault, M., Lucas, C., Goubault, M., Chevrier, C., Bressac, C. & Lécureuil, C. (2015). A
583 combined approach to heat stress effect on male fertility in *Nasonia vitripennis*: from
584 the physiological consequences on spermatogenesis to the reproductive adjustment of
585 females mated with stressed males. *PLoS One*, 10, e0120656.
586 Coyte, K.Z., Schluter, J. & Foster, K.R. (2015). The ecology of the microbiome: networks,
587 competition, and stability. *Science*, 350, 663–666.

588 Crean, A.J. & Immler, S. (2021). Evolutionary consequences of environmental effects on
589 gamete performance. *Philos. Trans. R. Soc. B*, 376, 20200122.
590 Dai, W., Holmstrup, M., Slotsbo, S., Bakker, R., Damgaard, C. & van Gestel, C.A. (2023).
591 Heat stress delays detoxification of phenanthrene in the springtail *Folsomia candida*.
592 *Chemosphere*, 311, 137119.
593 Daisley, B.A., Trinder, M., McDowell, T.W., Welle, H., Dube, J.S., Ali, S.N., *et al.* (2017).
594 Neonicotinoid-induced pathogen susceptibility is mitigated by *Lactobacillus plantarum*
595 immune stimulation in a *Drosophila melanogaster* model. *Sci. Rep.*, 7, 2703.
596 Dampc, J., Mołoń, M., Durak, T. & Durak, R. (2021). Changes in aphid—plant interactions
597 under increased temperature. *Biology*, 10, 480.
598 David, J., Araripe, L., Chakir, M., Legout, H., Lemos, B., Pétavy, G., *et al.* (2005). Male
599 sterility at extreme temperatures: a significant but neglected phenomenon for
600 understanding *Drosophila* climatic adaptations. *J. Evol. Biol.*, 18, 838–846.
601 De França, S.M., Breda, M.O., Barbosa, D.R., Araujo, A., Guedes, C.A. & Shields, V. (2017).
602 The sublethal effects of insecticides in insects. *Biological Control of Pest and Vector*
603 *Insects.,10 ,23-39.*
604 DeCourten, B.M. & Brander, S.M. (2017). Combined effects of increased temperature and
605 endocrine disrupting pollutants on sex determination, survival, and development across
606 generations. *Sci. Rep.*, 7, 9310.
607 Delnat, V., Janssens, L. & Stoks, R. (2019). Whether warming magnifies the toxicity of a
608 pesticide is strongly dependent on the concentration and the null model. *Aquat. Toxicol.*,
609 211, 38–45.
610 Deng, Z., Zhang, F., Wu, Z., Yu, Z. & Wu, G. (2016). Chlorpyrifos-induced hormesis in
611 insecticide-resistant and-susceptible *Plutella xylostella* under normal and high
612 temperatures. *Bull. Entomol. Res.*, 106, 378–386.

613 Desneux, N., Decourtye, A. & Delpuech, J.-M. (2007). The sublethal effects of pesticides on
614 beneficial arthropods. *Annu Rev Entomol*, 52, 81–106.
615 Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., *et*
616 *al.* (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc.*
617 *Natl. Acad. Sci. U. S. A.*, 105, 6668–6672.
618 Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C., Huey, R.B., *et al.*
619 (2018). Increase in crop losses to insect pests in a warming climate. *Science*, 361, 916–
620 919.
621 Dougherty, L.R., Frost, F., Maenpaa, M.I., Rowe, M., Cole, B.J., Vasudeva, R., *et al.* (2024).
622 A systematic map of studies testing the relationship between temperature and animal
623 reproduction. *Ecol. Solut. Evid.*, 5, e12303.
624 Douglas, A.E. (2009). The microbial dimension in insect nutritional ecology. *Funct. Ecol.*, 23,
625 38–47.
626 Dunbar, H.E., Wilson, A.C.C., Ferguson, N.R. & Moran, N.A. (2007). Aphid thermal tolerance
627 is governed by a point mutation in bacterial symbionts. *PLoS Biol.*, 5, e96.
628 Fasolo, A.G. & Krebs, R.A. (2004). A comparison of behavioural change in *Drosophila* during
629 exposure to thermal stress. *Biol. J. Linn. Soc.*, 83, 197–205.
630 Fathy, R.F. (2024). Divergent perspectives on the synergistic impacts of thermal-chemical
631 stress on aquatic biota within the framework of climate change scenarios.
632 *Chemosphere*, 355, 141810.
633 Feldhaar, H. (2011). Bacterial symbionts as mediators of ecologically important traits of insect
634 hosts. *Ecol. Entomol.*, 36, 533–543.
635 Ferrer, A., Dorn, S. & Mazzi, D. (2013). Cross-generational effects of temperature on flight
636 performance, and associated life-history traits in an insect. *J. Evol. Biol.*, 26, 2321–
637 2330.

638 Fitzpatrick, J.L. & Lüpold, S. (2014). Sexual selection and the evolution of sperm quality. *Mol.*
639 *Hum. Reprod.*, 20, 1180–1189.
640 Folt, C., Chen, C., Moore, M. & Burnaford, J. (1999). Synergism and antagonism among
641 multiple stressors. *Limnol. Oceanogr.*, 44, 864–877.
642 Fournier-Level, A., Neumann-Mondlak, A., Good, R., Green, L., Schmidt, J.M. & Robin, C.
643 (2016). Behavioural response to combined insecticide and temperature stress in natural
644 populations of *Drosophila melanogaster*. *J. Evol. Biol.*, 29, 1030–1044.
645 Frantzios, G., Paptsiki, K., Sidiropoulou, B., Lazaridis, I., Theophilidis, G. & Mavragani-
646 Tsipidou, P. (2008). Evaluation of insecticidal and genotoxic effects of imidacloprid
647 and acetochlor in *Drosophila melanogaster*. *J. Appl. Entomol.*, 132, 583–590.
648 Freilich, S., Zarecki, R., Eilam, O., Segal, E.S., Henry, C.S., Kupiec, M., *et al.* (2011).
649 Competitive and cooperative metabolic interactions in bacterial communities. *Nat.*
650 *Commun.*, 2, 589.
651 Gandara, A.C.P. & Drummond-Barbosa, D. (2022). Warm and cold temperatures have distinct
652 germline stem cell lineage effects during *Drosophila oogenesis*. *Development*, 149,
653 dev200149.
654 Gandara, A.C.P. & Drummond-Barbosa, D. (2023). Chronic exposure to warm temperature
655 causes low sperm abundance and quality in *Drosophila melanogaster*. *Sci. Rep.*, 13,
656 12331.
657 Gardner, J.L., Peters, A., Kearney, M.R., Joseph, L. & Heinsohn, R. (2011). Declining body
658 size: a third universal response to warming? *Trends Ecol. Evol.*, 26, 285–291.
659 Ge, L., Huang, L., Yang, G., Song, Q., Stanley, D., Gurr, G., *et al.* (2013). Molecular basis for
660 insecticide-enhanced thermotolerance in the brown planthopper *Nilaparvata lugens*
661 *Stål* (Hemiptera: Delphacidae). *Mol. Ecol.*, 22, 5624–5634.

662 Gould, A.L., Zhang, V., Lamberti, L., Jones, E.W., Obadia, B., Korasidis, N., *et al.* (2018).
663 Microbiome interactions shape host fitness. *Proc. Natl. Acad. Sci.*, 115, E11951–
664 E11960.
665 Gruntenko, N.E., Ilinsky, Y.Yu., Adonyeva, N.V., Burdina, E.V., Bykov, R.A., Menshanov,
666 P.N., *et al.* (2017). Various *Wolbachia* genotypes differently influence host *Drosophila*
667 dopamine metabolism and survival under heat stress conditions. *BMC Evol. Biol.*, 17,
668 252.
669 Gu, X., Tian, S., Wang, D., Fei, G. & Wei, H. (2010). Interaction between short-term heat
670 pretreatment and fipronil on 2nd instar larvae of diamondback moth, *Plutella xylostella*
671 (Linn). *Dose-Response*, 8, 331-346.
672 Guilhot, R., Xuéreb, A. & Fellous, S. (2025). Transmission of yeast and bacterial symbionts
673 between sexual partners in *Drosophila suzukii* and *Drosophila melanogaster*. *R. Soc.*
674 *Open Sci.*, 12, 241149.
675 Guillaume, A.S., Monro, K. & Marshall, D.J. (2016). Transgenerational plasticity and
676 environmental stress: do paternal effects act as a conduit or a buffer? *Funct. Ecol.*, 30,
677 1175–1184.
678 Hallman, T.A. & Brooks, M.L. (2015). The deal with diel: Temperature fluctuations,
679 asymmetrical warming, and ubiquitous metals contaminants. *Environ. Pollut. Barking*
680 *Essex 1987*, 206, 88–94.
681 He, Y., Zhao, J., Wu, D., Wyckhuys, K.A. & Wu, K. (2011). Sublethal effects of imidacloprid
682 on *Bemisia tabaci* (Hemiptera: Aleyrodidae) under laboratory conditions. *J. Econ.*
683 *Entomol.*, 104, 833–838.
684 Heino, J., Virkkala, R. & Toivonen, H. (2009). Climate change and freshwater biodiversity:
685 detected patterns, future trends and adaptations in northern regions. *Biol. Rev.*, 84, 39–
686 54.

687 Henry, Y., Overgaard, J. & Colinet, H. (2020). Dietary nutrient balance shapes phenotypic
688 traits of *Drosophila melanogaster* in interaction with gut microbiota. *Comp. Biochem.*
689 *Physiol. A. Mol. Integr. Physiol.*, 241, 110626.
690 Hoekstra, L.A., Siddiq, M.A. & Montooth, K.L. (2013). Pleiotropic effects of a mitochondrial–
691 nuclear incompatibility depend upon the accelerating effect of temperature in
692 *Drosophila*. *Genetics*, 195, 1129–1139.
693 Holmstrup, M., Bindesbøl, A.-M., Oostingh, G.J., Duschl, A., Scheil, V., Köhler, H.-R., *et al.*
694 (2010). Interactions between effects of environmental chemicals and natural stressors:
695 a review. *Sci. Total Environ.*, 408, 3746–3762.
696 Hooper, M.J., Ankley, G.T., Cristol, D.A., Maryoung, L.A., Noyes, P.D. & Pinkerton, K.E.
697 (2013). Interactions between chemical and climate stressors: A role for mechanistic
698 toxicology in assessing climate change risks. *Environ. Toxicol. Chem.*, 32, 32–48.
699 Ihara, M., D. Buckingham, S., Matsuda, K. & B. Sattelle, D. (2017). Modes of action, resistance
700 and toxicity of insecticides targeting nicotinic acetylcholine receptors. *Curr. Med.*
701 *Chem.*, 24, 2925–2934.
702 Iossa, G. (2019). Sex-specific differences in thermal fertility Limits. *Trends Ecol. Evol.*, 34,
703 490–492.
704 Jaramillo, A. & Castañeda, L.E. (2021). Gut microbiota of *Drosophila subobscura* contributes
705 to its heat tolerance and is sensitive to transient thermal stress. *Front. Microbiol.*, 12,
706 654108.
707 Jones, R.M., Desai, C., Darby, T.M., Luo, L., Wolfarth, A.A., Scharer, C.D., *et al.* (2015).
708 *Lactobacilli* modulate epithelial cytoprotection through the Nrf2 pathway. *Cell Rep.*,
709 12, 1217–1225.

710 Jutfelt, F., Norin, T., Åsheim, E.R., Rowsey, L.E., Andreassen, A.H., Morgan, R., *et al.* (2021).
711 'Aerobic scope protection'reduces ectotherm growth under warming. *Funct. Ecol.*, 35,
712 1397–1407.
713 Kang, Z.-W., Liu, F.-H., Pang, R.-P., Tian, H.-G. & Liu, T.-X. (2018). Effect of sublethal doses
714 of imidacloprid on the biological performance of aphid endoparasitoid *Aphidius*
715 *gifuensis* (Hymenoptera: Aphidiidae) and influence on its related gene expression.
716 *Front. Physiol.*, 9, 1729.
717 Karimzadeh, R., Javanshir, M. & Hejazi, M.J. (2020). Individual and combined effects of
718 insecticides, inert dusts and high temperatures on *Callosobruchus maculatus*
719 (Coleoptera: Chrysomelidae). *J. Stored Prod. Res.*, 89, 101693.
720 Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K. & Fukatsu, T. (2012).
721 Symbiont-mediated insecticide resistance. *Proc. Natl. Acad. Sci.*, 109, 8618–8622.
722 Kim, H.Y., Yu, S., Jeong, T. & Kim, S.D. (2014). Relationship between trans-generational
723 effects of tetracycline on *Daphnia magna* at the physiological and whole organism
724 level. *Environ. Pollut.*, 191, 111–118.
725 Kimberly, D.A. & Salice, C.J. (2015). Multigenerational contaminant exposures produce non-
726 monotonic, transgenerational responses in *Daphnia magna*. *Environ. Pollut. Barking*
727 *Essex 1987*, 207, 176–182.
728 Kokou, F., Sasson, G., Nitzan, T., Doron-Faigenboim, A., Harpaz, S., Cnaani, A., *et al.* (2018).
729 Host genetic selection for cold tolerance shapes microbiome composition and
730 modulates its response to temperature. *elife*, 7, e36398.
731 Kooijman, S.A.L.M. (2000). *Dynamic energy and mass budgets in biological systems*.
732 Cambridge University Press.

Koyle, M.L., Veloz, M., Judd, A.M., Wong, A.C.-N., Newell, P.D., Douglas, A.E., *et al.* (2016). Rearing the fruit fly *Drosophila melanogaster* under axenic and gnotobiotic conditions. *JoVE J. Vis. Exp.*, e54219.

Lenth, R.V. (2025). emmeans: Estimated marginal means, aka least-squares means. R package version 1.11.1, <https://CRAN.R-project.org/package=emmeans>.

Levy, R. & Borenstein, E. (2013). Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules. *Proc. Natl. Acad. Sci.*, 110, 12804–12809.

Li, S., Tan, H.-Y., Wang, N., Zhang, Z.-J., Lao, L., Wong, C.-W., *et al.* (2015). The role of oxidative stress and antioxidants in liver diseases. *Int. J. Mol. Sci.*, 16, 26087–26124.

Li, W., Lu, Z., Li, L., Yu, Y., Dong, S., Men, X., *et al.* (2018). Sublethal effects of imidacloprid on the performance of the bird cherry-oat aphid *Rhopalosiphum padi*. *PLOS ONE*, 13, e0204097.

Liu, H., He, J., Chi, C. & Shao, J. (2014). Differential HSP70 expression in *Mytilus coruscus* under various stressors. *Gene*, 543, 166–173.

Lüdecke, D., Ben-Shachar, M., Patil, I., Waggoner, P. & Makowski, D. (2021). "performance: An R package for assessment, comparison and testing of statistical models." *J. Open Source Softw.*, 6, 3139.

Lüpold, S., Manier, M.K., Berben, K.S., Smith, K.J., Daley, B.D., Buckley, S.H., *et al.* (2012). How multivariate ejaculate traits determine competitive fertilization success in *Drosophila melanogaster*. *Curr. Biol.*, 22, 1667–1672.

Lüpold, S., Reil, J.B., Manier, M.K., Zeender, V., Belote, J.M. & Pitnick, S. (2020). How female × male and male × male interactions influence competitive fertilization in *Drosophila melanogaster*. *Evol. Lett.*, 4, 416–429.

757 Macaulay, S.J., Buchwalter, D.B. & Matthaei, C.D. (2020). Water temperature interacts with
758 the insecticide imidacloprid to alter acute lethal and sublethal toxicity to mayfly larvae.
759 *N. Z. J. Mar. Freshw. Res.*, 54, 115–130.
760 Maggu, K., Meena, A., De Nardo, A.N., Sbilordo, S.H., Roy, J. & Lüpold, S. (2025). Male
761 gut microbiome mediates postmating sexual selection in *Drosophila*. *bioRxiv*. doi:
762 10.1101/2025.05.23.655737.
763 Magnúsdóttir, S., Heinken, A., Kutt, L., Ravcheev, D.A., Bauer, E., Noronha, A., *et al.* (2017).
764 Generation of genome-scale metabolic reconstructions for 773 members of the human
765 gut microbiota. *Nat. Biotechnol.*, 35, 81–89.
766 Manzi, C., Vergara-Amado, J., Franco, L.M. & Silva, A.X. (2020). The effect of temperature
767 on candidate gene expression in the brain of honey bee *Apis mellifera* (Hymenoptera:
768 Apidae) workers exposed to neonicotinoid imidacloprid. *J. Therm. Biol.*, 93, 102696.
769 Mao, K., Jin, R., Li, W., Ren, Z., Qin, X., He, S., *et al.* (2019). The influence of temperature
770 on the toxicity of insecticides to *Nilaparvata lugens* (Stål). *Pestic. Biochem. Physiol.*,
771 156, 80–86.
772 Marshall, D.J., Heppell, S.S., Munch, S.B. & Warner, R.R. (2010). The relationship between
773 maternal phenotype and offspring quality: do older mothers really produce the best
774 offspring? *Ecology*, 91, 2862–2873.
775 Martelli, F., Zhongyuan, Z., Wang, J., Wong, C.-O., Karagas, N.E., Roessner, U., *et al.* (2020).
776 Low doses of the neonicotinoid insecticide imidacloprid induce ROS triggering
777 neurological and metabolic impairments in *Drosophila*. *Proc. Natl. Acad. Sci.*, 117,
778 25840–25850.
779 Massot, M., Bagni, T., Maria, A., Couzi, P., Drozdz, T., Malbert-Colas, A., *et al.* (2021).
780 Combined influences of transgenerational effects, temperature and insecticide on the
781 moth *Spodoptera littoralis*. *Environ. Pollut.*, 289, 117889.

782 Meena, A., De Nardo, A.N., Maggu, K., Sbilordo, S.H., Roy, J., Snook, R.R., *et al.* (2024a).
783 Fertility loss and recovery dynamics after repeated heat stress across life stages in male
784 *Drosophila melanogaster*: patterns and processes. *R. Soc. Open Sci.*, 11, 241082.
785 Meena, A., Maggu, K., De Nardo, A.N., Sbilordo, S.H., Eggs, B., Al Toma Sho, R., *et al.*
786 (2024b). Life stage-specific effects of heat stress on spermatogenesis and oogenesis in
787 *Drosophila melanogaster*. *J. Therm. Biol.*, 125, 104001.
788 Meng, S., Tran, T.T., Van Dinh, K., Delnat, V. & Stoks, R. (2022). Acute warming increases
789 pesticide toxicity more than transgenerational warming by reducing the energy budget.
790 *Sci. Total Environ.*, 805, 150373.
791 Moeller, A.H., Ivey, K., Cornwall, M.B., Herr, K., Rede, J., Taylor, E.N., *et al.* (2020). The
792 lizard gut microbiome changes with temperature and is associated with heat tolerance.
793 *Appl. Environ. Microbiol.*, 86, e01181-20.
794 Moghadam, N.N., Thorshauge, P.M., Kristensen, T.N., de Jonge, N., Bahrndorff, S., Kjeldal,
795 H., *et al.* (2018). Strong responses of *Drosophila melanogaster* microbiota to
796 developmental temperature. *Fly (Austin)*, 12, 1–12.
797 Montllor, C.B., Maxmen, A. & Purcell, A.H. (2002). Facultative bacterial endosymbionts
798 benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecol. Entomol.*, 27, 189–195.
799 Moore, M.P., Whiteman, H.H. & Martin, R.A. (2019). A mother's legacy: the strength of
800 maternal effects in animal populations. *Ecol. Lett.*, 22, 1620–1628.
801 Moreira, D.R., de Souza, T.H.S., Galhardo, D., Puentes, S.M.D., Figueira, C.L., Silva, B.G. da,
802 *et al.* (2022). Imidacloprid induces histopathological damage in the midgut, ovary, and
803 spermathecal stored spermatozoa of queens after chronic colony exposure. *Environ.*
804 *Toxicol. Chem.*, 41, 1637–1648.
805 Morgan, H.L. & Watkins, A.J. (2019). Transgenerational impact of environmental change. In:
806 *Reproductive Sciences in Animal Conservation*, Advances in Experimental Medicine

807 and Biology (eds. Comizzoli, P., Brown, J.L. & Holt, W.V.). Springer International
808 Publishing, Cham, pp. 71–89.
809 Mousseau, T.A., Uller, T., Wapstra, E. & Badyaev, A.V. (2009). Evolution of maternal effects:
810 past and present. *Philos. Trans. R. Soc. B Biol. Sci.*, 364, 1035–1038.
811 Navarro-Ortega, A., Acuña, V., Bellin, A., Burek, P., Cassiani, G., Choukr-Allah, R., *et al.*
812 (2015). Managing the effects of multiple stressors on aquatic ecosystems under water
813 scarcity. The GLOBAQUA project. *Sci. Total Environ.*, 503–504, 3–9.
814 Neven, L.G. (2000). Physiological responses of insects to heat. *Postharvest Biol. Technol.*, 21,
815 103–111.
816 Newell, P.D. & Douglas, A.E. (2014). Interspecies interactions determine the impact of the gut
817 microbiota on nutrient allocation in *Drosophila melanogaster*. *Appl. Environ.*
818 *Microbiol.*, 80, 788–796.
819 Noecker, C., Chiu, H.-C., McNally, C.P. & Borenstein, E. (2019). Defining and evaluating
820 microbial contributions to metabolite variation in microbiome-metabolome association
821 studies. *mSystems*, 4, 10–1128.
822 Noyes, P.D. & Lema, S.C. (2015). Forecasting the impacts of chemical pollution and climate
823 change interactions on the health of wildlife. *Curr. Zool.*, 61, 669–689.
824 Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L.A., Walcott, K.C., *et al.*
825 (2009). The toxicology of climate change: environmental contaminants in a warming
826 world. *Environ. Int.*, 35, 971–986.
827 Obadia, B., Güvener, Z.T., Zhang, V., Ceja-Navarro, J.A., Brodie, E.L., William, W.J., *et al.*
828 (2017). Probabilistic invasion underlies natural gut microbiome stability. *Curr. Biol.*,
829 27, 1999–2006.
830 Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., *et al.* (2025).
831 vegan: Community ecology package. R package version 2.7-1.

832 Osborne, B., Siboni, N., Seymour, J.R., Ralph, P. & Pernice, M. (2023). Exploring the potential
833 of algae-bacteria interactions in the biocontrol of the marine pathogen *Vibrio*
834 *parahaemolyticus*. *J. Appl. Phycol.*, 35, 2731–2743.
835 Overgaard, J., Malmendal, A., Sørensen, J.G., Bundy, J.G., Loeschcke, V., Nielsen, N.C., *et*
836 *al.* (2007). Metabolomic profiling of rapid cold hardening and cold shock in *Drosophila*
837 *melanogaster*. *J. Insect Physiol.*, 53, 1218–1232.
838 Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C., *et al.*
839 (2013). Temperature variation makes ectotherms more sensitive to climate change.
840 *Glob. Change Biol.*, 19, 2373–2380.
841 Pang, R., Chen, M., Yue, L., Xing, K., Li, T., Kang, K., *et al.* (2018). A distinct strain of
842 Arsenophonus symbiont decreases insecticide resistance in its insect host. *PLoS Genet.*,
843 14, e1007725.
844 Parker, G.A. & Pizzari, T. (2010). Sperm competition and ejaculate economics. *Biol. Rev.*, 85,
845 897–934.
846 Piggott, J.J., Niyogi, D.K., Townsend, C.R. & Matthaei, C.D. (2015a). Multiple stressors and
847 stream ecosystem functioning: climate warming and agricultural stressors interact to
848 affect processing of organic matter. *J. Appl. Ecol.*, 52, 1126–1134.
849 Piggott, J.J., Townsend, C.R. & Matthaei, C.D. (2015b). Climate warming and agricultural
850 stressors interact to determine stream macroinvertebrate community dynamics. *Glob.*
851 *Change Biol.*, 21, 1887–1906.
852 Pilakouta, N. & Baillet, A. (2022). Effects of temperature on mating behaviour and mating
853 success: A meta-analysis. *J. Anim. Ecol.*, 91, 1642–1650.
854 Pizzari, T. & Parker, G.A. (2009). Sperm competition and sperm phenotype. In: *Sperm Biology*.
855 Elsevier, pp. 207–245.

856 Pölkki, M., Kangassalo, K. & Rantala, M.J. (2012). Transgenerational effects of heavy metal
857 pollution on immune defense of the blow fly *Protophormia terraenovae*. *PLoS One*, 7,
858 e38832.
859 Pörtner, H.-O., Bock, C. & Mark, F.C. (2017). Oxygen-and capacity-limited thermal tolerance:
860 bridging ecology and physiology. *J. Exp. Biol.*, 220, 2685–2696.
861 Przeslawski, R., Byrne, M. & Mellin, C. (2015). A review and meta-analysis of the effects of
862 multiple abiotic stressors on marine embryos and larvae. *Glob. Change Biol.*, 21, 2122–
863 2140.
864 Reátegui-Zirena, E.G., Fidder, B.N., Olson, A.D., Dawson, D.E., Bilbo, T.R. & Salice, C.J.
865 (2017). Transgenerational endpoints provide increased sensitivity and insight into
866 multigenerational responses of *Lymnaea stagnalis* exposed to cadmium. *Environ.*
867 *Pollut.*, 224, 572–580.
868 Renoz, F., Pons, I. & Hance, T. (2019). Evolutionary responses of mutualistic insect–bacterial
869 symbioses in a world of fluctuating temperatures. *Curr. Opin. Insect Sci.*, 35, 20–26.
870 Ricupero, M., Abbes, K., Haddi, K., Kurtulus, A., Desneux, N., Russo, A., *et al.* (2020).
871 Combined thermal and insecticidal stresses on the generalist predator *Macrolophus*
872 *pygmaeus*. *Sci. Total Environ.*, 729, 138922.
873 Ridley, E.V., Wong, A.C. & Douglas, A.E. (2013). Microbe-dependent and nonspecific effects
874 of procedures to eliminate the resident microbiota from *Drosophila melanogaster*. *Appl.*
875 *Environ. Microbiol.*, 79, 3209–3214.
876 Ridley, E.V., Wong, A.C., Westmiller, S. & Douglas, A.E. (2012). Impact of the resident
877 microbiota on the nutritional phenotype of *Drosophila melanogaster*. *PloS One*, 7,
878 e36765.
879 Roy, P., Phukan, P.K., Changmai, D. & Boruah, S. (2017). Pesticides, insecticides and male
880 infertility. *Int J Reprod Contracept Obs Gynecol*, 6, 3387–3391.

881 Russell, J.A. & Moran, N.A. (2006). Costs and benefits of symbiont infection in aphids:
882 variation among symbionts and across temperatures. *Proc. Biol. Sci.*, 273, 603–610.
883 Sales, K., Thomas, P., Gage, M.J. & Vasudeva, R. (2024). Experimental heatwaves reduce the
884 effectiveness of ejaculates at occupying female reproductive tracts in a model insect. *R.*
885 *Soc. Open Sci.*, 11, 231949.
886 Sales, K., Vasudeva, R., Dickinson, M.E., Godwin, J.L., Lumley, A.J., Michalczyk, Ł., *et al.*
887 (2018). Experimental heatwaves compromise sperm function and cause
888 transgenerational damage in a model insect. *Nat. Commun.*, 9, 4771.
889 Schultz, C.L., Wamucho, A., Tsyusko, O.V., Unrine, J.M., Crossley, A., Svendsen, C., *et al.*
890 (2016). Multigenerational exposure to silver ions and silver nanoparticles reveals
891 heightened sensitivity and epigenetic memory in *Caenorhabditis elegans*. *Proc. R. Soc.*
892 *B Biol. Sci.*, 283, 20152911.
893 Schwasinger-schmidt, T.E., Kachman, S.D. & Harshman, L.G. (2012). Evolution of starvation
894 resistance in *Drosophila melanogaster*: measurement of direct and correlated responses
895 to artificial selection. *J. Evol. Biol.*, 25, 378–387.
896 Segner, H., Schmitt-Jansen, M. & Sabater, S. (2014). Assessing the impact of multiple stressors
897 on aquatic biota: The receptor's side matters. *Environ. Sci. Technol.*, 48, 7690–7696.
898 Semsar-Kazerouni, M., Boerrigter, J.G. & Verberk, W.C. (2020). Changes in heat stress
899 tolerance in a freshwater amphipod following starvation: The role of oxygen
900 availability, metabolic rate, heat shock proteins and energy reserves. *Comp. Biochem.*
901 *Physiol. A. Mol. Integr. Physiol.*, 245, 110697.
902 Sepulveda, J. & Moeller, A.H. (2020). The effects of temperature on animal gut microbiomes.
903 *Front. Microbiol.*, 11, 384.
904 Shahid, N., Siddique, A. & Liess, M. (2024). Synergistic interaction between a toxicant and
905 food stress is further exacerbated by temperature. *Environ. Pollut.*, 363, 125109.

Shama, L.N., Strobel, A., Mark, F.C. & Wegner, K.M. (2014). Transgenerational plasticity in marine sticklebacks: maternal effects mediate impacts of a warming ocean. *Funct. Ecol.*, 28, 1482–1493.

Shokal, U., Yadav, S., Atri, J., Accetta, J., Kenney, E., Banks, K., *et al.* (2016). Effects of co-occurring *Wolbachia* and *Spiroplasma* endosymbionts on the *Drosophila* immune response against insect pathogenic and non-pathogenic bacteria. *BMC Microbiol.*, 16, 16.

Silva, A.P.N., Andrade, E.S., Nascimento, V.L. & Haddi, K. (2025). Thermal modulation of insecticide-induced hormetic and oxidative responses in insect pests. *Chemosphere*, 370, 143920.

Silva, A.S.J., Kristiansen, S.M., Sengupta, S., van Gestel, C.A., Leinaas, H.P. & Borgå, K. (2023). Using dietary exposure to determine sub-lethal effects from imidacloprid in two springtail (*Collembola*) species. *Ecotoxicology*, 32, 1209–1220.

Simmons, L.W. & Fitzpatrick, J.L. (2012). Sperm wars and the evolution of male fertility. *Reproduction*, 144, 519–534.

Siviter, H., Bailes, E.J., Martin, C.D., Oliver, T.R., Koricheva, J., Leadbeater, E., *et al.* (2021). Agrochemicals interact synergistically to increase bee mortality. *Nature*, 596, 389–392.

Sokolova, I.M. (2013). Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integr. Comp. Biol.*, 53, 597–608.

Sørensen, J.G., Kristensen, T.N. & Loeschcke, V. (2003). The evolutionary and ecological role of heat shock proteins. *Ecol. Lett.*, 6, 1025–1037.

Stehle, S. & Schulz, R. (2015). Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci.*, 112, 5750–5755.

Tran, T.T., Janssens, L., Dinh, K.V. & Stoks, R. (2018). Transgenerational interactions between pesticide exposure and warming in a vector mosquito. *Evol. Appl.*, 11, 906–917.

Uller, T., Nakagawa, S. & English, S. (2013). Weak evidence for anticipatory parental effects in plants and animals. *J. Evol. Biol.*, 26, 2161–2170.

Verberk, W.C.E.P., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L., *et al.* (2016). Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.*, 192, 64–78.

Verheyen, J. & Stoks, R. (2019). Current and future daily temperature fluctuations make a pesticide more toxic: Contrasting effects on life history and physiology. *Environ. Pollut.*, 248, 209–218.

Verheyen, J. & Stoks, R. (2023). Thermal performance curves in a polluted world: too cold and too hot temperatures synergistically increase pesticide toxicity. *Environ. Sci. Technol.*, 57, 3270–3279.

Walsh, B.S., Parratt, S.R., Atkinson, D., Snook, R.R., Bretman, A. & Price, T.A. (2019). Integrated approaches to studying male and female thermal fertility limits. *Trends Ecol. Evol.*, 34, 492–493.

Walsh, M.R., Whittington, D. & Funkhouser, C. (2014). Thermal transgenerational plasticity in natural populations of *Daphnia*.

Wan, N.-F., Fu, L., Dainese, M., Kiær, L.P., Hu, Y.-Q., Xin, F., *et al.* (2025). Pesticides have negative effects on non-target organisms. *Nat. Commun.*, 16, 1360.

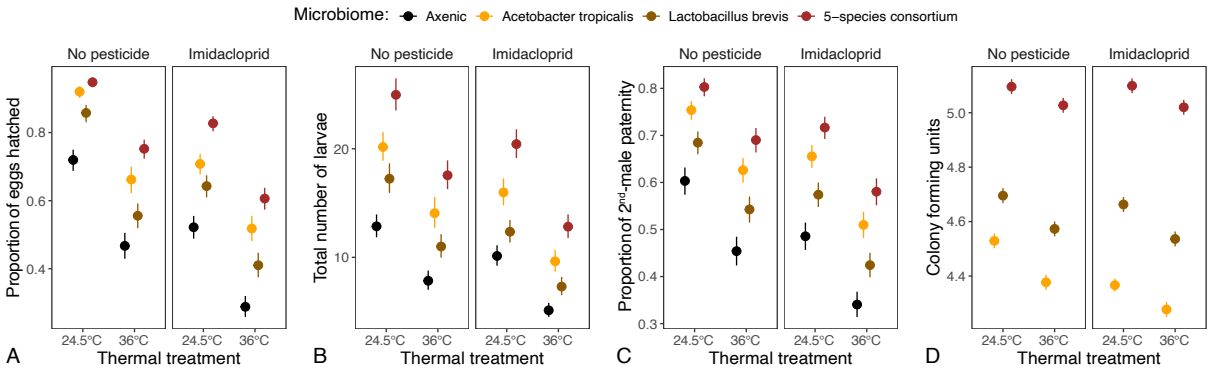
Wang, X., Ma, D., Jin, Q., Deng, S., Stančin, H., Tan, H., *et al.* (2019). Synergistic effects of biomass and polyurethane co-pyrolysis on the yield, reactivity, and heating value of biochar at high temperatures. *Fuel Process. Technol.*, 194, 106127.

953 Wang, Y.-C., Chang, Y.-W., Bai, J., Zhang, X.-X., Iqbal, J., Lu, M.-X., *et al.* (2021a). High
954 temperature stress induces expression of CYP450 genes and contributes to insecticide
955 tolerance in *Liriomyza trifolii*. *Pestic. Biochem. Physiol.*, 174, 104826.
956 Wang, Y.-C., Chang, Y.-W., Bai, J., Zhang, X.-X., Iqbal, J., Lu, M.-X., *et al.* (2021b).
957 Temperature affects the tolerance of *Liriomyza trifolii* to insecticide abamectin.
958 *Ecotoxicol. Environ. Saf.*, 218, 112307.
959 Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer, New York.
960 Xiao, D., Zhao, J., Guo, X., Chen, H., Qu, M., Zhai, W., *et al.* (2016). Sublethal effects of
961 imidacloprid on the predatory seven-spot ladybird beetle *Coccinella septempunctata*.
962 *Ecotoxicology*, 25, 1782–1793.
963 Yu, C.-W. & Liao, V.H.-C. (2016). Transgenerational Reproductive effects of arsenite are
964 associated with H3K4 dimethylation and SPR-5 downregulation in *Caenorhabditis*
965 *elegans*. *Environ. Sci. Technol.*, 50, 10673–10681.
966 Zelezniak, A., Andrejev, S., Ponomarova, O., Mende, D.R., Bork, P. & Patil, K.R. (2015).
967 Metabolic dependencies drive species co-occurrence in diverse microbial communities.
968 *Proc. Natl. Acad. Sci.*, 112, 6449–6454.
969 Zhang, X., Yu, X. & Chen, J. (2008). High temperature effects on yeast-like endosymbiotes
970 and pesticide resistance of the small brown planthopper, *Laodelphax striatellus*. *Rice*
971 *Sci.*, 15, 326–330.
972 Zhang, Y., Cai, T., Ren, Z., Liu, Y., Yuan, M., Cai, Y., *et al.* (2021a). Decline in symbiont-
973 dependent host detoxification metabolism contributes to increased insecticide
974 susceptibility of insects under high temperature. *ISME J.*, 15, 3693–3703.
975 Zhang, Y., Xu, G., Jiang, Y., Ma, C. & Yang, G. (2021b). Sublethal effects of Imidacloprid on
976 fecundity, apoptosis and virus transmission in the small brown planthopper *Laodelphax*
977 *striatellus*. *Insects*, 12, 1131.

Zhou, C., Liu, L., Yang, H., Wang, Z., Long, G. & Jin, D. (2017). Sublethal effects of imidacloprid on the development, reproduction, and susceptibility of the white-backed planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae). *J. Asia-Pac. Entomol.*, 20, 996–1000.

Zwoinska, M.K., Rodrigues, L.R., Slate, J. & Snook, R.R. (2020). Phenotypic responses to and genetic architecture of sterility following exposure to sub-lethal temperature during development. *Front. Genet.*, 11, 573.

986



987 **Figure 1** Effects of varying thermal and pesticide conditions and microbiome types on (A)
988 hatching success, (B) male-mediated female fecundity, and (C) competitive fertilization
989 success. (D) Bacterial abundance/colony forming units of different microbial treatments in
990 response to varying thermal and pesticide conditions. Each circle represents the mean of all
991 replicates in that treatment combination with error bars corresponding to 95% confidence
992 intervals.

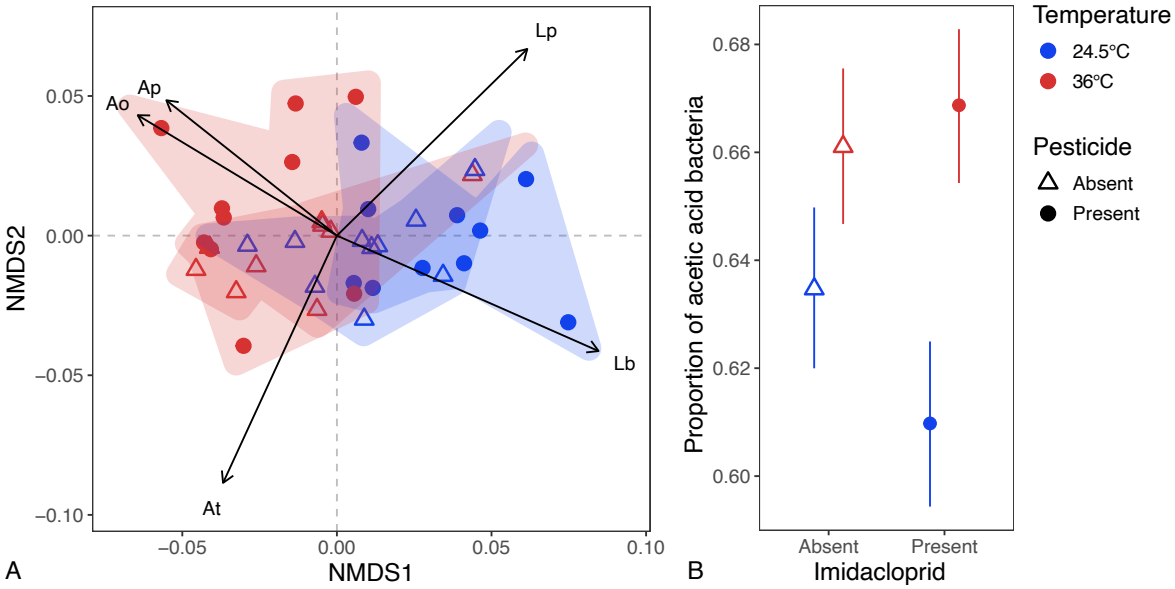


Figure 2 Bacterial abundances in the five-species consortium in response to thermal and pesticide treatments. A: Non-metric multidimensional scaling (NMDS) ordination (Bray-Curtis distance, $k = 4$, stress = 0.046) of microbial community composition across temperature and pesticide treatments. Shaded convex hulls denote 95% confidence areas for each temperature × pesticide group, and arrows the direction and strength of species (Hellinger-transformed) most strongly correlated with the ordination space, as identified by *envfit* (species abbreviations: Ao = *Acetobacter orientalis*, Ap = *A. pasteuriensis*, At = *A. tropicalis*, Lb = *Lactobacillus brevis*, Lp = *L. plantarum*). B: Proportion of acetic acid bacteria in the five-species consortium in response to heat and pesticide stress. Each circle represents the mean of all replicates in that treatment combination with error bars corresponding to 95% confidence intervals.

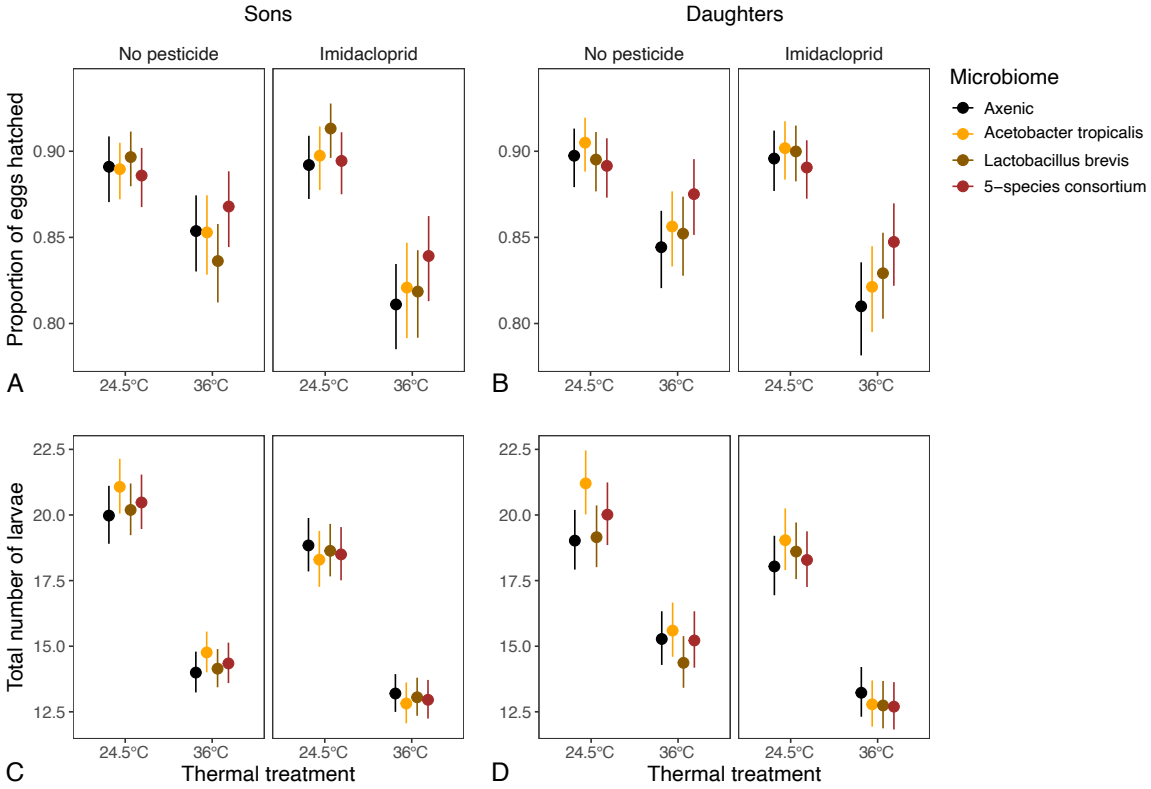


Figure 3 Fitness consequences of paternal thermal and pesticide treatments on fitness measures in male (A, C) and female (B, D) offspring. Panels A and B depict the proportion of eggs hatched out of total number of eggs fertilized, panels C and D the fecundity either of the sons' mates (C) or of the daughters (D). Each circle represents the mean of all the replicates in that treatment combination with error bars corresponding to 95% confidence intervals.

Supplementary material

Table S1: Results from a generalized linear model on hatching success. The model assessed the impact of male microbiome (axenic, *Acetobacter tropicalis*, *Lactobacillus brevis*, control), temperature (24.5°C/36°C), and pesticide exposure (absent/present). Total sample size: $N = 650$.

Predictor	*df*	χ^2	*P*
Microbiome	3	215.696	**<0.001**
Temperature	1	93.332	**<0.001**
Pesticide	1	67.065	**<0.001**
Block	1	6.955	**0.008**
Microbiome × Temperature	3	22.007	**<0.001**
Microbiome × Pesticide	3	18.286	**<0.001**
Temperature × Pesticide	1	0.292	0.589
Microbiome × Temperature × Pesticide	3	15.190	**0.002**

Coefficients of the model described above.

Term	*Estimate*	*SE*	*z*	*P*
(Intercept)	0.889	0.082	10.847	**<0.001**
Microbiome[At]	1.501	0.141	10.624	**<0.001**
Microbiome[Lb]	0.855	0.133	6.442	**<0.001**
Microbiome[Control]	1.960	0.154	12.713	**<0.001**
Temperature[36°C]	-1.074	0.111	-9.661	**<0.001**
Pesticide[Present]	-0.855	0.104	-8.189	**<0.001**
Block[2]	0.107	0.040	2.637	**0.008**
Microbiome[At] × Temperature[36°C]	-0.698	0.184	-3.793	**<0.001**
Microbiome[Lb] × Temperature[36°C]	-0.500	0.171	-2.915	**0.004**
Microbiome[Control] × Temperature[36°C]	-0.717	0.189	-3.796	**<0.001**
Microbiome[At] × Pesticide[Present]	-0.704	0.173	-4.062	**<0.001**
Microbiome[Lb] × Pesticide[Present]	-0.355	0.166	-2.144	**0.032**
Microbiome[Control] × Pesticide[Present]	-0.483	0.186	-2.589	**0.010**
Temperature[36°C] × Pesticide[Present]	0.082	0.151	0.540	0.589
Microbiome[At] × Temperature[36°C] × Pesticide[Present]	0.877	0.236	3.721	**<0.001**
Microbiome[Lb] × Temperature[36°C] × Pesticide[Present]	0.542	0.226	2.400	**0.016**
Microbiome[Control] × Temperature[36°C] × Pesticide[Present]	0.576	0.239	2.405	**0.016**

Table S2: Results from a generalized linear model on the total number of larvae produced. The model assessed the impact of male microbiome (axenic, *Acetobacter tropicalis*, *Lactobacillus brevis*, control), temperature (24.5°C/36°C), and pesticide exposure (absent/present). The three-way interaction was removed due to non-significance ($P = 0.88$). Total sample size: $N = 650$.

Predictor	*df*	χ^2	*P*
Microbiome	3	207.693	**<0.001**
Temperature	1	85.063	**<0.001**
Pesticide	1	23.921	**<0.001**
Block	1	0.718	0.397
Microbiome × Temperature	3	8.223	**0.042**
Microbiome × Pesticide	3	4.264	0.234
Temperature × Pesticide	1	8.061	**0.005**

Coefficients of the model described above.

Term	*Estimate*	*SE*	*z*	*P*
(Intercept)	2.554	0.040	63.187	**<0.001**
Microbiome[At]	0.443	0.049	9.039	**<0.001**
Microbiome[Lb]	0.275	0.053	5.206	**<0.001**
Microbiome[Control]	0.652	0.047	13.873	**<0.001**
Temperature[36°C]	-0.521	0.057	-9.223	**<0.001**
Pesticide[Present]	-0.260	0.053	-4.891	**<0.001**
Block[2]	0.018	0.021	0.848	0.397
Microbiome[At] × Temperature[36°C]	0.152	0.069	2.190	**0.029**
Microbiome[Lb] × Temperature[36°C]	0.094	0.072	1.311	0.190
Microbiome[Control] × Temperature[36°C]	0.175	0.064	2.724	**0.006**
Microbiome[At] × Pesticide[Present]	0.021	0.066	0.318	0.750
Microbiome[Lb] × Pesticide[Present]	-0.054	0.069	-0.788	0.431
Microbiome[Control] × Pesticide[Present]	0.065	0.062	1.042	0.297
Temperature[36°C] × Pesticide[Present]	-0.126	0.045	-2.839	**0.005**

Table S3: Results from a generalized linear model on the proportion of second-male offspring (sperm offense ability). The model assessed the impact of male microbiome (axenic, *Acetobacter tropicalis*, *Lactobacillus brevis*, control), temperature (24.5°C/36°C), and pesticide exposure (absent/present). All interactions were sequentially removed due to non-significance ($P > 0.15$). Total sample size: $N = 331$.

Predictor	*df*	χ^2	*P*
Microbiome	3	218.453	**<0.001**
Temperature	1	162.250	**<0.001**
Pesticide	1	99.596	**<0.001**
Block	1	0.827	0.363

Coefficients of the model described above.

Term	*Estimate*	*SE*	*z*	*P*
(Intercept)	0.397	0.067	5.902	**<0.001**
Microbiome[At]	0.700	0.068	10.320	**<0.001**
Microbiome[Lb]	0.356	0.068	5.252	**<0.001**
Microbiome[Control]	0.985	0.072	13.716	**<0.001**
Temperature[36°C]	-0.604	0.047	-12.738	**<0.001**
Pesticide[Present]	-0.476	0.048	-9.980	**<0.001**
Block[2]	0.043	0.048	0.910	0.363

Table S4: Results from a linear model on colony forming units. The model assessed the impact of male microbiome (axenic, *Acetobacter tropicalis*, *Lactobacillus brevis*, control), temperature (24.5°C/36°C), and pesticide exposure (absent/present). Total sample size: $N = 120$.

Predictor	*df*	*F*	*P*
Temperature	1	135.611	**<0.001**
Pesticide	1	146.557	**<0.001**
Microbiome	2	991.897	**<0.001**
Block	2	0.156	0.856
Temperature × Pesticide	1	11.281	**0.001**
Temperature × Microbiome	2	10.543	**<0.001**
Pesticide × Microbiome	2	43.073	**<0.001**
Temperature × Pesticide × Microbiome	2	4.778	**0.010**
Residuals	106		

Coefficients of the model described above.

Term	*Estimate*	*SE*	*t*	*P*
(Intercept)	4.530	0.010	470.489	**<0.001**
Temperature[36°C]	-0.152	0.013	-11.645	**<0.001**
Pesticide[Present]	-0.163	0.013	-12.106	**<0.001**
Microbiome[Lb]	0.166	0.013	12.706	**<0.001**
Microbiome[Control]	0.567	0.013	43.323	**<0.001**
Block[2]	0.003	0.005	0.510	0.611
Block[3]	-0.006	0.031	-0.187	0.852
Temperature[36°C] × Pesticide[Present]	0.063	0.019	3.359	**0.001**
Temperature[36°C] × Microbiome[Lb]	0.030	0.018	1.624	0.107
Temperature[36°C] × Microbiome[Control]	0.084	0.018	4.532	**<0.001**
Pesticide[Present] × Microbiome[Lb]	0.130	0.019	6.944	**<0.001**
Pesticide[Present] × Microbiome[Control]	0.166	0.019	8.851	**<0.001**
Temperature[36°C] × Pesticide[Present] × Microbiome[Lb]	-0.067	0.026	-2.559	**0.012**
Temperature[36°C] × Pesticide[Present] × Microbiome[Control]	-0.074	0.026	-2.792	**0.006**

Table S5: Results of a PERMANOVA examining the temperature and pesticide effects on relative bacterial abundances (based on colony-forming units) in the five-species consortium.

Predictor	*df*	*Sum of squares*	R^2	*F*	*P*
Temperature	1	0.004	0.263	13.882	**<0.001**
Pesticide	1	<0.001	0.022	1.157	0.314
Block	1	<0.001	0.004	0.202	0.874
Temperature × Pesticide	1	<0.001	0.048	2.553	*0.068*
Residual	35	0.011	0.663		
Total	39	0.017	1.000		

Table S6: Ordination of the Bray-Curtis dissimilarities between the relative abundances of the five bacterial species via non-metric multidimensional scaling (NMDS).

Species	*NMDS1*	*NMDS2*	R^2	*P*
A. tropicalis	-0.384	-0.923	0.920	**<0.001**
A. orientalis	-0.831	0.556	0.601	**<0.001**
A. pasteurianus	-0.751	0.660	0.539	**<0.001**
L. brevis	0.899	-0.439	0.887	**<0.001**
L. plantarum	0.678	0.735	0.826	**<0.001**

Table S7: Results from a binomial generalized linear mixed-effects model on hatching success among the eggs laid by the mates of the sons. The model assessed the impact of male microbiome (axenic, *Acetobacter tropicalis*, *Lactobacillus brevis*, control), temperature (24.5°C/36°C), and pesticide exposure (absent/present), incorporating sire to account for non-independence among siblings. The three-way interaction was removed due to non-significance ($P = 0.48$). Total sample size: $N = 725$.

Predictor	*df*	χ^2	*P*
Microbiome	3	0.903	0.825
Temperature	1	8.359	**0.004**
Pesticide	1	0.008	0.929
Block	1	5.924	**0.015**
Microbiome × Temperature	3	6.380	*0.095*
Microbiome × Pesticide	3	1.681	0.641
Temperature × Pesticide	1	9.451	**0.002**

Random effects variance components from the model described above.

Group	*Term*	*SD*	*Variance*
Sire	(Intercept)	0	0

Fixed effects estimates from the model described above.

Term	*Estimate*	*SE*	*z*	*P*
(Intercept)	2.163	0.103	21.076	**<0.001**
Microbiome[At]	-0.014	0.126	-0.113	0.910
Microbiome[Lb]	0.059	0.127	0.467	0.640
Microbiome[Control]	-0.052	0.127	-0.406	0.685
Temperature[36°C]	-0.337	0.117	-2.891	**0.004**
Pesticide[Present]	0.010	0.118	0.089	0.929
Block[2]	-0.125	0.051	-2.434	**0.015**
Microbiome[At] × Temperature[36°C]	0.008	0.147	0.054	0.957
Microbiome[Lb] × Temperature[36°C]	-0.192	0.143	-1.340	0.180
Microbiome[Control] × Temperature[36°C]	0.170	0.146	1.165	0.244
Microbiome[At] × Pesticide[Present]	0.072	0.147	0.489	0.625
Microbiome[Lb] × Pesticide[Present]	0.183	0.142	1.283	0.199
Microbiome[Control] × Pesticide[Present]	0.076	0.146	0.524	0.600
Temperature[36°C] × Pesticide[Present]	-0.317	0.103	-3.074	**0.002**

Table S8: Results from a binomial generalized linear mixed-effects model on hatching success among the eggs laid by the daughters. The model assessed the impact of male microbiome (axenic, *Acetobacter tropicalis*, *Lactobacillus brevis*, control), temperature (24.5°C/36°C), and pesticide exposure (absent/present), incorporating sire to account for non-independence among siblings. The three-way interaction was removed due to non-significance ($P = 0.87$). Total sample size: $N = 740$.

Predictor	*df*	χ^2	*P*
Microbiome	3	1.500	0.682
Temperature	1	17.866	**<0.001**
Pesticide	1	0.023	0.878
Block	1	7.816	**0.005**
Microbiome × Temperature	3	6.151	0.104
Microbiome × Pesticide	3	0.420	0.936
Temperature × Pesticide	1	4.733	**0.030**

Random effects variance components from the model described above.

Group	*Term*	*SD*	*Variance*
Sire	(Intercept)	0	0

Fixed effects estimates from the model described above.

Term	*Estimate*	*SE*	*z*	*P*
(Intercept)	2.240	0.097	23.120	**<0.001**
Microbiome[At]	0.085	0.126	0.675	0.499
Microbiome[Lb]	-0.024	0.127	-0.188	0.851
Microbiome[Control]	-0.062	0.126	-0.496	0.620
Temperature[36°C]	-0.478	0.113	-4.227	**<0.001**
Pesticide[Present]	-0.018	0.116	-0.153	0.878
Block[2]	-0.143	0.051	-2.796	**0.005**
Microbiome[At] × Temperature[36°C]	0.009	0.144	0.063	0.950
Microbiome[Lb] × Temperature[36°C]	0.085	0.144	0.591	0.555
Microbiome[Control] × Temperature[36°C]	0.319	0.146	2.184	**0.029**
Microbiome[At] × Pesticide[Present]	-0.019	0.143	-0.133	0.894
Microbiome[Lb] × Pesticide[Present]	0.069	0.144	0.480	0.631
Microbiome[Control] × Pesticide[Present]	0.008	0.146	0.054	0.957
Temperature[36°C] × Pesticide[Present]	-0.223	0.103	-2.176	**0.030**

Table S9: Results from a Poisson generalized linear mixed-effects model on the total number of larvae produced by the daughters. The model assessed the impact of male microbiome (axenic, *Acetobacter tropicalis*, *Lactobacillus brevis*, control), temperature (24.5°C/36°C), and pesticide exposure (absent/present), incorporating sire to account for non-independence among siblings. The three-way interaction was removed due to non-significance ($P = 0.22$). Total sample size: $N = 740$.

Predictor	*df*	χ^2	*P*
Microbiome	3	8.911	**0.030**
Temperature	1	29.042	**<0.001**
Pesticide	1	1.776	0.183
Block	1	15.554	**<0.001**
Microbiome × Temperature	3	3.148	0.369
Microbiome × Pesticide	3	2.930	0.403
Temperature × Pesticide	1	6.106	**0.013**

Random effects variance components from the model described above.

Group	*Term*	*SD*	*Variance*
Sire	(Intercept)	0	0

Fixed effects estimates from the model described above.

Term	*Estimate*	*SE*	*z*	*P*
(Intercept)	2.981	0.032	94.536	**<0.001**
Microbiome[At]	0.109	0.041	2.645	**0.008**
Microbiome[Lb]	0.007	0.042	0.162	0.872
Microbiome[Control]	0.051	0.042	1.214	0.225
Temperature[36°C]	-0.219	0.041	-5.389	**<0.001**
Pesticide[Present]	-0.053	0.040	-1.333	0.183
Block[2]	-0.071	0.018	-3.944	**<0.001**
Microbiome[At] × Temperature[36°C]	-0.088	0.052	-1.698	*0.089*
Microbiome[Lb] × Temperature[36°C]	-0.068	0.052	-1.303	0.192
Microbiome[Control] × Temperature[36°C]	-0.054	0.052	-1.038	0.299
Microbiome[At] × Pesticide[Present]	-0.055	0.051	-1.069	0.285
Microbiome[Lb] × Pesticide[Present]	0.024	0.052	0.467	0.641
Microbiome[Control] × Pesticide[Present]	-0.037	0.051	-0.722	0.470
Temperature[36°C] × Pesticide[Present]	-0.091	0.037	-2.471	**0.013**

Table S10: Results from a Poisson generalized linear mixed-effects model on the total number of larvae produced by the mates of the sons. The model assessed the impact of male microbiome (axenic, *Acetobacter tropicalis*, *Lactobacillus brevis*, control), temperature (24.5°C/36°C), and pesticide exposure (absent/present), incorporating sire to account for non-independence among siblings. The interactions were sequentially removed due to non-significance ($P \geq 0.20$). Total sample size: $N = 725$.

Predictor	*df*	χ^2	*P*
Microbiome	3	0.516	0.915
Temperature	1	367.958	**<0.001**
Pesticide	1	26.840	**<0.001**
Block	1	11.132	**<0.001**

Random effects variance components from the model described above.

Group	*Term*	*SD*	*Variance*
Sire	(Intercept)	0	0

Fixed effects estimates from the model described above.

Term	*Estimate*	*SE*	*z*	*P*
(Intercept)	3.044	0.024	128.264	**<0.001**
Microbiome[At]	0.016	0.026	0.589	0.556
Microbiome[Lb]	-0.001	0.026	-0.042	0.967
Microbiome[Control]	0.003	0.026	0.122	0.903
Temperature[36°C]	-0.356	0.019	-19.182	**<0.001**
Pesticide[Present]	-0.095	0.018	-5.181	**<0.001**
Block[2]	-0.061	0.018	-3.337	**<0.001**