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1 **A sex-specific trade-off between pesticide resistance and thermal**
2 **tolerance in *Tetranychus urticae***

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17

18 **Abstract**

19 Current pest management relies extensively on pesticide application worldwide, despite the frequent
20 rise of pesticide resistance in crop pests. This is particularly worrisome because resistance is often not
21 costly enough to be lost in populations after pesticide application, resulting in increased dependency
22 on pesticide application. As climate warming increases, effort should be put into understanding how
23 thermal tolerance will affect the persistence of pesticide resistance in populations. To address this, we
24 measured thermal tolerance in two populations of the spider mite crop pest *Tetranychus urticae* that
25 differ in the presence or absence of a target-site mutation conferring resistance to etoxazole pesticide.

26 We found that developmental time and fertility, but not survival, were negatively affected by
27 increasing temperatures in the susceptible population. Furthermore, we found no difference between
28 resistant and susceptible populations in all life-history traits when both sexes developed at control
29 temperature, nor when females developed at high temperature. Resistant heat-stressed males, in
30 contrast, showed lower fertility than susceptible ones, indicating a sex-specific trade-off between
31 thermal tolerance and pesticide resistance. This suggests that global warming can lead to reduced
32 pesticide resistance in natural populations. However, resistant females, being as affected by high
33 temperature as susceptible individuals, may buffer the toll in resistant male fertility. In addition, the
34 decrease in developmental time of both sexes at high temperature may accelerate adaptation to both
35 temperature and pesticide, which can promote the maintenance of resistant populations under climate
36 warming.

37 **Keywords:** reproductive success; climate warming; crop pests; fertility

38

39 **Author Contribution Statement**

40 LRR conceived the study, with input from SM; LRR and SGC designed the experimental
41 methodology; FZ conceived the backcross procedure and created the populations that were used in this
42 study with the help of LRR and IS. IS was in charge of the maintenance of the populations after their
43 creation. SGC collected and analysed the experimental data; SGC and LRR led the writing of the
44 manuscript, with significant contributions from SM and FZ. All authors gave final approval for
45 publication. The authors declare no conflict of interest.

46

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57 **Introduction**

58 Crop pests are responsible for enormous economic losses worldwide (Culliney 2014; Oliveira et al.
59 2014). Particularly, insects and mites account for most of the damage to crops, with estimated losses
60 of 10-20% pre-harvest and 5-10% post-harvest (Culliney 2014). Current pest management relies
61 extensively on pesticide application, despite its unambiguous adverse effects (IPBES 2019; Janssen
62 and van Rijn 2021; Jacquet et al. 2022). Indeed, the use of pesticides is one of the main drivers of
63 biodiversity loss, including of biological control agents (Geiger et al. 2010; Sánchez-Bayo and
64 Wyckhuys 2019; IPBES 2019), and it is highly detrimental to the environment and human health
65 (Bourguet and Guillemaud 2016; Kim et al. 2017). The evolution of pesticide resistance in crop pests
66 (Bass et al. 2015; Hawkins et al. 2019) along with the loss of biocontrol agents and other ecosystem
67 services, in turn, increases the dependency on pesticide application to ensure pest control, resulting in
68 the emergence of a “pesticide treadmill” (van den Bosch 1989).

69 Current climate warming is leading to a continuous increase in intensity and frequency of
70 temperature extremes on a global scale (Seneviratne et al. 2021). The response of arthropod crop pests
71 to high temperature can lead to increased or decreased crop losses depending on the pest species and
72 other varying biotic and abiotic factors (Skendžić et al. 2021). For instance, decreases in crop losses
73 have been attributed to reduced physiological performance at extreme temperatures and to range
74 contraction of insect pests (Lehmann et al. 2020). In these cases, one could expect the need for pest
75 control measures to be reduced. In turn, many crop pests respond to high temperatures by increasing in
76 number (Lehmann et al. 2020) due, for example, to a rise in metabolic rate and population growth
77 (Deutsch et al. 2018), or to the disruption of biological control as biocontrol agents show lower
78 tolerance to high temperatures (Montserrat et al. 2013). When this increase in crop number occurs, it
79 may be necessary to increase pesticide use (Delcour et al. 2015).

80 Given that pest management greatly depends on pesticide application, effort should be put into
81 understanding the reciprocal interaction between thermal tolerance and pesticide resistance, such as to
82 determine how persistent will pesticide resistance be under climate warming. In fact, even if

83 agricultural practices are moving towards a reduction in the use of pesticides, resistant genotypes can
84 remain in the environment for many generations (Kontsedalov et al. 1998; Bielza et al. 2008),
85 especially those that are not costly in pesticide-free environments (Bielza et al. 2008; Abbas et al.
86 2014). How these genotypes will respond to climate change is thus key to understand the effect of
87 climate change on crop pest populations. Up until now, various studies have shown that resistance to
88 pesticide changes with temperature, often becoming reduced under heat (reviewed in Matzrafi 2019).
89 However, evidence on whether thermal tolerance differs between resistant and susceptible individuals
90 in a pesticide-free environment is not abundant (but see Fulton et al. 2021; Langmüller et al. 2020; Li
91 et al. 2007; Ma et al. 2021; Yang et al. 2018; Zhang et al. 2015). This is unfortunate because such
92 knowledge is crucial to predict the dynamics of crop pests under climate warming given that,
93 depending on the outcome, one could expect the maintenance or loss of pesticide resistance in a
94 population and thus the failure or success of pesticide applications, respectively.

95 Increase in the efficacy of pesticides under global warming would be expected when pesticide
96 resistance and thermal tolerance trade-off, given the expected decline in the number of resistant
97 individuals in such a scenario. This has been shown in a few studies (Li et al. 2007; Zhang et al. 2015;
98 Yang et al. 2018). For example, in the diamondback moth *Plutella xylostella*, chlorpyrifos-resistant
99 individuals have significantly lower survival, adult emergence rates and higher damages of wing veins
100 under heat stress than susceptible ones (Zhang et al. 2015). In turn, the efficacy of pesticides would be
101 expected to decrease whenever resistant individuals are also more tolerant to heat stress than
102 susceptible ones, meaning they will spread quicker in the population under climate warming. For
103 example, in *Drosophila simulans*, differences in viability between haplotypes susceptible and resistant
104 to carbamate and organophosphate are reduced at high temperatures, meaning the resistant haplotypes
105 have lost their disadvantage compared to susceptible ones under these conditions (Langmüller et al.
106 2020).

107 The majority of studies focusing on the costs of pesticide resistance disregard the possibility of
108 sex-specific responses. This is unfortunate, as the meagre evidence of such a phenomenon call
109 attention to its important implications. Indeed, in *Drosophila melanogaster*, resistance to DDT is

110 costly in males but increases female reproductive output in the absence of pesticide (Smith et al.
111 2011), which helps explaining why the resistance allele was present in populations at low frequency
112 prior to the widespread use of DDT (Rostant et al. 2015). Likewise, extensive work has demonstrated
113 that understanding the effect of temperature in each sex is a crucial step to accurately predict the
114 impact of climate change on organisms. For instance, evidence of sex-specific thermal sensitivity in
115 different taxa accumulate (Vasudeva et al. 2014; Iossa 2019), with reproductive traits being affected at
116 lower temperatures in males than in females (e.g., David et al. 2005; Iossa 2019; Rodrigues et al.
117 2022b; Sales et al. 2018; van Heerwaarden and Sgrò 2021). As such, male, not female, thermal
118 fertility limits have been identified as the best predictors of the response of populations to climate
119 change (Parratt et al. 2021; van Heerwaarden and Sgrò 2021). In haplodiploid species, heat-induced
120 sterility can also lead to variation in sex ratio and productivity, depending on the sex that is being
121 affected (Costa et al. 2023). Indeed, because fertilized eggs only generate daughters, whereas sons
122 develop from unfertilized eggs, male sterility should lead to a more male-biased sex ratio and female
123 sterility to fewer offspring. Such sex-specific responses are bound to affect plastic and/or evolutionary
124 responses of populations (Costa et al. 2023). Therefore, disregarding sex-specific responses
125 compromises our ability to accurately predict the impact of climate warming on the persistence of
126 pesticide-resistance in populations. Yet, to date no study has investigated the existence of such sex-
127 specific responses.

128 Here, we tested the effect of high temperature and its interaction with pesticide resistance on
129 three life-history traits (survival, developmental time and fertility) in *Tetranychus urticae*. This spider
130 mite is a haplodiploid polyphagous agricultural pest that occurs on a wide variety of plant species
131 across the world, some of which of great economic significance (Migeon et al. 2010). Pesticides are
132 commonly used to control the spread of mite population in crops, but the success of this control
133 strategy is often compromised by the spider mites' exceptional ability to rapidly evolve resistance
134 (Van Leeuwen et al. 2010). We have created two populations with the same genetic background but a
135 different resistance status (susceptible *vs* resistant) to etoxazole, an acaricide commonly used on
136 populations of this species worldwide (Kim et al. 2008). This allows us to attribute any observed

137 differences between populations to the resistance allele or to evolutionary changes in the genome due
138 to its presence. Etoxazole resistance has been thoroughly characterized in *T. urticae* (Van Leeuwen et
139 al. 2009, 2012): this pesticide exhibits high efficacy as a chitin biosynthesis inhibitor in *T. urticae*
140 during the developmental stages, being lethal to eggs and juveniles, but not adults (Nauen and
141 Smaghe 2006; Van Leeuwen et al. 2012) and resistance is recessive and conferred by a single
142 nonsynonymous mutation in the chitin synthase (Van Leeuwen et al. 2012). Despite this wealth of
143 information, no study has yet tested if the resistance status of each sex elicits specific responses to
144 temperature. Here, we set out to fill this gap.

145

146 **Materials and Methods**

147 **Spider mite populations**

148 Two populations of the spider mite *Tetranychus urticae* red form (a.k.a. *T. cinnabarinus*; Cruz et al.,
149 2021) that share the same genetic background, except for the presence or absence of an allele
150 conferring resistance to the pesticide etoxazole (*i.e.*, a single nucleotide mutation on the chitin
151 synthase 1 gene, CHS1; Van Leeuwen et al. 2012), were used in the experiments. The procedure used
152 to create these populations is depicted in Fig. 1 and fully described in the sections below.

153 *Creation of the Wi.SS populations*

154 Five *Wolbachia*-infected homozygous etoxazole-susceptible (“Wi.SS”) population replicates were
155 initially created in 2015, each by merging six compatible *Wolbachia*-infected populations collected in
156 the region of Lisbon, Portugal, in 2013 (Zélé et al. 2018a, 2020b). Each population replicate was
157 initially created with a census population size of 200 mated females and subsequently maintained with
158 discrete generations at this same population size (*cf.* detailed procedure for the creation and
159 maintenance of these populations, called “Infected controls; iC”, in Rodrigues et al. 2022a). Full
160 homozygosity for the etoxazole susceptible allele was subsequently confirmed by PCR-RFLP (as

161 described in Van Leeuwen et al. 2012) for each of the field-derived populations and each of the *Wi.SS*
162 replicates.

163 *Creation of the Wi.RR populations*

164 Five *Wolbachia*-infected homozygous etoxazole-resistant (“*Wi.RR*”) population replicates were
165 created in 2018 by introgression of an etoxazole-resistance allele into each of the *Wi.SS* population
166 replicates (Fig. 1 and detailed procedure in Suppl. Fig. 1). The etoxazole-resistance allele originated
167 from the homozygous etoxazole-resistant (RR) red lab strain *SB9*, collected in Crete, Greece, in 2006
168 (Van Leeuwen et al. 2012). As coinfection with *Wolbachia* and *Rickettsia* was detected in this strain
169 (using the multiplex PCR procedure described in Zélé et al. 2018b), and because these endosymbionts
170 may cause reproductive incompatibilities with the *Wolbachia* strain that is present in the *Wi.SS*
171 populations, *SB9* was treated with a rifampicin solution at 0.05%, w/v for one generation as described
172 in Zélé et al. (2020a) to create the symbiont-free donor parent strain *SB9.rif*. We then used a backcross
173 procedure ensuring the complete introgression of the etoxazole-resistance allele from the *SB9.rif* donor
174 strain into each of the *Wi.SS* recurrent parent populations, with an estimated retention of 99.6% of
175 their nuclear background, and complete retention of their mitochondrial background and *Wolbachia*
176 infection.

177 At the onset of the backcross procedure, *SB9.rif* males were crossed with females from each of
178 the *Wi.SS* population replicates to obtain a 100% heterozygous F1 female progeny (hence all “*Wi.RS*”:
179 *Wolbachia*-infected with a resistant and a susceptible allele). Given haplodiploidy in spider mites and
180 because the allele conferring resistance to etoxazole is recessive, these females were isolated as virgin
181 to produce “*Wi.S*” and “*Wi.R*” F2 haploid males (note that this step replaces the usual self-cross step
182 for the introgression of recessive alleles in diploid organisms). Resistant F2 males (*Wi.R*) were then
183 selected by being placed on etoxazole-soaked cotton (diluted in water at 0.5g/L) during the
184 development of the male offspring, and subsequently backcrossed with females from the recurrent
185 parent population *Wi.SS* to produce a new generation of heterozygous females (*i.e.*, the first generation
186 of backcrossed females; BC₁). Heterozygous BC₁ females were, as their grandmothers, isolated as
187 virgin to produce a new generation of males (*i.e.*, the progeny of “non crossed” females; NC₁), which

188 were again selected for etoxazole resistance during their development. This procedure was repeated
189 for six additional backcrosses (BC₂NC₂ to BC₇NC₇). At the end of the procedure, a subset of the
190 progeny resulting from the last generation of backcross (*Wi.RS* females and *Wi.S* males; BC₇) was self-
191 crossed (SC₁) to obtain *Wi.RS* and *Wi.SS* females, which were crossed with NC₇ selected cousins.
192 Their offspring (SC₂) was then exposed to etoxazole to select the homozygous resistant *Wi.RR* females
193 and *Wi.R* males that founded the replicated populations “*Wi.RR*”. A census population size of at least
194 200 females and 100 males was used at each step of the procedure (*cf.* detailed procedure in Suppl.
195 Fig. 1). The concentration of etoxazole used is higher than the LC100 seen in Van Leeuwen et al.
196 (2012), having the same effect on our populations as it led them to be 100% homozygous resistant.

197 *Creation and merge of the Wu.SS and Wu.RR populations*

198 The five *Wi.SS* and the five *Wi.RR* population replicates (each with a population size of 200 females)
199 were subsequently treated for one generation with rifampicin (0.05% w/v), following the procedure
200 described in Zélé et al. (2020a). Each newly created *Wu.SS* and *Wu.RR* population replicates was
201 subsequently maintained in discrete generations (population size of 200 females) in absence of
202 antibiotics (*i.e.*, under the same conditions as the untreated populations). Three generations later, the
203 presence/absence of *Wolbachia* and that of the etoxazole-resistance allele were confirmed in a pool of
204 100 females from each *Wi.SS*, *Wi.RR*, *Wu.SS* and *Wu.RR* population replicate by multiplex PCR (see
205 Zélé et al. 2018b) and PCR-RFLP (see Van Leeuwen et al. 2012), respectively. All replicates from
206 either the *Wu.SS* or the *Wu.RR* populations, were then merged in September 2020 to create the
207 susceptible and resistant base populations used in this study, respectively.

208

209 **Rearing conditions**

210 Since their creation and merging, both *Wu.SS* and *Wu.RR* were maintained in large numbers (> 2000)
211 under continuous generations on entire plants. These populations were kept on bean leaves (*Phaseolus*
212 *vulgaris*, variety Contender, provided by Germisem, Portugal) under controlled conditions (25°C;
213 photoperiod of 16L:8D). Bean plants were germinated and grown in an isolated and herbivore-free

214 room for 14 days under controlled conditions (photoperiod of 16L at 25°C: 8D at 20°C) before being
215 infested with spider mites.

216

217 **Experimental setup**

218 *Effect of high developmental temperatures on life-history traits*

219 First, we characterized the impact of four temperatures above the control temperature in the
220 laboratory (control temperature: 25°C, high temperatures: 33, 35, 36 and 37°C) during development
221 on the developmental time, survival and fertility of pesticide-susceptible spider mites (from *Wu.SS*).

222 To assess the effect of temperature on developmental time and juvenile survival, mite cohorts
223 were created by placing on a bean leaf patch (ca. 75 cm²) 100 mated females for 2 days at 25°C, so
224 they could oviposit synchronously. Once adult, the offspring of those females was transferred to one
225 of the tested temperatures (25, 33, 35, 36 or 37°C) for three days, after which a maximum of 32
226 offspring females were individually placed on bean leaf discs (2.55 cm²) to oviposit for 24 hours at the
227 same temperature. Egg development was followed daily, and the number of days elapsed until the first
228 adult male and female offspring emerged (*i.e.*, developmental time), as well as the number of eggs that
229 reached adulthood (*i.e.*, number of surviving juveniles) were recorded.

230 Due to logistic constraints, three assays were performed, always including the control
231 temperature (25°C): i) 25°C, 33°C and 37°C; ii) 25°C and 35°C; iii) 25°C and 36°C. In all cases,
232 replicates with damaged (*i.e.*, hurt during manipulation) mothers were excluded from the analyses.
233 Additionally, in the analysis of all independent variables, patches with females that did not lay eggs
234 were excluded. Overall, we analysed 67, 65 and 82 replicates (*i.e.*, patches) of female developmental
235 time, male developmental time and number of surviving juveniles, respectively (see Table S1 for more
236 details). Due to the low number of adult females surviving and ovipositing at 37°C, this temperature
237 was not included in the statistical analyses of developmental time and number of surviving juveniles
238 (see Table S2).

239 To determine the impact of heat on fertility, we focused only on female fertility, as we
240 assumed that male fertility would also be hindered at the temperature females are affected, since males
241 generally suffer from a reduction in fertility at lower high temperatures than females in several
242 arthropod species (David et al. 2005; Sales et al. 2018; Iossa 2019; Zwoinska et al. 2020; van
243 Heerwaarden and Sgrò 2021). Cohorts were created with mated females as before, but they were
244 directly maintained at one of the tested temperatures (25, 33, 35, 36 or 37°C). Before the offspring
245 reached adulthood, deutonymph quiescent males and females were isolated separately to ensure
246 virginity. Once adults (ca. 24 hours later), a maximum of 40 pairs of virgin females and males were
247 isolated on bean leaf discs (2.55 cm²) to mate and oviposit. Five treatments were established: pairs
248 where both individuals developed at 25°C (control; ♀25 x ♂25) and pairs where only the female
249 developed at one of the high temperatures (♀33 x ♂25, ♀35 x ♂25, ♀36 x ♂25 and ♀37 x ♂25). Pairs
250 were maintained during 24 hours, after which males were discarded. Females were left to lay eggs for
251 two more days, after which they were also discarded, and the eggs were counted. Leaf discs were kept
252 at the female developmental temperature from pairing until the female was removed, being placed at
253 25°C afterwards. The survival status of both male and female was recorded until removal. Once
254 reaching adulthood, offspring was counted, sexed and removed 12 and 14 days after the onset of
255 oviposition. Daily fecundity and offspring number were thus used as proxies for female fertility.

256 As in the previous experiment, temperature treatments were distributed in three assays: (i) 25°C, 33°C
257 and 37°C; (ii) 25°C and 35°C; (iii) 25°C and 36°C. For the first assay, we performed two blocks,
258 which occurred in two consecutive days. The other two assays were done in a single block. In all
259 cases, replicates with individuals forming the mating pairs that were damaged during manipulation
260 were excluded from the analyses. In addition, patches with females that did not lay eggs were
261 excluded from the analysis of offspring number. Overall, we analysed 140 and 117 replicates (*i.e.*,
262 patches) of daily fecundity and offspring number, respectively (see Table S1 for more details).

263 *Interaction between pesticide resistance and response to high developmental temperature*

264 To test if the effect of high developmental temperature on developmental time, survival and fertility
265 differed depending on the resistance status of individuals, we exposed both resistant (*Wu.RR*) and

266 susceptible (*Wu.SS*) populations to either 25 or 36°C and repeated the experiments described above. In
267 this case, when testing fertility, we measured both sexes, with offspring number being used to access
268 female fertility and sex ratio to access male fertility, as only daughters contain the genetic material of
269 the father. Here, females were given four days to oviposit instead of three, and four treatments were
270 established to separately test the response of males and females to high developmental temperature:
271 pairs where both individuals developed at 25°C (control; ♀25 x ♂25), and pairs where each or both
272 sexes developed at 36°C (♀36 x ♂25, ♀25 x ♂36 and ♀36 x ♂36, respectively). Pairs were
273 maintained at 25°C when both individuals developed at that temperature. Otherwise, they were kept at
274 36°C until the sex that developed at 36°C was removed, at which time they were moved to 25°C.
275 When both sexes developed at 36°C, the transfer to 25°C occurred when the last individual of the pair
276 was removed. All other experimental details are as above.

277 Replicates were excluded according to the same criteria as described above. Overall, we
278 analysed the developmental time of females and males from 97 and 95 patches, respectively, the
279 number of surviving juveniles from 114 patches and the number and sex of offspring from 233 patches
280 (see Table S1 for more details).

281

282 **Statistical analyses**

283 All statistical analyses were performed using the software R (version 4.0.3; R Core Team 2020).
284 Depending on the data and error structure, we performed linear models (LM), linear mixed-effects
285 models (LMM), generalized linear models (GLM) and generalized mixed-effects models (GLMM)
286 implemented in *lme4* (version 1.1.26; Bates et al. 2015) and *glmmTMB* (version 1.0.2.1; Brooks et al.
287 2017; see Table S3 and S4). Maximal models were simplified by removing non-significant terms ($p >$
288 0.05) from the highest- to the lowest-order interaction (Crawley 2012). The significance of each
289 explanatory variable was determined by chi-squared tests for discrete distributions, and Wald F tests
290 for continuous distributions (Bolker et al. 2009). Graphic representations of the data were produced
291 with the software package *ggplot2* (version 3.3.3; Wickham 2016).

292 *Effect of high developmental temperatures on life-history traits*

293 The effect of developmental high temperatures on juvenile traits was assessed by analysing two traits:
294 the number of days from egg until adulthood (*i.e.*, number of days the first male or female took to
295 reach adulthood in a patch), used as a proxy for developmental time of each sex; and the number of
296 surviving juveniles (*i.e.*, number of juveniles surviving until the adult stage; Table S3). The effect of
297 developmental heat stress on female fertility, was determined by analysing daily fecundity and
298 offspring number (Table S3).

299 As temperatures were not tested simultaneously, we analysed the differences of each high
300 temperature to the control temperature. For that, within each assay, the mean trait value measured at
301 the control temperature was subtracted to the individual trait values measured at the high temperature
302 tested (trait value at the high temperature - \bar{x} trait value at 25°C). When the assay was done in more
303 than one block, the mean of the control temperature was calculated within each block, to account for
304 that variability. The new variable was used as dependent variable in the subsequent models.

305 All variables were analysed using LM models with a gaussian error distribution, except for the
306 number of surviving juveniles that was analysed using a LMM model (Table S3). The data were box
307 cox transformed (female developmental time: $\lambda = 1.94$; male developmental time: $\lambda = 0.73$; number of
308 surviving juveniles: $\lambda = 0.91$; *MASS* package, *boxcox* function; version 7.3.53; Venables and Ripley
309 2002) to improve the fit of the model in each variable, except for daily fecundity and offspring
310 number. To analyse the impact of temperature on developmental time and number of surviving
311 juveniles, the developmental temperature (25, 33, 35 or 36°C) was included in the models as fixed
312 factor (Table S3). Fecundity was added as random factor in the model testing the impact of
313 temperature on the number of surviving juveniles, to account for the variation in the number of eggs
314 laid (Table S3). In the analyses of daily fecundity and offspring number, the type of cross between
315 males and females from different developmental temperatures ($\text{♀}_{25} \times \text{♂}_{25}$, $\text{♀}_{33} \times \text{♂}_{25}$, $\text{♀}_{35} \times \text{♂}_{25}$,
316 $\text{♀}_{36} \times \text{♂}_{25}$ or $\text{♀}_{37} \times \text{♂}_{25}$) was included in the model as fixed factor (Table S3).

317 To determine if the difference of each temperature to the control was significant, we used the
318 function *summary* and took the value that reports the difference of the first temperature listed on the
319 dataset to an intercept of zero (which in this case corresponds to the mean trait value at control
320 temperature). This was repeated for all temperatures by reordering this variable on the dataset. A
321 *posteriori* contrasts with Bonferroni corrections of significant explanatory variables were made to test
322 differences among the different high temperatures using the *emmeans* package (version 1.5.3; Length
323 2020; Table 2).

324 *Interaction between pesticide resistance and response to high developmental temperature*

325 To test whether developmental heat stress differentially affected individuals with different resistance
326 status in a sex-specific manner, we analysed four traits: developmental time, the number of surviving
327 juveniles, the offspring number and the offspring sex ratio (Table S4).

328 Developmental time and the number of surviving juveniles were analysed using GLM and
329 GLMM models, respectively, with a Poisson error distribution (Table S4). The developmental
330 temperature of the individuals (25°C or 36°C), their resistance status (susceptible or resistant) and the
331 interaction between these two factors were included in all models as fixed factors. Fecundity was
332 included as a random factor in the analysis of the number of surviving juveniles.

333 The offspring number was analysed using a GLMM model with a quasi-Poisson error
334 distribution (Table S4). Offspring sex ratio, analysed as the proportion of daughters, was computed
335 using the function *cbind* with the number of daughters and sons as arguments (Table S4). This variable
336 was analysed with a GLMM model with a beta-binomial error distribution and a parameter to account
337 for zero inflation (*ziformula* ~1; package *glmmTMB*). In the analysis of both variables, the
338 developmental temperature of the female and of the male (25°C or 36°C, in both variables), the
339 resistance status of the pair (susceptible or resistant), as well as all possible interactions, were included
340 as fixed factors. We paired males and females from two different cohorts (*i.e.*, males and females
341 could be 9 or 10 day-old when developed at 25°C, or 7 or 8 day-old when developed at 36°C), so the

342 age of each sex was included as a random factor (Table S4). *A posteriori* contrasts with Bonferroni
343 corrections were made using the *emmeans* package (Length 2020; Table 4).

344

345 **Results**

346 **Effect of high developmental temperatures on life-history traits**

347 The developmental time of males and females was affected by the temperature they developed at
348 (Table 1). Indeed, the development of both sexes was faster at any of the high temperatures than at the
349 control temperature, and the individuals developed significantly faster at 35°C than at 36°C (Table 2;
350 Fig. 2a and b). In contrast, the number of surviving juveniles, accounting for the total number of eggs
351 produced, was not affected by the developmental temperature of individuals (Table 1; Fig. 2c).

352 Female fertility was affected by developmental temperature (Table 1), with both daily
353 fecundity and the offspring number being higher at 33°C and lower at 35, 36 and 37°C, compared to
354 the control temperature (Table 2; Fig. 2d and e). In both cases, 36 and 37°C showed the lowest values
355 compared to the other temperatures (Table 2; Fig. 2d and e).

356 In sum, fertility was most affected at 36 and 37°C. However, at 37°C adult survival was
357 drastically hindered (ca. 10% survival after 3 days of exposure; Table S2). Therefore, we used 36°C as
358 the sublethal temperature in subsequent tests.

359

360 **Interaction between pesticide resistance and response to high developmental** 361 **temperature**

362 The developmental time and number of surviving juveniles were not affected by the interaction
363 between developmental temperature and resistance status (female developmental time: $\chi^2_1 = 0.118$, $p =$
364 0.731 ; male developmental time: $\chi^2_1 = 0.359$, $p = 0.549$; number of surviving juveniles: $\chi^2_1 = 0.072$, p

365 = 0.789). However, these traits were equally affected by high developmental temperature (36°C) in
366 individuals from susceptible and resistant populations (Table 3; Fig. 3a, b and c; Fig. S2).

367 Fertility was affected by the interaction between the developmental temperature of the male
368 and the resistance status of the pair (Table 3). Indeed, resistant males produced fewer offspring and a
369 lower proportion of daughters than susceptible ones, but only when they developed at 36°C. (Table 4;
370 Fig. 3d and e). The female developmental temperature affected both traits similarly in the two
371 populations, as all pairs with females developed at 36°C produced fewer offspring and a lower
372 proportion of daughters than all pairs with females developed at 25°C (Table 3; Fig. 3d and e).
373 However, its interaction with the other two factors did not affect the offspring number (♀ temperature
374 * ♂ temperature: $\chi^2_1 = 3.170$, $p = 0.075$; ♀ temperature * Resistance status: $\chi^2_1 = 1.471$, $p = 0.225$; ♀
375 temperature * ♂ temperature * Resistance status: $\chi^2_1 = 0.207$, $p = 0.649$) or sex ratio (♀ temperature *
376 ♂ temperature: $\chi^2_1 = 0.322$, $p = 0.571$; ♀ temperature * Resistance status: $\chi^2_1 = 1.661$, $p = 0.197$; ♀
377 temperature * ♂ temperature * Resistance status: $\chi^2_1 = 3.062$, $p = 0.080$).

378

379 Discussion

380 Our results show that all life-history traits measured in the individuals from the susceptible population,
381 but the number of surviving juveniles, responded to high developmental temperatures in a non-linear
382 manner. At 36°C, fertility was affected the most, while survival was as yet not compromised. We thus
383 selected this temperature to test for sex-specific effects of temperature on individuals with different
384 resistant status. Developmental time and number of surviving juveniles were affected by temperature
385 but not by resistance status. In contrast, reproductive traits of resistant and susceptible individuals
386 were differentially affected by high developmental temperatures in a sex-specific manner. Indeed,
387 matings involving resistant males developed at high temperature resulted in fewer offspring and more
388 sons than matings involving susceptible males.

389 We found that the response of several traits to temperature was non-linear, with daily
390 fecundity and offspring number peaking at 33°C and the developmental time of both sexes reaching its

391 lowest at 35°C. Evidence of non-linear responses in this and other arthropods towards increasing
392 temperatures (Umoeok Akpassam et al. 2017; Zou et al. 2018; Sales et al. 2018; Zwoinska et al.
393 2020) and other environmental variables (Godinho et al. 2018, 2023; Guedes et al. 2022) are not
394 uncommon. In *Tetranychus*, some studies show a decrease in developmental time with temperature
395 (Kasap 2004; Praslička and Huszár 2004; Bayu et al. 2017; Zou et al. 2018), while other studies notice
396 a decrease followed by an increase in developmental time with rising temperatures (Riahi et al. 2013;
397 Hasanvand et al. 2020; Farazmand 2020). A peak in daily fecundity has also been recorded in previous
398 work, but the temperatures leading to such peak differ between studies (Kasap 2004; Riahi et al. 2013;
399 Zou et al. 2018). These differences may be related to the origin of the populations tested, given that
400 populations from different climates are adapted to specific sets of temperatures (Deutsch et al. 2008;
401 García-Robledo et al. 2016). Clearly, more studies are needed to understand the origin and
402 consequences of non-linear responses to temperature.

403 Several studies show that resistance to pesticide can trade off with life-history traits in
404 pesticide-free environments (e.g., Carriere et al. 1994; Okuma et al. 2018; Yan et al. 2014), including
405 in *T. urticae* (Stumpf and Nauen 2002; Sato et al. 2005; Nicastro et al. 2010, 2011), and specifically
406 when resistant to etoxazole (Stocco et al. 2016; Bajda et al. 2018). However, in our study we found no
407 cost of etoxazole resistance at control temperature for any of the traits measured, which should result
408 in the maintenance of pesticide resistance in these populations long after pesticide use. One possible
409 explanation for the discrepancy between studies could be the existence of different genetic bases of
410 resistance to the same pesticide (e.g., Van Leeuwen et al. 2012; Ilias et al. 2014; Adesanya et al.
411 2018), some entailing costs and others not. Another possibility could be that the observed costs are
412 associated with, but not due to, resistance. First, there could be non-genetic differences between
413 resistant and susceptible populations that are responsible for the costs observed. For instance, pesticide
414 resistant mosquitoes (*Culex pipiens*) are often more heavily infected with *Wolbachia* than susceptible
415 ones, with the costs associated with the resistance phenotype being due in part to the infection and not
416 to resistance (Duron et al. 2006). In turn, the differences between resistant and susceptible populations
417 could be genetic, the costs being due to differences in the genetic background. This was observed in

418 *Culex quinquefasciatus*, in which the fitness costs associated with resistance to organophosphate
419 identified when comparing strains from different locations were strongly reduced after backcrossing
420 the resistant gene into the genetic background of the susceptible strain (Amin and White 1984). Here,
421 we have eliminated the possibility of non-genetic differences due to symbionts by treating the
422 populations with antibiotics. Conversely to some previous studies (e.g., Okuma et al. 2018; Stocco et
423 al. 2016; Yan et al. 2014), we have also minimized genetic differences among resistant and susceptible
424 populations, by following an introgression procedure. Still, because we are studying genetically
425 variable populations, the resistant population may have evolved after introgression to compensate for
426 an existing cost of resistance. Such hypothesis would explain the discrepancy between our study and
427 that of Bajda et al. (2018), who found a cost of the same resistance gene in *T. urticae* after performing
428 a similar introgression procedure but on isogenic lines, thereby preventing evolution. All in all, this
429 suggests that the genetic background in which resistance to etoxazole is inserted plays a role in the
430 expression of costs, but more studies should be performed to confirm this possibility.

431 While resistance was not costly at control temperature, with the individuals from resistant and
432 susceptible populations showing similar values for all life-history traits, fertility was differently
433 affected at 36°C depending on the resistance status of the individuals. Indeed, the reduction in the
434 number of offspring and in the proportion of daughters felt after exposure to the developmental high
435 temperature was steeper in pairs involving resistant individuals than in those involving susceptible
436 individuals. These results are in accordance with a few other studies done on other crop pests such as
437 *Plutella xylostella* and *Nilaparvata lugens* (Li et al. 2007; Zhang et al. 2015; Yang et al. 2018),
438 showing that pesticide resistance trades-off with thermal tolerance. Such a cost of pesticide resistance
439 on fertility at high temperatures should reduce the selective advantage of resistant individuals, which
440 in turn should increase the efficacy of crop management by pesticide application. However, to the best
441 of our knowledge, none of these previous studies has investigated possible sex-specific responses, thus
442 risking overestimating the cost of resistance.

443 Here we find that the trade-off between pesticide resistance and thermal tolerance can be
444 observed in males but not in females. Several studies have shown that male thermal fertility limits are

445 the best predictors of extinction in several species (van Heerwaarden and Sgrò 2021). This suggests
446 that the fertility toll suffered by resistant spider mite males can foster the exclusion of resistance from
447 the population. On the other hand, the observed sex-specific response to temperature may buffer the
448 costs of pesticide resistance, assuming resistant females have the chance to mate with migrant males
449 from cooler places or mate multiply to compensate sterility. This last strategy has been observed in
450 several arthropod species, including spider mites (Berger et al. 2011; Sutter et al. 2019; Vasudeva et
451 al. 2021; Baur et al. 2022; Costa et al. 2023).

452 Given sex-specific responses, the sex-determination system of the species will also influence
453 the outcome of resistance in the population. First, because of haplodiploidy in spider mites, high
454 temperature affected not only the number, but also the sex ratio of the offspring. Each of these traits
455 entails different consequences for population dynamics. For instance, while both fecundity and sex
456 ratio influence population growth rate, they may do so in different ways, with the effects of sex ratio
457 depending on population structure but those of fecundity not being influenced by this factor (Metz and
458 Gyllenberg 2001). Second, resistance to etoxazole in spider mites is recessive (Van Leeuwen et al.
459 2012). Therefore, haploid males will express the resistance cost as soon as they carry one resistance
460 allele, promoting a quick elimination of resistance (Carrière 2003), which would not be the case in
461 diploid organisms, including diploid females from haplodiploid species.

462 Overall, we show that climate change can delay the growth of spider mite populations as
463 temperatures rise, negatively affecting several life-history traits, with pesticide resistant males being
464 more affected by temperature than susceptible males. While this is good news from a pest
465 management standpoint, the complex expectations stemming from its sex-determination system and
466 the possible buffering effect of females, coupled with the decrease in developmental time of both
467 sexes at high temperature may counter such forces. Indeed, decreased developmental time should
468 result in an increased number of generations per crop period, which in turn should accelerate
469 adaptation to both high temperature and pesticide, and lead to a faster expansion of populations. Effort
470 should be put into studying the evolution of populations exposed to both stressors, temperature and
471 pesticide, to better understand and ultimately predict the dynamics of crop pests in a changing world.

472

473 **Data Availability**

474 The data that support the findings of this study will be openly available at Figshare. For reviewing
475 purposes, we provide the corresponding private link: <https://figshare.com/s/6ea0871d134c948bd250>.

476

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724

725 **Table 1 Statistical results of the effect of high developmental temperatures on life-history traits.**

726 “Df”: degrees of freedom. “F”: the sum of squares obtained from the F-test. “Developmental time (♀)”
 727 and “Developmental time (♂)”: number of days the first female or male, respectively, took to reach
 728 adulthood in a patch; “Number of surviving juveniles”: number of juveniles surviving until the adult
 729 stage; “Daily fecundity”: mean number of eggs laid per day, representing female fertility; “Offspring
 730 number”: number of eggs that reached adulthood laid by females exposed to different temperatures
 731 (proxy for female fertility). “Developmental temperature”: temperature at which eggs were exposed
 732 until adulthood; “Temperature of pair”: developmental temperature of both females and males paired
 733 to mate and oviposit (offspring developed at 25°C). Statistically significant terms ($p \leq 0.05$) are
 734 represented in bold.

735

Var. of interest	Explanatory var.	Df (Df residuals)	F	P-value
Developmental time (♀)	Developmental temperature	2 (10.651)	4.08	<0.001
Developmental time (♂)	Developmental temperature	2 (6.153)	4.491	0.004
Number of surviving juveniles	Developmental temperature	2 (31.880)	2.2084	0.126
Daily fecundity	Temperature of pair	3 (51.478)	2074.07	<0.001
Offspring number	Temperature of pair	3 (70.384)	14176.4	<0.001

736

737

738 **Table 2** *A posteriori* contrasts of significant explanatory variables for the effect of high
739 **developmental temperatures on life-history traits.** *A posteriori* contrasts with Bonferroni
740 corrections were done to interpret the significant effect of the fixed factors. “T ratio”: the T-test value
741 obtained in each comparison. Comparisons were made between offspring developed at 25, 33, 35 or
742 36°C (“25°C”, “33°C”, “35°C” and “36°C”), and between pairs with males developed at 25°C and
743 females developed at 25, 33, 35, 36 or 37°C (“♀25°C”, “♀33°C”, “♀35°C”, “♀36°C” and “♀37°C”).
744 Statistically significant terms ($p \leq 0.05$) are represented in bold. *, represents marginally significant
745 values.

746

Var. of interest	Comparison	T ratio	P-value
Developmental time (♀)	25°C – 33°C	-40.712	<0.001
	25°C – 35°C	-46.158	<0.001
	25°C – 36°C	-39.64	<0.001
	33°C – 35°C	2.454	0.051*
	33°C – 36°C	-2.050	0.133
	35°C – 36°C	-4.610	<0.001
Developmental time (♂)	25°C – 33°C	-27.097	<0.001
	25°C – 35°C	-31.599	<0.001
	25°C – 36°C	-25.998	<0.001
	33°C – 35°C	1.733	0.264
	33°C – 36°C	-1.671	0.299
	35°C – 36°C	-3.507	0.003
Daily fecundity	♀25°C - ♀33°C	7.067	<0.001
	♀25°C - ♀35°C	-3.250	0.001
	♀25°C - ♀36°C	-6.114	<0.001
	♀25°C - ♀37°C	-9.871	<0.001
	♀33°C - ♀35°C	7.315	<0.001
	♀33°C - ♀36°C	9.325	<0.001
	♀33°C - ♀37°C	11.883	<0.001
	♀35°C - ♀36°C	2.025	0.269
	♀35°C - ♀37°C	4.361	<0.001
♀36°C - ♀37°C	2.268	0.150	
Offspring number	♀25°C - ♀33°C	6.156	<0.001
	♀25°C - ♀35°C	-3.631	<0.001
	♀25°C - ♀36°C	-12.327	<0.001

$\bar{Q}25^{\circ}\text{C} - \bar{Q}37^{\circ}\text{C}$	-9.810	<0.001
$\bar{Q}33^{\circ}\text{C} - \bar{Q}35^{\circ}\text{C}$	6.841	<0.001
$\bar{Q}33^{\circ}\text{C} - \bar{Q}36^{\circ}\text{C}$	13.136	<0.001
$\bar{Q}33^{\circ}\text{C} - \bar{Q}37^{\circ}\text{C}$	11.441	<0.001
$\bar{Q}35^{\circ}\text{C} - \bar{Q}36^{\circ}\text{C}$	5.860	<0.001
$\bar{Q}35^{\circ}\text{C} - \bar{Q}37^{\circ}\text{C}$	4.637	<0.001
$\bar{Q}36^{\circ}\text{C} - \bar{Q}37^{\circ}\text{C}$	-0.937	1.000

747

748

749 **Table 3 Significant statistical results of the interaction between pesticide resistance and response**
750 **to high developmental temperature.** “Df”: degrees of freedom. “ χ^2 ”: Chi-square value obtained in
751 each analysis. “Developmental time (♀)” and “Developmental time (♂)”: number of days the first
752 female or male, respectively, took to reach adulthood in a patch; “Number of surviving juveniles”:
753 number of juveniles surviving until the adult stage; “Offspring number”: number of eggs that reached
754 adulthood coming from crosses with females and males developed at high or control temperatures
755 (proxy for female fertility); “Offspring sex ratio”: proportion of adult daughters in the offspring (proxy
756 for male fertility). “Developmental temperature”: temperature at which eggs were exposed until
757 adulthood; “♀ temperature”: developmental temperature of the females paired; “♂ temperature”:
758 developmental temperature of the males paired; “Resistance status”: resistance or susceptibility to
759 etoxazole; “Fecundity”: number of eggs laid for 4 days. Statistically significant terms ($p \leq 0.05$) are
760 represented in bold.
761

Var. of interest	Explanatory var.	Df	χ^2	P-value
Developmental time (♀)	Developmental temperature	1	23.578	<0.001
	Resistance status	1	0.001	0.977
Developmental time (♂)	Developmental temperature	1	22.175	<0.001
	Resistance status	1	0.032	0.857
Number of surviving juveniles	Developmental temperature	1	62.199	<0.001
	Resistance status	1	0.047	0.829
Offspring number	♀ temperature	1	243.924	<0.001
	♂ temperature	1	2.492	0.114
	Resistance status	1	0.291	0.590
	♂ temperature * Resistance status	1	5.227	0.022
Offspring sex ratio	♀ temperature	1	10.957	<0.001
	♂ temperature	1	66.215	<0.001
	Resistance status	1	0.181	0.670
	♂ temperature * Resistance status	1	4.895	0.027

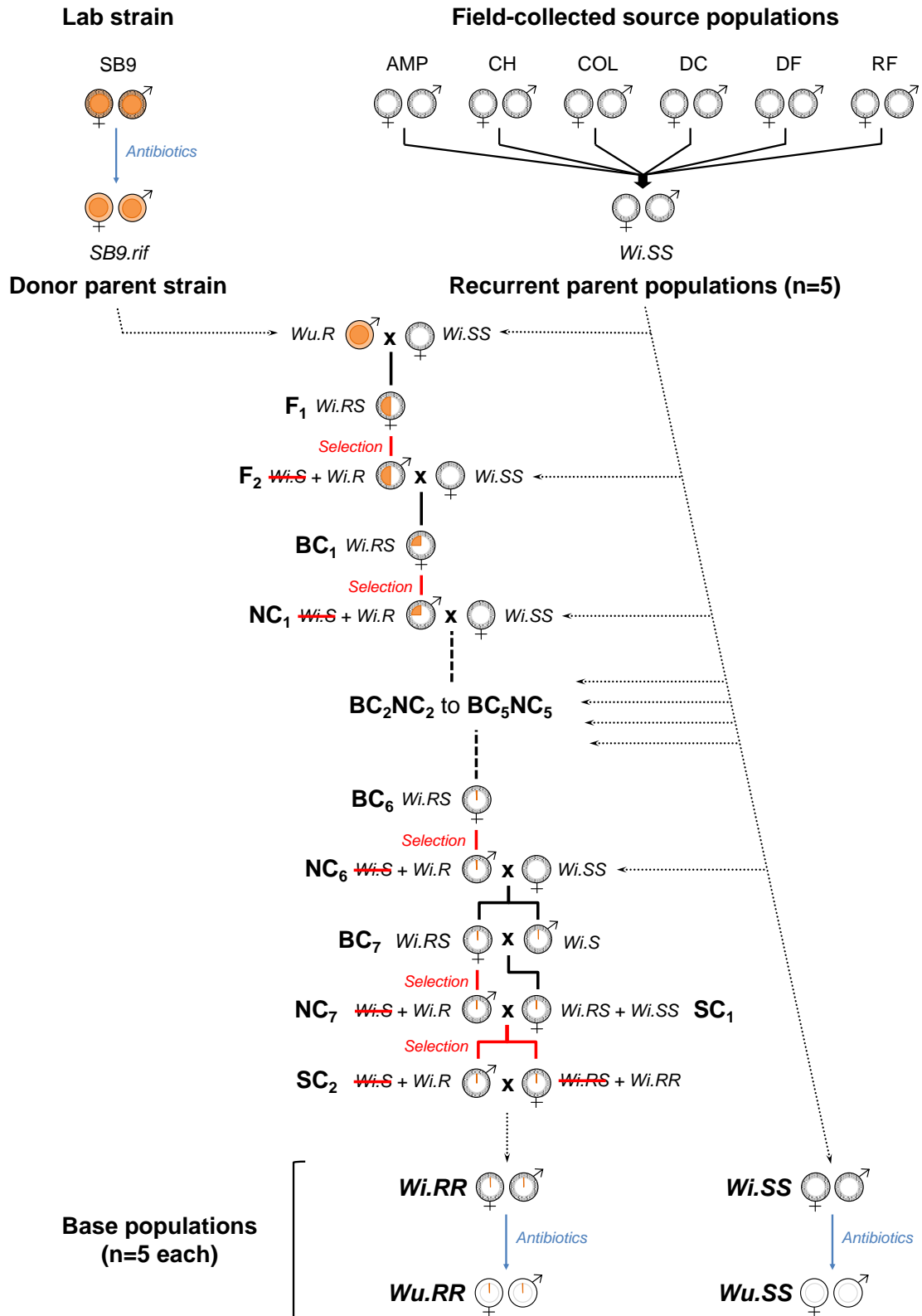
762 **Table 4** *A posteriori* contrasts of significant explanatory variables for the interaction between
763 **pesticide resistance and response to high developmental temperature.** *A posteriori* contrasts with
764 Bonferroni corrections were done to interpret the significant effect of the fixed factors. “T ratio”: the
765 T-test value obtained in each comparison. Comparisons were made between males developed at 25°C
766 or 36°C (“♂25” and “♂36”) and that were susceptible or resistant to etoxazole (“Susceptible” and
767 “Resistant”). Statistically significant terms ($p \leq 0.05$) are represented in bold.

768

Var. of interest	Comparison	T ratio	P-value
Offspring number	Susceptible ♂25°C – Susceptible ♂36°C	-1.659	0.394
	Resistant ♂25°C – Resistant ♂36°C	1.579	0.463
	Resistant ♂25°C – Susceptible ♂25°C	0.540	1.000
	Resistant ♂36°C – Susceptible ♂36°C	-2.705	0.029
Offspring sex ratio	Susceptible ♂25°C – Susceptible ♂36°C	8.560	<0.001
	Resistant ♂25°C – Resistant ♂36°C	8.137	<0.001
	Resistant ♂25°C – Susceptible ♂25°C	-0.426	1.000
	Resistant ♂36°C – Susceptible ♂36°C	-2.641	0.035

769

770



771

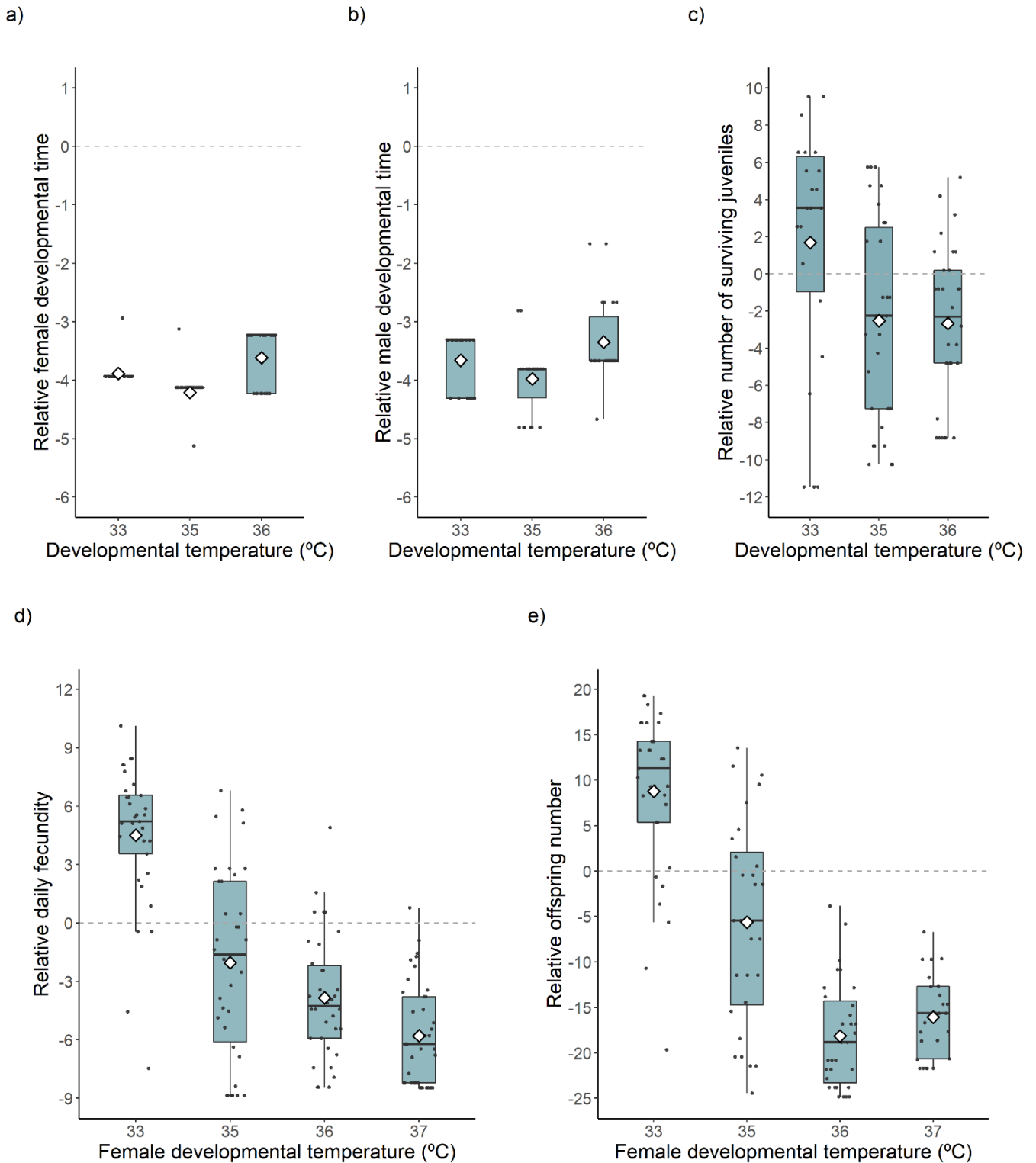
772 **Fig. 1** Backcross procedure used to introgress the etoxazole-resistant allele into the susceptible

773 *Wolbachia*-infected populations *Wi.SS* to create the resistant populations *Wi.RR*, and subsequent

774 antibiotic treatment to obtain the *Wolbachia*-uninfected populations *Wu.RR* and *Wu.SS*. The

775 entire procedure, from the creation of the *Wi.SS* populations to the obtention of all base populations,
776 was performed in five independent replicates. *Wu.RR* and *Wu.SS* replicates were then merged before
777 being used in the current study. Antibiotic treatments are represented in blue and selection for
778 etoxazole resistance is in red. Inner and outer circles within male/female symbols represent,
779 respectively, the proportion of nuclear and mitochondrial genomes from the donor (orange) and
780 recurrent (white) parent populations. Dotted-filled outer circles represent *Wolbachia*-infected
781 cytoplasm. BC: back-cross progeny; NC: no-cross progeny; SC: self-cross progeny.

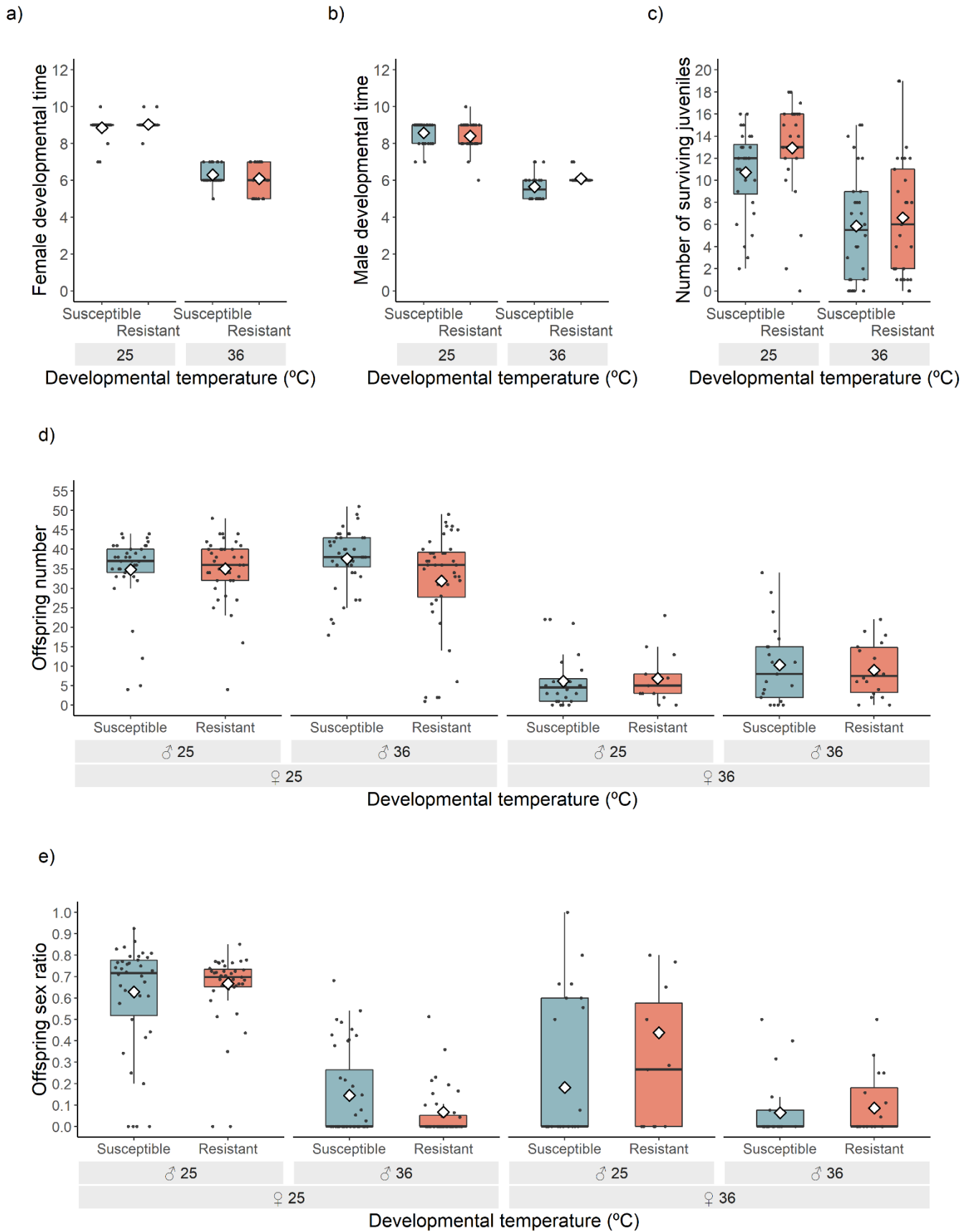
782



783 **Fig. 2 Effect of high developmental temperatures on life-history traits.** Difference between each
 784 tested temperature (33, 35, 36 and 37°C) and the mean value at 25°C (control; dashed line).
 785 Developmental time, defined as the day the first female (a) and male (b) in a patch reached adulthood,
 786 and number of surviving juveniles, defined as the number of eggs that reached adulthood (c), were

787 tested by allowing females that had developed at 25°C to oviposit at each indicated temperature. Data
788 obtained at 37°C were excluded due to lack of replication. Female fertility was tested by placing
789 females developed at each indicated temperature with males developed at 25°C, and allowing them to
790 oviposit at 25°C to assess their mean daily fecundity as the number of eggs laid per day (d), and
791 offspring production as the number of eggs that reached adulthood (e). Boxplots display a median line,
792 interquartile range (IQR) boxes, 1.5*IQR whiskers and data points. Black dots represent individual
793 replicates, white diamonds represent the mean values per conditions tested.

794



795 **Fig. 3 Sex-specific effects of the interaction between pesticide resistance and high developmental**
 796 **temperature.** Effect of 36°C during development on populations susceptible (blue bars) or resistant

797 (red bars) to the pesticide etoxazole. Developmental time was measured by the day the first female (a)
798 and male (b) in a patch reached adulthood, the number of surviving juveniles by the number of eggs
799 that reached adulthood (c), female fertility by the offspring number (d), and male fertility by the
800 offspring sex ratio (proportion of daughters; e). Boxplots display a median line, interquartile range
801 (IQR) boxes, 1.5*IQR whiskers and data points. Black dots represent individual replicates, white
802 diamonds represent the mean values per conditions tested.