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

Komal Maggu*, Abhishek Meena, Alessio N. De Nardo, Benjamin Eggs, Sonja H. Sbilordo, Rawaa Al Toma Sho & Stefan Lüpold*

Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Running title: Microbiome mediation of environmental stress

Keywords: Microbiota, thermal fertility, pesticide exposure, inter-generational effects, fruit fly, stress resistance

Correspondence: komal024maggu@gmail.com, stefan.luepold@uzh.ch

Conflict of interest declaration: We declare that authors have no competing interests.

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; 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
60 (microbiomes) are increasingly recognized as mediators for environmental stress tolerance
61 (Feldhaar 2011; Osborne *et al.* 2023; Sepulveda & Moeller 2020; Shokal *et al.* 2016).
62 Commensal microbes can enhance host thermal performance (Renoz *et al.* 2019; Sepulveda &
63 Moeller 2020), either as obligate or facultative symbionts (Dunbar *et al.* 2007; Gruntenko *et*
64 *al.* 2017; Montllor *et al.* 2002; Russell & Moran 2006; Zhang *et al.* 2008), and a healthy
65 microbiome can bolster insect resistance to insecticides through direct pesticide metabolism or
66 by inducing the expression of host detoxification genes (Cheng *et al.* 2017; Jones *et al.* 2015;
67 Kikuchi *et al.* 2012; Pang *et al.* 2018). Despite progress in studying individual stressors, how
68 microbiomes modulate *combined* effects remains largely unknown, but microbiome disruption

69 by one stressor could amplify susceptibility to another, potentially explaining observed
70 synergisms (Karimzadeh *et al.* 2020; Mao *et al.* 2019; Zhang *et al.* 2008; Zhang, Cai, *et al.*
71 2021).

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

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

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

113 Based on the evidence outlined above, we tested the following five predictions: (1)
114 Combined sublethal heat and imidacloprid exposure will synergistically reduce male
115 reproductive fitness. (2) Gut microbes will partially mitigate the negative impacts of individual
116 and combined stressors on male reproductive fitness compared to germ-free males. (3)
117 Different bacterial compositions will vary in their protective capacity, with the complex
118 community offering greater resilience than either mono-association. (4) Paternal exposure to

individual and combined stressors will negatively impact offspring fitness (cross-generational effects), with potential synergistic amplification. (5) The paternal microbiome composition will influence the magnitude of these cross-generational effects.

Material and Methods

Detailed methods are available in the supplementary information. The following is a concise description of the experimental procedures.

Experimental populations

We used an outbred *D. melanogaster* (LH_m wild-type) population maintained at 25°C, 60% humidity, and a 12h:12h light:dark cycle. To generate axenic (germ-free) focal flies, eggs collected within 6h of oviposition were dechorionated using bleach and ethanol washes, then transferred to sterile glass vials containing autoclaved standard fly food supplemented with ampicillin, kanamycin, erythromycin, and tetracycline. Axenic status of flies was confirmed by plating homogenates on LB and MRS agar and by PCR for bacterial DNA using universal 16S rRNA primers.

Bacterial cultures and gnotobiotic associations

Axenic males were associated with defined gut microbiota isolated from wild-type flies (Obadia *et al.* 2017): mono-associations of *Acetobacter tropicalis* (*At*) or *Lactobacillus brevis* (*Lb*), or a five-species community (*A. tropicalis*, *A. orientalis, A. pasteurianus, L. plantarum, L. brevis*). Bacterial strains were cultured overnight at 30°C in appropriate media (MRS + mannitol and MRS broth), centrifuged, and resuspended in sterile phosphate-buffered saline (PBS) to a concentration of 10^8 cells/mL (Koyle *et al.* 2016; Newell & Douglas 2014).

144 Sterile food vials were inoculated with 50µL of bacterial suspension (approx. 5×10^6 CFUs,
145 with equal representation in the five-species consortium) (Gould *et al.* 2018; Newell & Douglas
146 2014). Ten 1-day-old axenic males were added to each vial and transferred to fresh inoculated
147 vials every 48h for 4d to ensure stable colonization. Successful colonization was confirmed by
148 plating homogenates and checking for characteristic colony morphologies.
149
150 ***Stress treatments***
151 After 4d of colonization, males were transferred to sterile vials and subjected to a fully factorial
152 design of pesticide and heat stress. Half of the males in each microbial group were exposed for
153 18h to food containing 1ppm imidacloprid, a concentration low enough to avoid immediate
154 lethality while inducing sublethal effects on behavior, reproduction, and physiology
155 (Charpentier *et al.* 2014; Frantzios *et al.* 2008; Martelli *et al.* 2020). Subsequently, half of the
156 males from each microbiome and pesticide treatment were exposed to acute heat stress at 36°C
157 for 4 hours in vials containing 1% agar to prevent dehydration, while the remaining males were
158 kept at 24.5°C as thermal controls. All males were then allowed to recover overnight in
159 standard food vials under standard conditions.
160
161 ***Hatching success and fertility***
162 The day after stress treatments, individual experimental males were mated with virgin standard
163 females reared under axenic conditions. Following mating, females laid eggs for 24h, and 30h
164 later, oviposition vials were frozen. Hatching success (proportion of hatched eggs) and male-
165 mediated fecundity (total number of larvae) were then quantified. This assay was performed in
166 two blocks with 25 males per treatment combination (i.e., total 800 males).
167
168

169 ***Sperm competitiveness***

170 Sperm competitiveness was assessed using a similar experimental design. Standard females
171 were pre-mated with competitor males ubiquitously expressing green fluorescent protein (GFP)
172 (Lüpold *et al.* 2012, 2020) 2d before mating with the experimental males. Matings events were
173 recorded, and before isolating remated females to determine offspring paternity (focal vs.
174 competitor) by GFP fluorescence to calculate P_2 (proportion of offspring sired by the focal
175 male). Non-remating females were excluded. The design included 30 trials per treatment
176 combination in each of two blocks (i.e., total 960 trials).

177

178 ***Bacterial abundance in males***

179 To assess the impact of stress on bacterial load, colony forming units (CFUs) were compared
180 in ten random males per treatment. Surface-sterilized males were homogenized, serially
181 diluted, and plated on MRS media. For groups with both *Acetobacter* and *Lactobacillus*, MRS
182 agar was supplemented with ampicillin or incubated in a CO_2 atmosphere for selective
183 counting. Colony morphologies were used to identify bacterial species, and total species-
184 specific CFUs per fly were calculated (Koyle *et al.* 2016).

185

186 ***Cross-generational effects***

187 To assess cross-generational effects of paternal stress, hatching success and fecundity were
188 quantified in F_1 offspring. From each block, up to 20 freshly hatched larvae from each of 10
189 randomly selected males per paternal treatment combination were transferred to vials
190 containing sterile food and reared under standard conditions. Two to three days upon eclosion,
191 three virgin F_1 males and females from each lineage were individually mated with axenically
192 reared standard flies of the opposite sex. Hatching success and fecundity were then measured

193 in these F_1 offspring as for their fathers (above). This experiment involved two independent
194 blocks of 480 offspring per sex from 160 F_0 males (i.e. total 1,920 mating trials).
195
196 ***Statistical analysis***
197 Data were analyzed in R version 4.4.0 (R Core Ream, 2024), using linear and generalized linear
198 mixed-effects models (LMMs and GLMMs). Models included microbiome composition,
199 thermal and pesticide treatments (and their interactions) as fixed effects, and experimental
200 block as a random effect. Hatching success was analyzed with a quasibinomial GLMM, male-
201 mediated female fecundity with a zero-inflated truncated Poisson model (glmmTMB; Brooks
202 *et al.* 2017), and sperm competitiveness (P_2) with a Tweedie GLMM (glmmTMB). Pairwise
203 comparisons were performed using the emmeans package (Searle *et al.* 1980).
204 Bacterial abundance was analyzed using a linear mixed-effects model. Microbiome
205 community composition in the five-species consortium was analyzed using Hellinger, Bray-
206 Curtis distances (*vegdist* in vegan; Oksanen *et al.* 2023), and PERMANOVA (*adonis2* in
207 vegan), with non-metric multidimensional scaling (NMDS) (*metaMDS*) for visualization.
208 Additionally, relative abundances were compared at the genus level using a binomial GLMM.
209 Finally, offspring fitness data were analyzed using GLMMs with F_0 treatments and their
210 interactions as fixed effects, and block and sire ID as random effects. Model performance was
211 checked using the DHARMa package (Florian 2016).
212
213 **Results**
214 ***Hatching success***
215 To assess the combined effects of heat stress, pesticide exposure, and microbiome composition
216 on male reproductive function, we first investigated fertilization success, estimated as the
217 proportion of eggs hatched out of all eggs laid by females. A GLM revealed a significant three-

218 way interaction between microbiome, temperature, and pesticide (N = 650, χ^2 = 16.07, df = 3,
219 p = 0.0011; Fig. 1A, Supplementary Table S1), highlighting that the combined effects of these
220 stressors depended on the microbial composition of the focal males. Overall, males associated
221 with all five bacterial strains exhibited the highest hatching success, followed by *At*- and *Lb*-
222 associated males, with axenic males being most severely affected. These general patterns were
223 also confirmed by the significant two-way interactions of temperature × microbiome (χ^2 =
224 12.13, df = 3, p = 0.007) and temperature × pesticide (χ^2 = 43.32, df = 1, p < 0.0001), as well
225 as by all main effects (all $\chi^2 \geq 520.76$, $df \geq 3$, $p \leq 0.001$; Supplementary Table S1).
226
227 ***Male-mediated fecundity***
228 Next, we investigated male-mediated fecundity, quantified as the total number of larvae
229 produced per female. In contrast to hatching success, the three-way interaction was not
230 significant (χ^2 = 0.319, df = 3, p = 0.956; Supplementary Table S2) for this metric and was
231 removed from the final model. The simplified model revealed significant two-way interactions
232 between microbiome and temperature (N = 650, χ^2 = 7.85, df = 3, p = 0.049) and between
233 temperature and pesticide (χ^2 = 5.41, df = 1, p = 0.020; Supplementary Table S2). All main
234 effects were again significant (all $\chi^2 \geq 5.41$, p ≤ 0.001). The relative differences in fecundity
235 between males with different microbiome statuses largely mirrored the patterns observed for
236 hatching success (Fig. 1B).
237
238 ***Sperm competitiveness***
239 Given reports of adverse effects of increased temperatures and pesticide exposure on sperm
240 quality and function (Chirault *et al.* 2015; Meena *et al.* 2024a; Moreira *et al.* 2022; Roy *et al.*
241 2017; Sales *et al.* 2018, 2024), we evaluated the combined effects of our treatments on the
242 proportion of progeny sired by focal males relative to their standard competitors (i.e., P_2).

243 Although the three-way interaction was not significant ($N = 331$, $\chi^2 = 1.003$, df = 3, $p = 0.800$),
244 all two-way interactions were (Supplementary Table S3): temperature × microbiome (χ^2 =
245 22.92, df = 3, $p < 0.001$), microbiome × pesticide (χ^2 = 11.89, df = 3, $p < 0.001$), and
246 temperature × pesticide ($\chi^2 = 27.33$, df = 1, $p < 0.001$). Similarly, all main effects on P_2 were
247 significant (all $\chi^2 \geq 472.33$, $p \leq 0.001$). These results indicate strong interdependent effects of
248 the treatments on sperm competitiveness, with the relative performance across different
249 microbiome groups again showing similar patterns to those observed for hatching success and
250 fecundity (Fig. 1C).
251
252 ***Bacterial abundance in males in response to heat stress and pesticide exposure***
253 We also analyzed the effect of heat stress, pesticide exposure and their interactions on the
254 growth and viability of commensal bacteria inside their host under the different stress
255 treatments by quantifying their colony forming units in homogenates of focal males
256 immediately after mating. For total CFU counts, we found a significant three-way interaction
257 ($\chi^2 = 5.06$, df = 2, $p = 0.008$; Supplementary Table S4), suggesting that the bacterial strains
258 differed in their accumulation patterns in adult flies depending on the combined effects of stress
259 treatments. Additionally, we documented significant two-way interactions and main effects of
260 all three factors (all $\chi^2 \geq 42.31$, $p \leq 0.001$), except for the two-way interaction between the two
261 stressors ($\chi^2 = 2.36$, df = 2, $p = 0.127$). Among these and relative to the control microbiome,
262 higher temperature had a stronger negative effect on the total number of CFUs than pesticide
263 exposure (Fig. 2; Supplementary Table S4).
264 A PERMANOVA controlling for block effects ($F_{1,35} = 0.20$, $p = 0.874$, Supplementary
265 Table S5) revealed that temperature was also the dominant driver of variation in the relative
266 abundances of the five bacterial species constituting the consortium, explaining 26.3% of the
267 variation in community composition ($F_{1,35} = 13.88$, $p = 0.0001$; Supplementary Table S5). In

contrast, the main effect 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. 3A) 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 $R^2 \geq 0.54$, $p \leq 0.0001$), in that both *Lactobacillus* species showed positive (≥ 0.68), 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 (≥ 0.56). 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.

293 We found a significant interactive effect between paternal temperature and pesticide
294 treatments on the hatching success of both male (N = 726, χ^2 = 19.39, df = 1, p < 0.001;
295 Supplementary Table S7) and female offspring (N = 750, χ^2 = 10.084, df = 1, p < 0.001;
296 Supplementary Table S8), indicating that the effects of paternal stress exposure were
297 transmitted to the next generation. However, the paternal microbiome did not significantly
298 influence offspring hatching success ($\chi^2 \leq 4.76$, df = 3, $p \geq 0.19$). The interactive effect of
299 paternal temperature and pesticide exposure was not significant for the fecundity induced by
300 sons (χ^2 = 0.36, df = 1, p = 0.55; Supplementary Table S9), but the same interaction showed
301 significant effect on fecundity of F_1 daughters (N = 750, χ^2 = 3.990, df = 1, p = 0.006;
302 Supplementary Table S10). There was also a non-significant trend for a main effect of paternal
303 microbiome composition on offspring fecundity in daughters (χ^2 = 7.26, df = 3, p = 0.06;
304 Supplementary Table S10).
305
306 **Discussion**
307 We investigated the role of the gut microbiome in modulating male reproductive success in *D.*
308 *melanogaster* under individual and combined exposure to acute heat stress and the pesticide
309 imidacloprid. Our results confirm and extend previous work demonstrating the detrimental
310 effects of sublethal temperatures (Canal Domenech & Fricke 2022; David *et al.* 2005; Fasolo
311 & Krebs 2004; Gandara & Drummond-Barbosa 2022, 2023; Meena *et al.* 2024a; Pilakouta &
312 Baillet 2022; Sales *et al.* 2024; Walsh *et al.* 2019) or imidacloprid (Charpentier *et al.* 2014;
313 Xiao *et al.* 2016; Zhang *et al.* 2021b; Zhou *et al.* 2017) on reproductive functions. Importantly,
314 our study revealed both the combined effects of these stressors and the critical role of the
315 microbiome in mediating the response of stressed males, highlighting the importance of the gut
316 microbiome in mitigating fitness losses. *Acetobacter* generally provided stronger protection
317 than *Lactobacillus*, especially in males with a complex microbial community. Furthermore, we

318 demonstrated that paternal stress exposure imposes fitness costs on the next generation,
319 although these cross-generational effects were not significantly mediated by the paternal
320 microbiome under our experimental conditions designed to minimize direct microbial
321 transmission.
322
323 ***Interactive effects of combined stressors on male reproduction***
324 Our study revealed that initial exposure to imidacloprid followed by heat shock reduced male
325 reproductive functions to a greater extent than either stressor alone, consistent with previous
326 studies indicating complex, often order-dependent interactions between thermal stress and
327 pesticide toxicity (Ahmed *et al.* 2023; Deng *et al.* 2016; Meng *et al.* 2022; Ricupero *et al.* 2020;
328 Wang *et al.* 2021). While heat stress has been shown to increase the tolerance to subsequent
329 insecticide exposure in diverse insect pests (Cao *et al.* 2019; Ge *et al.* 2013; Gu *et al.* 2010),
330 the reverse order of stress exposure can have opposing effects (Delnat *et al.* 2019; Wang *et al.*
331 2021), emphasizing the importance of stress sequence in cross-protection.
332 Pre-exposure to insecticides can also decrease heat stress tolerance, suggesting that
333 warmer temperatures may exacerbate insecticide toxicity depending on the exposure sequence.
334 Increased imidacloprid toxicity at higher temperatures has been documented in mayflies
335 (Macaulay *et al.* 2020), earthworms and collembolans (Bandeira *et al.* 2020), and the terrestrial
336 ectotherm *Hypogastrura viatica* (Silva *et al.* 2023). Our study supports these findings, showing
337 decreased male reproductive success when pesticide exposure preceded heat stress. These
338 multi-stressor studies highlight the complex temperature-toxicity interplay, which can be
339 positive or negative depending on the insecticide mode of action, exposure route, and insect
340 species. Therefore, the sequence of exposure must be carefully considered when evaluating
341 combined stressor effects on insect performance.

342 The mechanisms underlying these sequence-dependent interactions likely involve
343 intricate physiological adjustments. Exposure to one stressor might compromise pathways
344 needed to cope with the subsequent stressor (lack of cross-tolerance) or trigger shared signaling
345 pathways that amplify susceptibility (negative cross-talk). Resource allocation trade-offs are
346 also probable (Sokolova 2013), where coping with pesticide toxicity may deplete energy
347 reserves or specific molecules (e.g., antioxidants, chaperones) needed for an effective heat
348 stress response. Generally, stressful environments can alter resource allocation among essential
349 processes like growth, reproduction or immune defense to optimize fitness outcomes
350 (Kooijman 2000). As such, elevated temperature may increase somatic maintenance costs
351 (Verberk *et al.* 2016), potentially reducing resources for defense and repair (Hallman & Brooks
352 2015).

353 While the precise mechanisms underlying increased toxicity at high temperatures remain
354 unclear, they may overlap with those explaining the increased toxicity of organophosphate
355 pesticides at higher mean temperatures (Noyes *et al.* 2009; Noyes & Lema 2015). Increased
356 temperatures can enhance the activity of detoxification enzymes like cytochrome P450s (Wang
357 *et al.* 2021), accelerating pesticide breakdown (Ahmad 2007). Increased uptake of chemicals
358 at higher temperatures (Holmstrup *et al.* 2010) has been shown to increase membrane
359 permeability (Noyes & Lema 2015) or inhibit growth (Gardner *et al.* 2011), possibly due to
360 higher basal metabolism (Hooper *et al.* 2013). Stressful conditions can promote the
361 accumulation of reactive oxygen species (ROS) due to reduced respiration and increased water
362 loss, thereby negatively affecting development and reproduction (Dampc *et al.* 2021). Low
363 doses of imidacloprid, for example, can cause ROS formation and metabolic impairment in *D.*
364 *melanogaster* (Martelli *et al.* 2020). Both pesticide tolerance (Bagchi *et al.* 1996) and heat
365 stress (Neven 2000) are also associated with overexpression of heat-shock proteins. In
366 honeybees, Hsp70 and Hsp90 are upregulated in response to both thermal stress and

367 imidacloprid (Manzi *et al.* 2020). Such chaperone activation could also protect *D.*
368 *melanogaster* males against combined stressors. If so, the observed fitness loss under both
369 stressors might at least in part be attributable to the costs associated with enhanced Hsp
370 expression (Hoekstra *et al.* 2013; Li *et al.* 2015), which may become overwhelming under
371 combined stress.
372
373 ***Microbiome as a modulator of stress resilience***
374 A key finding of our study was the significant role of the gut microbiome in mitigating the
375 detrimental reproductive consequences of heat and pesticide stress. Axenic males consistently
376 performed worse than colonized males across all measured fitness components, highlighting
377 the baseline importance of the microbiome for reproduction and its crucial role under stress.
378 The reduced fitness of axenic males under combined stress suggests an impaired ability to
379 metabolize and excrete imidacloprid, potentially leading to higher internal toxin loads and
380 increased vulnerability to subsequent heat stress.
381 Interestingly, the degree of protection depended on microbial composition. While both
382 *Lactobacillus brevis* and *Acetobacter tropicalis* mono-associations offered benefits over the
383 axenic state, the five-species community consistently provided the greatest resilience,
384 underscoring the potential importance of microbial diversity and inter-species interactions for
385 robust host function (Coyte *et al.* 2015; Freilich *et al.* 2011; Levy & Borenstein 2013;
386 Magnúsdóttir *et al.* 2017; Noecker *et al.* 2019; Zelezniak *et al.* 2015). Mono-associations might
387 lack the metabolic versatility or synergistic cross-feeding capabilities present in more complex
388 consortia, limiting their ability to buffer the host against multiple stressors.
389 Our results partially contrast with Daisley *et al.* (2017), who reported that *Lactobacillus*
390 *plantarum* (Lp39) supplementation rescued *D. melanogaster* from harmful imidacloprid
391 effects, accompanied by increased *Acetobacter* and *Lactobacillus* abundance. We did not

392 observe such a strong rescue with *L. brevis*, nor an increased abundance of either bacterial
393 genus. This discrepancy could stem from strain-specific functions, similar to opposing effects
394 on chlorpyrifos (CP) toxicity between *L. plantarum* ISO and *Lactobacillus ATCC* in *D.*
395 *melanogaster* (Daisley *et al.* 2017), or differences in experimental protocols (e.g., our acute
396 18h imidacloprid exposure versus Daisley *et al.*'s (2017) continued exposure).

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

406 The mechanisms by which the microbiome enhances stress tolerance likely involve
407 multiple pathways. Beyond direct detoxification (Cheng *et al.* 2017; Jones *et al.* 2015; Kikuchi
408 *et al.* 2012; Pang *et al.* 2018), gut microbes significantly impact host nutrition and metabolism
409 (Douglas 2009; Ridley *et al.* 2012), which are intrinsically linked to thermal tolerance in
410 ectotherms (Henry *et al.* 2020; Moeller *et al.* 2020; Moghadam *et al.* 2018; Semsar-Kazerouni
411 *et al.* 2020). In *D. melanogaster*, the gut microbiota influences nutrient allocation and the
412 thermo-protectant trehalose (Overgaard *et al.* 2007; Ridley *et al.* 2012; Schwasinger-Schmidt
413 *et al.* 2012). It can also modulate host immune responses and potentially Hsp expression in the
414 gut epithelium (Arnal & Lalles 2016; Liu *et al.* 2014). Given the critical role of Hsps in
415 mitigating thermal stress in *D. melanogaster* (Calabria *et al.* 2012; Sørensen *et al.* 2003), and
416 their rapid induction after heat exposure (Hoekstra *et al.* 2013), the microbiota may enhance

417 heat tolerance by promoting higher Hsp levels. Disrupting the microbiome can alter these
418 processes, as seen with heat-induced reductions in *Buchnera aphidicola* in pea aphids lowering
419 heat resistance (Dunbar *et al.* 2007), and temperature-driven microbiome shifts increasing
420 imidacloprid susceptibility in *Nilaparvata lugens* (Zhang *et al.* 2021a).
421
422 ***Cross-generational impacts of parental stress***
423 We found clear evidence for cross-generational fitness costs of paternal stress exposure, with
424 reduced hatching success and fecundity in offspring of stressed fathers. Cross-generational
425 effects can either increase or decrease offspring vulnerability to stressors (Brausch & Salice
426 2011; Kim *et al.* 2014; Pölkki *et al.* 2012; Reátegui-Zirena *et al.* 2017; Schultz *et al.* 2016).
427 Our results corroborate studies reporting detrimental cross- or transgenerational effects of
428 paternal heat stress (Ferrer *et al.* 2013; Guillaume *et al.* 2016; Shama *et al.* 2014; Walsh *et al.*
429 2014) or toxicant exposure (Bhandari *et al.* 2015; Kimberly & Salice 2015; Schultz *et al.* 2016;
430 Yu & Liao 2016), possibly through epigenetic processes (Schultz *et al.* 2016). The observed
431 synergism between paternal heat and pesticide exposure in offspring fitness underscores the
432 potential for long-lasting consequences of multi-stressor events.
433 Offspring from stressed fathers exhibited reduced fecundity and hatching success,
434 indicating that paternal exposure does not buffer against these environmental stressors. Our
435 findings support the view that cross-generational effects of stress are often non-adaptive or
436 costly for offspring (Uller *et al.* 2013), highlighting the potential for cumulative fitness declines
437 across generations in populations facing recurrent multi-stressor events.
438 Intriguingly, the paternal microbiome did not significantly mediate these cross-
439 generational effects in our study. This could be attributable to our experimental design, which
440 deliberately minimized vertical transmission of microbes by using axenic mothers and
441 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.

467 **References**

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

470 Ahmed, T., Masood, H.A., Noman, M., Al-Huqail, A.A., Alghanem, S.M., Khan, M.M., *et al.*
471 (2023). Biogenic silicon nanoparticles mitigate cadmium (Cd) toxicity in rapeseed
472 (*Brassica napus* L.) by modulating the cellular oxidative stress metabolism and
473 reducing Cd translocation. *J. Hazard. Mater.*, 459, 132070.

474 Arambourou, H. & Stoks, R. (2015). Combined effects of larval exposure to a heat wave and
475 chlorpyrifos in northern and southern populations of the damselfly *Ischnura elegans*.
476 *Chemosphere*, 128, 148–154.

477 Arnal, M.-E. & Lalles, J.-P. (2016). Gut epithelial inducible heat-shock proteins and their
478 modulation by diet and the microbiota. *Nutr. Rev.*, 74, 181–197.

479 Aulsebrook, L.C., Bertram, M.G., Martin, J.M., Aulsebrook, A.E., Brodin, T., Evans, J.P., *et*
480 *al.* (2020). Reproduction in a polluted world: implications for wildlife. *Reproduction*,
481 160, R13–R23.

482 Bagchi, D., Bhaitacharya, G. & Stohs, S. (1996). Production of reactive oxygen species by
483 gastric cells in association with *Helicobacter pylori*. *Free Radic. Res.*, 24, 439–450.

484 Bandeira, F.O., Alves, P.R.L., Hennig, T.B., Toniolo, T., Natal-da-Luz, T. & Baretta, D.
485 (2020). Effect of temperature on the toxicity of imidacloprid to *Eisenia andrei* and
486 *Folsomia candida* in tropical soils. *Environ. Pollut.*, 267, 115565.

487 Beketov, M.A., Kefford, B.J., Schäfer, R.B. & Liess, M. (2013). Pesticides reduce regional
488 biodiversity of stream invertebrates. *Proc. Natl. Acad. Sci.*, 110, 11039–11043.

489 Bhandari, R.K., Vom Saal, F.S. & Tillitt, D.E. (2015). Transgenerational effects from early
490 developmental exposures to bisphenol A or 17α-ethinylestradiol in medaka, *Oryzias*
491 *latipes*. *Sci. Rep.*, 5, 9303.

492 Bonduriansky, R., Crean, A.J. & Day, T. (2012). The implications of nongenetic inheritance
493 for evolution in changing environments. *Evol. Appl.*, 5, 192–201.
494 Brausch, J.M. & Salice, C.J. (2011). Effects of an environmentally realistic pesticide mixture
495 on *Daphnia magna* exposed for two generations. *Arch. Environ. Contam. Toxicol.*, 61,
496 272–279.
497 Broderick, N.A. & Lemaitre, B. (2012). Gut-associated microbes of *Drosophila melanogaster*.
498 *Gut Microbes*, 3, 307–321.
499 Brooks, M.E., Kristensen, K., Van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., *et*
500 *al.* (2017). glmmTMB balances speed and flexibility among packages for zero-inflated
501 generalized linear mixed modeling.
502 Calabria, G., Dolgova, O., Rego, C., Castañeda, L., Rezende, E., Balanyà, J., *et al.* (2012).
503 Hsp70 protein levels and thermotolerance in *Drosophila subobscura*: a reassessment of
504 the thermal co-adaptation hypothesis. *J. Evol. Biol.*, 25, 691–700.
505 Canal Domenech, B. & Fricke, C. (2022). Recovery from heat-induced infertility—A study of
506 reproductive tissue responses and fitness consequences in male *Drosophila*
507 *melanogaster*. *Ecol. Evol.*, 12, e9563.
508 Cao, Y., Yang, H., Li, J., Wang, C., Li, C. & Gao, Y. (2019). Sublethal effects of imidacloprid
509 on the population development of western flower thrips *Frankliniella occidentalis*
510 (Thysanoptera: Thripidae). *Insects*, 10, 3.
511 Charpentier, G., Louat, F., Bonmatin, J.-M., Marchand, P.A., Vanier, F., Locker, D., *et al.*
512 (2014). Lethal and sublethal effects of imidacloprid, after chronic exposure, on the
513 insect model *Drosophila melanogaster*. *Environ. Sci. Technol.*, 48, 4096–4102.
514 Cheng, D., Guo, Z., Riegler, M., Xi, Z., Liang, G. & Xu, Y. (2017). Gut symbiont enhances
515 insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis*
516 (Hendel). *Microbiome*, 5, 1–12.

517 Chirault, M., Lucas, C., Goubault, M., Chevrier, C., Bressac, C. & Lécureuil, C. (2015). A
518 combined approach to heat stress effect on male fertility in *Nasonia vitripennis*: from
519 the physiological consequences on spermatogenesis to the reproductive adjustment of
520 females mated with stressed males. *PLoS One*, 10, e0120656.
521 Coyte, K.Z., Schluter, J. & Foster, K.R. (2015). The ecology of the microbiome: networks,
522 competition, and stability. *Science*, 350, 663–666.
523 Crean, A.J. & Immler, S. (2021). Evolutionary consequences of environmental effects on
524 gamete performance. *Philos. Trans. R. Soc. B*, 376, 20200122.
525 Dai, W., Holmstrup, M., Slotsbo, S., Bakker, R., Damgaard, C. & van Gestel, C.A. (2023).
526 Heat stress delays detoxification of phenanthrene in the springtail *Folsomia candida*.
527 *Chemosphere*, 311, 137119.
528 Daisley, B.A., Trinder, M., McDowell, T.W., Welle, H., Dube, J.S., Ali, S.N., *et al.* (2017).
529 Neonicotinoid-induced pathogen susceptibility is mitigated by *Lactobacillus plantarum*
530 immune stimulation in a *Drosophila melanogaster* model. *Sci. Rep.*, 7, 2703.
531 Dampc, J., Mołoń, M., Durak, T. & Durak, R. (2021). Changes in aphid—plant interactions
532 under increased temperature. *Biology*, 10, 480.
533 David, J., Araripe, L., Chakir, M., Legout, H., Lemos, B., Pétavy, G., *et al.* (2005). Male
534 sterility at extreme temperatures: a significant but neglected phenomenon for
535 understanding *Drosophila* climatic adaptations. *J. Evol. Biol.*, 18, 838–846.
536 De França, S.M., Breda, M.O., Barbosa, D.R., Araujo, A. & Guedes, C.A. (2017). The
537 sublethal effects of insecticides in insects. In: *Biological Control of Pest and Vector*
538 *Insects*. (ed. V.D.C. Shields). InTech, doi: 10.5772/66461.
539 DeCourten, B.M. & Brander, S.M. (2017). Combined effects of increased temperature and
540 endocrine disrupting pollutants on sex determination, survival, and development across
541 generations. *Sci. Rep.*, 7, 9310.

542 Delnat, V., Janssens, L. & Stoks, R. (2019). Whether warming magnifies the toxicity of a
543 pesticide is strongly dependent on the concentration and the null model. *Aquat.*
544 *Toxicol.*, 211, 38–45.
545 Deng, Z., Zhang, F., Wu, Z., Yu, Z. & Wu, G. (2016). Chlorpyrifos-induced hormesis in
546 insecticide-resistant and-susceptible *Plutella xylostella* under normal and high
547 temperatures. *Bull. Entomol. Res.*, 106, 378–386.
548 Desneux, N., Decourtye, A. & Delpuech, J.-M. (2007). The sublethal effects of pesticides on
549 beneficial arthropods. *Annu Rev Entomol*, 52, 81–106.
550 Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., *et*
551 *al.* (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc.*
552 *Natl. Acad. Sci. U. S. A.*, 105, 6668–6672.
553 Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C., Huey, R.B., *et al.*
554 (2018). Increase in crop losses to insect pests in a warming climate. *Science*, 361, 916–
555 919.
556 Dougherty, L.R., Frost, F., Maenpaa, M.I., Rowe, M., Cole, B.J., Vasudeva, R., *et al.* (2024).
557 A systematic map of studies testing the relationship between temperature and animal
558 reproduction. *Ecol. Solut. Evid.*, 5, e12303.
559 Douglas, A.E. (2009). The microbial dimension in insect nutritional ecology. *Funct. Ecol.*, 23,
560 38–47.
561 Dunbar, H.E., Wilson, A.C.C., Ferguson, N.R. & Moran, N.A. (2007). Aphid thermal tolerance
562 is governed by a point mutation in bacterial symbionts. *PLoS Biol.*, 5, e96.
563 Fasolo, A.G. & Krebs, R.A. (2004). A comparison of behavioural change in *Drosophila* during
564 exposure to thermal stress. *Biol. J. Linn. Soc.*, 83, 197–205.

565 Fathy, R.F. (2024). Divergent perspectives on the synergistic impacts of thermal-chemical
566 stress on aquatic biota within the framework of climate change scenarios.
567 *Chemosphere*, 355, 141810.
568 Feldhaar, H. (2011). Bacterial symbionts as mediators of ecologically important traits of insect
569 hosts. *Ecol. Entomol.*, 36, 533–543.
570 Ferrer, A., Dorn, S. & Mazzi, D. (2013). Cross-generational effects of temperature on flight
571 performance, and associated life-history traits in an insect. *J. Evol. Biol.*, 26, 2321–
572 2330.
573 Fitzpatrick, J.L. & Lüpold, S. (2014). Sexual selection and the evolution of sperm quality. *Mol.*
574 *Hum. Reprod.*, 20, 1180–1189.
575 Florian, H. (2016). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed)
576 Regression Models. *CRAN Contrib. Packag.*
577 Folt, C., Chen, C., Moore, M. & Burnaford, J. (1999). Synergism and antagonism among
578 multiple stressors. *Limnol. Oceanogr.*, 44, 864–877.
579 Fournier-Level, A., Neumann-Mondlak, A., Good, R., Green, L., Schmidt, J.M. & Robin, C.
580 (2016). Behavioural response to combined insecticide and temperature stress in natural
581 populations of *Drosophila melanogaster*. *J. Evol. Biol.*, 29, 1030–1044.
582 Frantzios, G., Paptsiki, K., Sidiropoulou, B., Lazaridis, I., Theophilidis, G. & Mavragani-
583 Tsipidou, P. (2008). Evaluation of insecticidal and genotoxic effects of imidacloprid
584 and acetochlor in *Drosophila melanogaster*. *J. Appl. Entomol.*, 132, 583–590.
585 Freilich, S., Zarecki, R., Eilam, O., Segal, E.S., Henry, C.S., Kupiec, M., *et al.* (2011).
586 Competitive and cooperative metabolic interactions in bacterial communities. *Nat.*
587 *Commun.*, 2, 589.

588 Gandara, A.C.P. & Drummond-Barbosa, D. (2022). Warm and cold temperatures have distinct
589 germline stem cell lineage effects during *Drosophila oogenesis*. *Development*, 149,
590 dev200149.
591 Gandara, A.C.P. & Drummond-Barbosa, D. (2023). Chronic exposure to warm temperature
592 causes low sperm abundance and quality in *Drosophila melanogaster*. *Sci. Rep.*, 13,
593 12331.
594 Gardner, J.L., Peters, A., Kearney, M.R., Joseph, L. & Heinsohn, R. (2011). Declining body
595 size: a third universal response to warming? *Trends Ecol. Evol.*, 26, 285–291.
596 Ge, L., Huang, L., Yang, G., Song, Q., Stanley, D., Gurr, G., *et al.* (2013). Molecular basis for
597 insecticide-enhanced thermotolerance in the brown planthopper *Nilaparvata lugens*
598 *Stål* (Hemiptera: Delphacidae). *Mol. Ecol.*, 22, 5624–5634.
599 Gould, A.L., Zhang, V., Lamberti, L., Jones, E.W., Obadia, B., Korasidis, N., *et al.* (2018).
600 Microbiome interactions shape host fitness. *Proc. Natl. Acad. Sci.*, 115, E11951–
601 E11960.
602 Gruntenko, N.E., Ilinsky, Y.Yu., Adonyeva, N.V., Burdina, E.V., Bykov, R.A., Menshanov,
603 P.N., *et al.* (2017). Various Wolbachia genotypes differently influence host *Drosophila*
604 dopamine metabolism and survival under heat stress conditions. *BMC Evol. Biol.*, 17,
605 252.
606 Gu, X., Tian, S., Wang, D., Fei, G. & Wei, H. (2010). Interaction between short-term heat
607 pretreatment and fipronil on 2nd instar larvae of diamondback moth, *Plutella xylostella*
608 (Linn). *Dose Response*, 8, 331–346.
609 Guilhot, R., Xuéreb, A. & Fellous, S. (2025). Transmission of yeast and bacterial symbionts
610 between sexual partners in *Drosophila suzukii* and *Drosophila melanogaster*. *R. Soc.*
611 *Open Sci.*, 12, 241149.

612 Guillaume, A.S., Monro, K. & Marshall, D.J. (2016). Transgenerational plasticity and
613 environmental stress: do paternal effects act as a conduit or a buffer? *Funct. Ecol.*, 30,
614 1175–1184.
615 Hallman, T.A. & Brooks, M.L. (2015). The deal with diel: Temperature fluctuations,
616 asymmetrical warming, and ubiquitous metals contaminants. *Environ. Pollut. Barking*
617 *Essex 1987*, 206, 88–94.
618 He, Y., Zhao, J., Wu, D., Wyckhuys, K.A. & Wu, K. (2011). Sublethal effects of imidacloprid
619 on *Bemisia tabaci* (Hemiptera: Aleyrodidae) under laboratory conditions. *J. Econ.*
620 *Entomol.*, 104, 833–838.
621 Heino, J., Virkkala, R. & Toivonen, H. (2009). Climate change and freshwater biodiversity:
622 detected patterns, future trends and adaptations in northern regions. *Biol. Rev.*, 84, 39–
623 54.
624 Henry, Y., Overgaard, J. & Colinet, H. (2020). Dietary nutrient balance shapes phenotypic
625 traits of *Drosophila melanogaster* in interaction with gut microbiota. *Comp. Biochem.*
626 *Physiol. A. Mol. Integr. Physiol.*, 241, 110626.
627 Hoekstra, L.A., Siddiq, M.A. & Montooth, K.L. (2013). Pleiotropic effects of a mitochondrial–
628 nuclear incompatibility depend upon the accelerating effect of temperature in
629 *Drosophila*. *Genetics*, 195, 1129–1139.
630 Holmstrup, M., Bindesbøl, A.-M., Oostingh, G.J., Duschl, A., Scheil, V., Köhler, H.-R., *et al.*
631 (2010). Interactions between effects of environmental chemicals and natural stressors:
632 a review. *Sci. Total Environ.*, 408, 3746–3762.
633 Hooper, M.J., Ankley, G.T., Cristol, D.A., Maryoung, L.A., Noyes, P.D. & Pinkerton, K.E.
634 (2013). Interactions between chemical and climate stressors: A role for mechanistic
635 toxicology in assessing climate change risks. *Environ. Toxicol. Chem.*, 32, 32–48.

636 Ihara, M., D. Buckingham, S., Matsuda, K. & B. Sattelle, D. (2017). Modes of action, resistance
637 and toxicity of insecticides targeting nicotinic acetylcholine receptors. *Curr. Med.*
638 *Chem.*, 24, 2925–2934.
639 Iossa, G. (2019). Sex-specific differences in thermal fertility Limits. *Trends Ecol. Evol.*, 34,
640 490–492.
641 Jaramillo, A. & Castañeda, L.E. (2021). Gut microbiota of *Drosophila subobscura* contributes
642 to its heat tolerance and is sensitive to transient thermal stress. *Front. Microbiol.*, 12,
643 654108.
644 Jones, R.M., Desai, C., Darby, T.M., Luo, L., Wolfarth, A.A., Scharer, C.D., *et al.* (2015).
645 *Lactobacilli* modulate epithelial cytoprotection through the Nrf2 pathway. *Cell Rep.*,
646 12, 1217–1225.
647 Jutfelt, F., Norin, T., Åsheim, E.R., Rowsey, L.E., Andreassen, A.H., Morgan, R., *et al.* (2021).
648 'Aerobic scope protection'reduces ectotherm growth under warming. *Funct. Ecol.*, 35,
649 1397–1407.
650 Kang, Z.-W., Liu, F.-H., Pang, R.-P., Tian, H.-G. & Liu, T.-X. (2018). Effect of sublethal doses
651 of imidacloprid on the biological performance of aphid endoparasitoid *Aphidius*
652 *gifuensis* (Hymenoptera: Aphidiidae) and influence on its related gene expression.
653 *Front. Physiol.*, 9, 1729.
654 Karimzadeh, R., Javanshir, M. & Hejazi, M.J. (2020). Individual and combined effects of
655 insecticides, inert dusts and high temperatures on *Callosobruchus maculatus*
656 (Coleoptera: Chrysomelidae). *J. Stored Prod. Res.*, 89, 101693.
657 Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K. & Fukatsu, T. (2012).
658 Symbiont-mediated insecticide resistance. *Proc. Natl. Acad. Sci.*, 109, 8618–8622.

659 Kim, H.Y., Yu, S., Jeong, T. & Kim, S.D. (2014). Relationship between trans-generational
660 effects of tetracycline on *Daphnia magna* at the physiological and whole organism
661 level. *Environ. Pollut.*, 191, 111–118.
662 Kimberly, D.A. & Salice, C.J. (2015). Multigenerational contaminant exposures produce non-
663 monotonic, transgenerational responses in *Daphnia magna*. *Environ. Pollut. Barking*
664 *Essex 1987*, 207, 176–182.
665 Kokou, F., Sasson, G., Nitzan, T., Doron-Faigenboim, A., Harpaz, S., Cnaani, A., *et al.* (2018).
666 Host genetic selection for cold tolerance shapes microbiome composition and
667 modulates its response to temperature. *elife*, 7, e36398.
668 Kooijman, S.A.L.M. (2000). *Dynamic energy and mass budgets in biological systems*.
669 Cambridge University Press.
670 Koyle, M.L., Veloz, M., Judd, A.M., Wong, A.C.-N., Newell, P.D., Douglas, A.E., *et al.*
671 (2016). Rearing the fruit fly *Drosophila melanogaster* under axenic and gnotobiotic
672 conditions. *JoVE J. Vis. Exp.*, e54219.
673 Levy, R. & Borenstein, E. (2013). Metabolic modeling of species interaction in the human
674 microbiome elucidates community-level assembly rules. *Proc. Natl. Acad. Sci.*, 110,
675 12804–12809.
676 Li, S., Tan, H.-Y., Wang, N., Zhang, Z.-J., Lao, L., Wong, C.-W., *et al.* (2015). The role of
677 oxidative stress and antioxidants in liver diseases. *Int. J. Mol. Sci.*, 16, 26087–26124.
678 Li, W., Lu, Z., Li, L., Yu, Y., Dong, S., Men, X., *et al.* (2018). Sublethal effects of imidacloprid
679 on the performance of the bird cherry-oat aphid *Rhopalosiphum padi*. *PLOS ONE*, 13,
680 e0204097.
681 Liu, H., He, J., Chi, C. & Shao, J. (2014). Differential HSP70 expression in *Mytilus coruscus*
682 under various stressors. *Gene*, 543, 166–173.

683 Lüpold, S., Manier, M.K., Berben, K.S., Smith, K.J., Daley, B.D., Buckley, S.H., *et al.* (2012).
684 How multivariate ejaculate traits determine competitive fertilization success in
685 *Drosophila melanogaster*. *Curr. Biol.*, 22, 1667–1672.
686 Lüpold, S., Reil, J.B., Manier, M.K., Zeender, V., Belote, J.M. & Pitnick, S. (2020). How
687 female × male and male × male interactions influence competitive fertilization in
688 *Drosophila melanogaster*. *Evol. Lett.*, 4, 416–429.
689 Macaulay, S.J., Buchwalter, D.B. & Matthaei, C.D. (2020). Water temperature interacts with
690 the insecticide imidacloprid to alter acute lethal and sublethal toxicity to mayfly larvae.
691 *N. Z. J. Mar. Freshw. Res.*, 54, 115–130.
692 Magnúsdóttir, S., Heinken, A., Kutt, L., Ravcheev, D.A., Bauer, E., Noronha, A., *et al.* (2017).
693 Generation of genome-scale metabolic reconstructions for 773 members of the human
694 gut microbiota. *Nat. Biotechnol.*, 35, 81–89.
695 Manzi, C., Vergara-Amado, J., Franco, L.M. & Silva, A.X. (2020). The effect of temperature
696 on candidate gene expression in the brain of honey bee *Apis mellifera* (Hymenoptera:
697 Apidae) workers exposed to neonicotinoid imidacloprid. *J. Therm. Biol.*, 93, 102696.
698 Mao, K., Jin, R., Li, W., Ren, Z., Qin, X., He, S., *et al.* (2019). The influence of temperature
699 on the toxicity of insecticides to *Nilaparvata lugens* (Stål). *Pestic. Biochem. Physiol.*,
700 156, 80–86.
701 Marshall, D.J., Heppell, S.S., Munch, S.B. & Warner, R.R. (2010). The relationship between
702 maternal phenotype and offspring quality: do older mothers really produce the best
703 offspring? *Ecology*, 91, 2862–2873.
704 Martelli, F., Zhongyuan, Z., Wang, J., Wong, C.-O., Karagas, N.E., Roessner, U., *et al.* (2020).
705 Low doses of the neonicotinoid insecticide imidacloprid induce ROS triggering
706 neurological and metabolic impairments in *Drosophila*. *Proc. Natl. Acad. Sci.*, 117,
707 25840–25850.

708 Massot, M., Bagni, T., Maria, A., Couzi, P., Drozdz, T., Malbert-Colas, A., *et al.* (2021).
709 Combined influences of transgenerational effects, temperature and insecticide on the
710 moth *Spodoptera littoralis*. *Environ. Pollut.*, 289, 117889.
711 Meena, A., De Nardo, A.N., Maggu, K., Sbilordo, S.H., Roy, J., Snook, R.R., *et al.* (2024a).
712 Fertility loss and recovery dynamics after repeated heat stress across life stages in male
713 *Drosophila melanogaster*: patterns and processes. *R. Soc. Open Sci.*, 11, 241082.
714 Meena, A., Maggu, K., De Nardo, A.N., Sbilordo, S.H., Eggs, B., Al Toma Sho, R., *et al.*
715 (2024b). Life stage-specific effects of heat stress on spermatogenesis and oogenesis in
716 *Drosophila melanogaster*. *J. Therm. Biol.*, 125, 104001.
717 Meng, S., Tran, T.T., Van Dinh, K., Delnat, V. & Stoks, R. (2022). Acute warming increases
718 pesticide toxicity more than transgenerational warming by reducing the energy budget.
719 *Sci. Total Environ.*, 805, 150373.
720 Moeller, A.H., Ivey, K., Cornwall, M.B., Herr, K., Rede, J., Taylor, E.N., *et al.* (2020). The
721 lizard gut microbiome changes with temperature and is associated with heat tolerance.
722 *Appl. Environ. Microbiol.*, 86, e01181-20.
723 Moghadam, N.N., Thorshauge, P.M., Kristensen, T.N., de Jonge, N., Bahrndorff, S., Kjeldal,
724 H., *et al.* (2018). Strong responses of *Drosophila melanogaster* microbiota to
725 developmental temperature. *Fly (Austin)*, 12, 1–12.
726 Montllor, C.B., Maxmen, A. & Purcell, A.H. (2002). Facultative bacterial endosymbionts
727 benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecol. Entomol.*, 27, 189–195.
728 Moore, M.P., Whiteman, H.H. & Martin, R.A. (2019). A mother's legacy: the strength of
729 maternal effects in animal populations. *Ecol. Lett.*, 22, 1620–1628.
730 Moreira, D.R., de Souza, T.H.S., Galhardo, D., Puentes, S.M.D., Figueira, C.L., Silva, B.G. da,
731 *et al.* (2022). Imidacloprid induces histopathological damage in the midgut, ovary, and

732 spermathecal stored spermatozoa of queens after chronic colony exposure. *Environ. Toxicol. Chem.*, 41, 1637–1648.

734 Morgan, H.L. & Watkins, A.J. (2019). Transgenerational impact of environmental change. In: *Reproductive Sciences in Animal Conservation*, Advances in Experimental Medicine and Biology (eds. Comizzoli, P., Brown, J.L. & Holt, W.V.). Springer International Publishing, Cham, pp. 71–89.

738 Mousseau, T.A., Uller, T., Wapstra, E. & Badyaev, A.V. (2009). Evolution of maternal effects: past and present. *Philos. Trans. R. Soc. B Biol. Sci.*, 364, 1035–1038.

740 Navarro-Ortega, A., Acuña, V., Bellin, A., Burek, P., Cassiani, G., Choukr-Allah, R., *et al.* (2015). Managing the effects of multiple stressors on aquatic ecosystems under water scarcity. The GLOBAQUA project. *Sci. Total Environ.*, 503–504, 3–9.

743 Neven, L.G. (2000). Physiological responses of insects to heat. *Postharvest Biol. Technol.*, 21, 103–111.

745 Newell, P.D. & Douglas, A.E. (2014). Interspecies interactions determine the impact of the gut microbiota on nutrient allocation in *Drosophila melanogaster*. *Appl. Environ. Microbiol.*, 80, 788–796.

748 Noecker, C., Chiu, H.-C., McNally, C.P. & Borenstein, E. (2019). Defining and evaluating microbial contributions to metabolite variation in microbiome-metabolome association studies. *mSystems*, 4, 10–1128.

751 Noyes, P.D. & Lema, S.C. (2015). Forecasting the impacts of chemical pollution and climate change interactions on the health of wildlife. *Curr. Zool.*, 61, 669–689.

753 Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L.A., Walcott, K.C., *et al.* (2009). The toxicology of climate change: environmental contaminants in a warming world. *Environ. Int.*, 35, 971–986.

Obadia, B., Güvener, Z.T., Zhang, V., Ceja-Navarro, J.A., Brodie, E.L., William, W.J., *et al.* (2017). Probabilistic invasion underlies natural gut microbiome stability. *Curr. Biol.*, 27, 1999–2006.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., *et al.* (2023). vegan: Community Ecology Package. R package version 2.6-4. 2022.

Osborne, B., Siboni, N., Seymour, J.R., Ralph, P. & Pernice, M. (2023). Exploring the potential of algae-bacteria interactions in the biocontrol of the marine pathogen *Vibrio parahaemolyticus*. *J. Appl. Phycol.*, 35, 2731–2743.

Overgaard, J., Malmendal, A., Sørensen, J.G., Bundy, J.G., Loeschcke, V., Nielsen, N.C., *et al.* (2007). Metabolomic profiling of rapid cold hardening and cold shock in *Drosophila melanogaster*. *J. Insect Physiol.*, 53, 1218–1232.

Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C., *et al.* (2013). Temperature variation makes ectotherms more sensitive to climate change. *Glob. Change Biol.*, 19, 2373–2380.

Pang, R., Chen, M., Yue, L., Xing, K., Li, T., Kang, K., *et al.* (2018). A distinct strain of Arsenophonus symbiont decreases insecticide resistance in its insect host. *PLoS Genet.*, 14, e1007725.

Parker, G.A. & Pizzari, T. (2010). Sperm competition and ejaculate economics. *Biol. Rev.*, 85, 897–934.

Piggott, J.J., Niyogi, D.K., Townsend, C.R. & Matthaei, C.D. (2015a). Multiple stressors and stream ecosystem functioning: climate warming and agricultural stressors interact to affect processing of organic matter. *J. Appl. Ecol.*, 52, 1126–1134.

Piggott, J.J., Townsend, C.R. & Matthaei, C.D. (2015b). Climate warming and agricultural stressors interact to determine stream macroinvertebrate community dynamics. *Glob. Change Biol.*, 21, 1887–1906.

Pilakouta, N. & Baillet, A. (2022). Effects of temperature on mating behaviour and mating success: A meta-analysis. *J. Anim. Ecol.*, 91, 1642–1650.

Pizzari, T. & Parker, G.A. (2009). Sperm competition and sperm phenotype. In: *Sperm Biology*. Elsevier, pp. 207–245.

Pölkki, M., Kangassalo, K. & Rantala, M.J. (2012). Transgenerational effects of heavy metal pollution on immune defense of the blow fly *Protophormia terraenovae*. *PLoS One*, 7, e38832.

Pörtner, H.-O., Bock, C. & Mark, F.C. (2017). Oxygen-and capacity-limited thermal tolerance: bridging ecology and physiology. *J. Exp. Biol.*, 220, 2685–2696.

Przeslawski, R., Byrne, M. & Mellin, C. (2015). A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Glob. Change Biol.*, 21, 2122–2140.

Reátegui-Zirena, E.G., Fidder, B.N., Olson, A.D., Dawson, D.E., Bilbo, T.R. & Salice, C.J. (2017). Transgenerational endpoints provide increased sensitivity and insight into multigenerational responses of *Lymnaea stagnalis* exposed to cadmium. *Environ. Pollut.*, 224, 572–580.

Renoz, F., Pons, I. & Hance, T. (2019). Evolutionary responses of mutualistic insect–bacterial symbioses in a world of fluctuating temperatures. *Curr. Opin. Insect Sci.*, 35, 20–26.

Ricupero, M., Abbes, K., Haddi, K., Kurtulus, A., Desneux, N., Russo, A., *et al.* (2020). Combined thermal and insecticidal stresses on the generalist predator *Macrolophus pygmaeus*. *Sci. Total Environ.*, 729, 138922.

Ridley, E.V., Wong, A.C. & Douglas, A.E. (2013). Microbe-dependent and nonspecific effects of procedures to eliminate the resident microbiota from *Drosophila melanogaster*. *Appl. Environ. Microbiol.*, 79, 3209–3214.

805 Ridley, E.V., Wong, A.C., Westmiller, S. & Douglas, A.E. (2012). Impact of the resident
806 microbiota on the nutritional phenotype of *Drosophila melanogaster*. *PloS One*, 7,
807 e36765.
808 Roy, P., Phukan, P.K., Changmai, D. & Boruah, S. (2017). Pesticides, insecticides and male
809 infertility. *Int J Reprod Contracept Obs Gynecol*, 6, 3387–3391.
810 Russell, J.A. & Moran, N.A. (2006). Costs and benefits of symbiont infection in aphids:
811 variation among symbionts and across temperatures. *Proc. Biol. Sci.*, 273, 603–610.
812 Sales, K., Thomas, P., Gage, M.J. & Vasudeva, R. (2024). Experimental heatwaves reduce the
813 effectiveness of ejaculates at occupying female reproductive tracts in a model insect. *R.*
814 *Soc. Open Sci.*, 11, 231949.
815 Sales, K., Vasudeva, R., Dickinson, M.E., Godwin, J.L., Lumley, A.J., Michalczyk, Ł., *et al.*
816 (2018). Experimental heatwaves compromise sperm function and cause
817 transgenerational damage in a model insect. *Nat. Commun.*, 9, 4771.
818 Schultz, C.L., Wamucho, A., Tsyusko, O.V., Unrine, J.M., Crossley, A., Svendsen, C., *et al.*
819 (2016). Multigenerational exposure to silver ions and silver nanoparticles reveals
820 heightened sensitivity and epigenetic memory in *Caenorhabditis elegans*. *Proc. R. Soc.*
821 *B Biol. Sci.*, 283, 20152911.
822 Schwasinger-schmidt, T.E., Kachman, S.D. & Harshman, L.G. (2012). Evolution of starvation
823 resistance in *Drosophila melanogaster*: measurement of direct and correlated responses
824 to artificial selection. *J. Evol. Biol.*, 25, 378–387.
825 Searle, S.R., Speed, F.M. & Milliken, G.A. (1980). Population Marginal means in the linear
826 model: An alternative to least squares means. *Am. Stat.*, 34, 216–221.
827 Segner, H., Schmitt-Jansen, M. & Sabater, S. (2014). Assessing the impact of multiple stressors
828 on aquatic biota: The receptor's side matters. *Environ. Sci. Technol.*, 48, 7690–7696.

829 Semsar-Kazerouni, M., Boerrigter, J.G. & Verberk, W.C. (2020). Changes in heat stress
830 tolerance in a freshwater amphipod following starvation: The role of oxygen
831 availability, metabolic rate, heat shock proteins and energy reserves. *Comp. Biochem.*
832 *Physiol. A. Mol. Integr. Physiol.*, 245, 110697.
833 Sepulveda, J. & Moeller, A.H. (2020). The effects of temperature on animal gut microbiomes.
834 *Front. Microbiol.*, 11, 384.
835 Shahid, N., Siddique, A. & Liess, M. (2024). Synergistic interaction between a toxicant and
836 food stress is further exacerbated by temperature. *Environ. Pollut.*, 363, 125109.
837 Shama, L.N., Strobel, A., Mark, F.C. & Wegner, K.M. (2014). Transgenerational plasticity in
838 marine sticklebacks: maternal effects mediate impacts of a warming ocean. *Funct.*
839 *Ecol.*, 28, 1482–1493.
840 Shokal, U., Yadav, S., Atri, J., Accetta, J., Kenney, E., Banks, K., *et al.* (2016). Effects of co-
841 occurring *Wolbachia* and *Spiroplasma* endosymbionts on the *Drosophila* immune
842 response against insect pathogenic and non-pathogenic bacteria. *BMC Microbiol.*, 16,
843 16.
844 Silva, A.P.N., Andrade, E.S., Nascimento, V.L. & Haddi, K. (2025). Thermal modulation of
845 insecticide-induced hormetic and oxidative responses in insect pests. *Chemosphere*,
846 370, 143920.
847 Silva, A.S.J., Kristiansen, S.M., Sengupta, S., van Gestel, C.A., Leinaas, H.P. & Borgå, K.
848 (2023). Using dietary exposure to determine sub-lethal effects from imidacloprid in two
849 springtail (*Collembola*) species. *Ecotoxicology*, 32, 1209–1220.
850 Simmons, L.W. & Fitzpatrick, J.L. (2012). Sperm wars and the evolution of male fertility.
851 *Reproduction*, 144, 519–534.
852 Siviter, H., Bailes, E.J., Martin, C.D., Oliver, T.R., Koricheva, J., Leadbeater, E., *et al.* (2021).
853 Agrochemicals interact synergistically to increase bee mortality. *Nature*, 596, 389–392.

854 Sokolova, I.M. (2013). Energy-limited tolerance to stress as a conceptual framework to
855 integrate the effects of multiple stressors. *Integr. Comp. Biol.*, 53, 597–608.
856 Sørensen, J.G., Kristensen, T.N. & Loeschcke, V. (2003). The evolutionary and ecological role
857 of heat shock proteins. *Ecol. Lett.*, 6, 1025–1037.
858 Stehle, S. & Schulz, R. (2015). Agricultural insecticides threaten surface waters at the global
859 scale. *Proc. Natl. Acad. Sci.*, 112, 5750–5755.
860 Tran, T.T., Janssens, L., Dinh, K.V. & Stoks, R. (2018). Transgenerational interactions
861 between pesticide exposure and warming in a vector mosquito. *Evol. Appl.*, 11, 906–
862 917.
863 Uller, T., Nakagawa, S. & English, S. (2013). Weak evidence for anticipatory parental effects
864 in plants and animals. *J. Evol. Biol.*, 26, 2161–2170.
865 Verberk, W.C.E.P., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L., *et al.* (2016).
866 Does oxygen limit thermal tolerance in arthropods? A critical review of current
867 evidence. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.*, 192, 64–78.
868 Verheyen, J. & Stoks, R. (2019). Current and future daily temperature fluctuations make a
869 pesticide more toxic: Contrasting effects on life history and physiology. *Environ.*
870 *Pollut.*, 248, 209–218.
871 Verheyen, J. & Stoks, R. (2023). Thermal performance curves in a polluted world: too cold
872 and too hot temperatures synergistically increase pesticide toxicity. *Environ. Sci.*
873 *Technol.*, 57, 3270–3279.
874 Walsh, B.S., Parratt, S.R., Atkinson, D., Snook, R.R., Bretman, A. & Price, T.A. (2019).
875 Integrated approaches to studying male and female thermal fertility limits. *Trends Ecol.*
876 *Evol.*, 34, 492–493.
877 Walsh, M.R., Whittington, D. & Funkhouser, C. (2014). Thermal transgenerational plasticity
878 in natural populations of *Daphnia*.

879 Wan, N.-F., Fu, L., Dainese, M., Kiær, L.P., Hu, Y.-Q., Xin, F., *et al.* (2025). Pesticides have
880 negative effects on non-target organisms. *Nat. Commun.*, 16, 1360.
881 Wang, X., Ma, D., Jin, Q., Deng, S., Stančin, H., Tan, H., *et al.* (2019). Synergistic effects of
882 biomass and polyurethane co-pyrolysis on the yield, reactivity, and heating value of
883 biochar at high temperatures. *Fuel Process. Technol.*, 194, 106127.
884 Wang, Y.-C., Chang, Y.-W., Bai, J., Zhang, X.-X., Iqbal, J., Lu, M.-X., *et al.* (2021a). High
885 temperature stress induces expression of CYP450 genes and contributes to insecticide
886 tolerance in *Liriomyza trifolii*. *Pestic. Biochem. Physiol.*, 174, 104826.
887 Wang, Y.-C., Chang, Y.-W., Bai, J., Zhang, X.-X., Iqbal, J., Lu, M.-X., *et al.* (2021b).
888 Temperature affects the tolerance of *Liriomyza trifolii* to insecticide abamectin.
889 *Ecotoxicol. Environ. Saf.*, 218, 112307.
890 Xiao, D., Zhao, J., Guo, X., Chen, H., Qu, M., Zhai, W., *et al.* (2016). Sublethal effects of
891 imidacloprid on the predatory seven-spot ladybird beetle *Coccinella septempunctata*.
892 *Ecotoxicology*, 25, 1782–1793.
893 Yu, C.-W. & Liao, V.H.-C. (2016). Transgenerational Reproductive effects of arsenite are
894 associated with H3K4 dimethylation and SPR-5 downregulation in *Caenorhabditis*
895 *elegans*. *Environ. Sci. Technol.*, 50, 10673–10681.
896 Zelezniak, A., Andrejev, S., Ponomarova, O., Mende, D.R., Bork, P. & Patil, K.R. (2015).
897 Metabolic dependencies drive species co-occurrence in diverse microbial communities.
898 *Proc. Natl. Acad. Sci.*, 112, 6449–6454.
899 Zhang, X., Yu, X. & Chen, J. (2008). High temperature effects on yeast-like endosymbiotes
900 and pesticide resistance of the small brown planthopper, *Laodelphax striatellus*. *Rice*
901 *Sci.*, 15, 326–330.

902 Zhang, Y., Cai, T., Ren, Z., Liu, Y., Yuan, M., Cai, Y., *et al.* (2021a). Decline in symbiont-
903 dependent host detoxification metabolism contributes to increased insecticide
904 susceptibility of insects under high temperature. *ISME J.*, 15, 3693–3703.
905 Zhang, Y., Xu, G., Jiang, Y., Ma, C. & Yang, G. (2021b). Sublethal effects of Imidacloprid on
906 fecundity, apoptosis and virus transmission in the small brown planthopper *Laodelphax*
907 *striatellus*. *Insects*, 12, 1131.
908 Zhou, C., Liu, L., Yang, H., Wang, Z., Long, G. & Jin, D. (2017). Sublethal effects of
909 imidacloprid on the development, reproduction, and susceptibility of the white-backed
910 planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae). *J. Asia-Pac. Entomol.*, 20,
911 996–1000.
912 Zwoinska, M.K., Rodrigues, L.R., Slate, J. & Snook, R.R. (2020). Phenotypic Responses to
913 and Genetic Architecture of Sterility Following Exposure to Sub-Lethal Temperature
914 During Development. *Front. Genet.*, 11, 573.
915

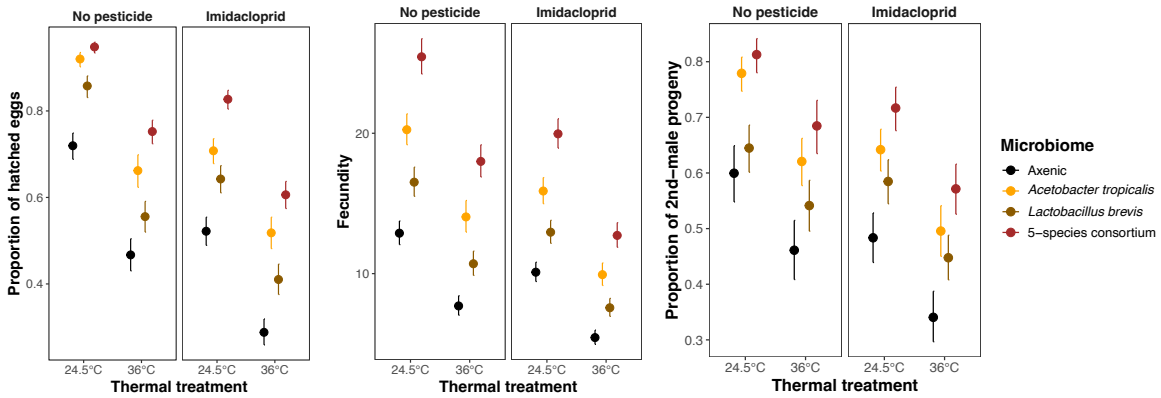

Figure 1 Effects of varying thermal and pesticide conditions and microbiome types on (A) hatching success, (B) male-mediated female fecundity, and (C) competitive fertilization success. Each circle represents the mean of all replicates in that treatment combination with error bars corresponding to 95% confidence intervals.

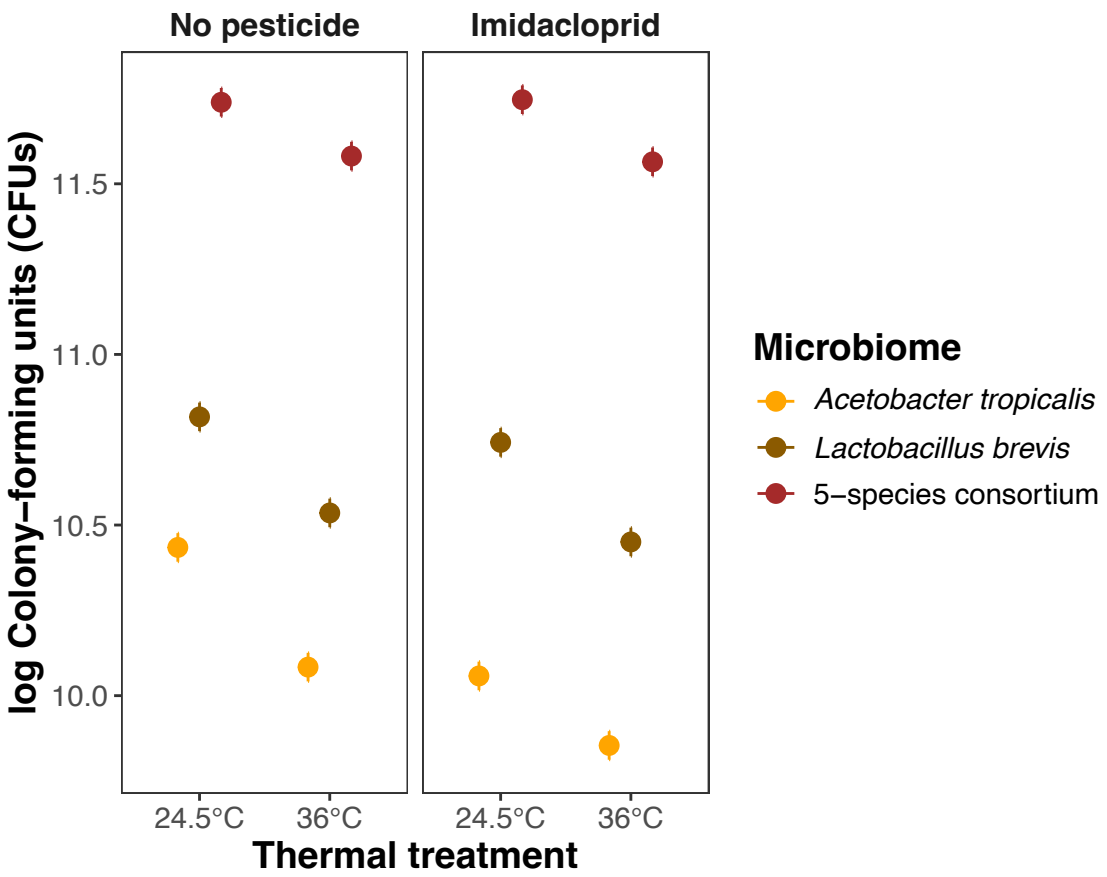

Figure 2 Bacterial abundance/colony forming units of different microbial treatments in response to varying thermal and pesticide conditions. Each circle represents the mean of all replicates in that treatment combination with error bars corresponding to 95% confidence intervals.

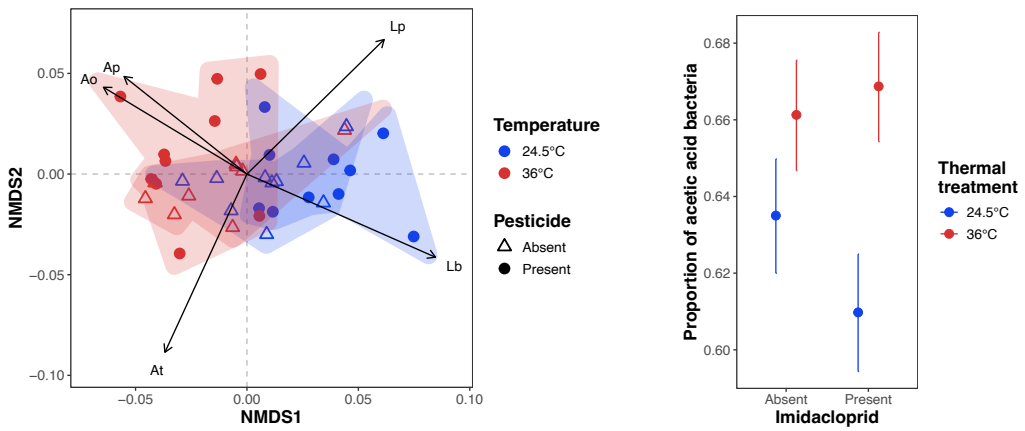

Figure 3 Bacterial abundances in the five-species consortium in response to thermal and pesticide treatments. A: Non-metric multidimensional scaling (NMDS) ordination (Bray-Curtis distance, $k = 4$, stress = 0.046) of microbial community composition across temperature and pesticide treatments. Shaded 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.

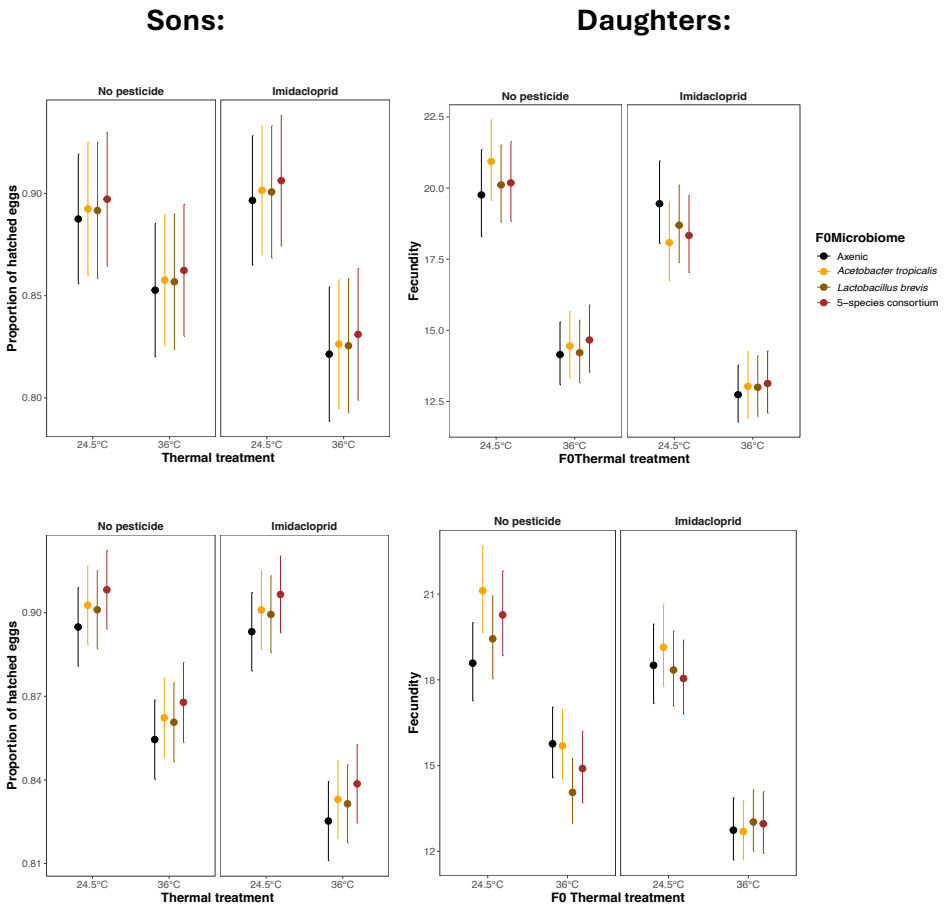

Figure 4 Fitness consequences of paternal thermal and pesticide treatments on fitness measures in male (A, B) and female (C, D) offspring. Panels A and C depict the proportion of eggs hatched out of total number of eggs fertilized, panels B and D the fecundity either of the sons' mates (B) or of the daughters (D). Each circle represents the mean of all the replicates in that treatment combination with error bars corresponding to 95% confidence intervals.