The mating system affects the temperature sensitivity of male and female fertility.

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Key words: climate change, temperature, heat shock, fertility, fecundity, sperm, sexual conflict, sexual selection, mating system, thermal fertility limits.

Author contributions: J.B., D.B. and M.K. conceived the ideas and designed the experiments; J.B., D.J. and P.M.M. collected the data; J.B. and D.B. analysed the data; J.B. and D.B. led the writing of the manuscript. J.B., D.B. and M.K. contributed critically to the drafts and all authors gave final approval for publication.

Data availability: All data presented in this manuscript will be archived and made publicly available on Dryad upon acceptance.

Abstract (323 words)

1. To mitigate effects of climate change it is important to understand species responses to increasing temperatures. This has often been done by studying survival or activity at temperature extremes. Before such extremes are reached, however, effects on fertility may already be apparent.

2. Sex differences in the thermal sensitivity of fertility (TSF) could impact species persistence under climate warming because female fertility is typically more limiting to population growth than male fertility. However, little is known about sex differences in TSF.

3. Here we first demonstrate that the mating system can strongly influence TSF using the seed beetle *Callosobruchus maculatus*. We exposed populations carrying artificially induced mutations to two generations of short-term experimental evolution under alternative mating systems, manipulating the opportunity for natural and sexual selection on the mutations. We then measured TSF in males and females subjected to juvenile or adult heat stress.

4. Populations kept under natural and sexual selection had higher fitness, but similar TSF, compared to control populations kept under relaxed selection. However, females had higher TSF than males, and strikingly, this sex difference had increased over only two generations in populations evolving under sexual selection.

5. We hypothesized that an increase in male-induced harm to females during mating had played a central role in driving this evolved sex difference, and indeed, remating under conditions limiting male harassment of females reduced both male and female TSF. Moreover, we show that manipulation of mating system parameters in *C. maculatus* generates intraspecific variation in the sex difference in TSF equal to that found among a diverse set of studies on insects.

6. Our study provides a causal link between the mating system and TSF. Sexual conflict, (re)mating rates, and genetic responses to sexual selection differ among ecological settings, mating systems and species. Our study therefore also provides mechanistic understanding for the variability in previously reported TSFs which can inform future experimental assays and predictions of species responses to climate warming.

Introduction

To predict evolutionary trajectories of natural populations experiencing warming climates, it is necessary to understand selection on, and the genetic architecture of, traits which expression depend heavily on temperature (Bubliy et al., 2012; Chevin et al., 2013; Hoffmann & Sgrò, 2011; Walters et al., 2012). Typical estimates of thermal sensitivity describe the temperatures at which individuals fail to maintain basic physiological functions such as controlled locomotion and respiration (Lutterschmidt & Hutchison, 1997). Approaches to predict organismal responses relying exclusively on such measures are however bound to neglect a variety of sublethal effects that will arise at less extreme temperatures, the most important being reductions in fertility (Angilletta, 2009; Chirgwin et al., 2021; Walsh et al., 2019; Parratt et al., 2021; Hoffmann et al., 2013; Kellermann & Heerwaarden, 2019). Indeed, already a slight decrease in fertility can have dramatic effects on population viability (Degioanni et al., 2019) and thermal plasticity in reproductive traits is widespread (Dell et al., 2011; Deutsch et al., 2008; Frazier et al., 2006) and often observable at a significantly lower temperature threshold than responses in viability (Angilletta, 2009; Gerking & Lee, 1983; Hoffmann, 2010; Loisel et al., 2019; van Heerwaarden & Sgrò, 2021). Hence, it is crucial to incorporate estimates of the thermal sensitivity of fertility (from hereon: TSF) into predictions of population persistence (Angilletta, 2009; Parratt et al., 2021; Walsh et al., 2019).

In many sexually reproducing species, population growth is mainly dependent on female fertility (Caswell, 2006). Female TSF may therefore be more consequential for population viability under climate warming, highlighting the need for a more thorough understanding of sex-differences in TSF (Iossa, 2019). For example, if male fertility is more sensitive to elevated temperatures, but assuming some shared genetic basis for TSF in the two sexes, genetic variation with deleterious effects on female TSF could effectively be purged at elevated temperatures while limiting the cost of adaptation (*sensu* Haldane 1957) mainly to males. Such male-biased purging of deleterious alleles affecting TSF could thus pursue with little reduction in population growth, which would aid evolutionary rescue of sexually reproducing species facing warming climates (Godwin et al., 2020; Manning, 1984; Martinossi-Allibert et al., 2019; Plesnar-Bielak et al., 2012; Whitlock & Agrawal, 2009). It has been suggested that male reproduction is more affected by elevated temperature in both

89   endotherms (Hansen, 2009) and ectotherms (David et al., 2005; Jørgensen et al., 2006).

90   However, male and female reproductive physiologies are vastly different (García-Roa et al.,

91   2020; Kodric-Brown & Brown, 1987), questioning whether genetic responses to selection on

92   TSF in one sex would be consequential for TSF in the other. On the other hand, some

93   general buffering mechanisms against elevated temperature, such as antioxidant defences

94   (Dowling & Simmons, 2009) or molecular chaperones aiding protein translation and folding

95   (Feder et al., 2000), are costly to produce and may depend strongly on the overall condition

96   and genetic quality of the individual. Such responses may therefore be much more likely to

97   share a genetic basis between the sexes (Andersson, 1994; Rowe & Houle, 1996; Tomkins et

98   al., 2004).

99   Sex differences are often rooted in the operation of sexual selection and mating systems,

100   and it is possible that sex-specificity in TSF could trace back to general differences in male

101   and female reproductive physiologies ingrained in the evolution of anisogamy. However,

102   fine-grained variation in sexual selection and mating systems is also likely to play an

103   important role in shaping male and female TSF (García-Roa et al., 2020; Gómez-Llano et al.,

104   2020; Martinossi-Allibert et al., 2019; Pilakouta & Ålund, 2021; Svensson et al., 2020). For

105   example, success under post-copulatory sexual selection (i.e. sperm competition) can

106   depend on both gamete quality (Gage et al., 2004; Hosken et al., 2003; McNamara et al.,

107   2014) and the overall genetic quality of the male (Hosken et al., 2003), suggesting that

108   sexual selection for genetic quality could increase tolerance to thermal stress. However,

109   sperms' tolerance of oxidative stress, and therefore likely also high temperature, can be

110   affected by investment in precopulatory traits (Dowling & Simmons, 2009; Helfenstein et al.,

111   2010) and studies have suggested that male gamete quality may trade-off with investment

112   into reproductive competition (Baur & Berger, 2020; Silva et al., 2019). Female reproduction

113   is also sensitive to temperature, especially since egg maturation and oviposition are two

114   highly temperature-dependent processes (Angilletta, 2009; Berger et al., 2008; Kingsolver &

115   Huey, 2008), and it is likely that female TSF could be modulated further by pre- and post-

116   fertilization mating interactions. For example, physical harm inflicted via male harassment

117   of females during copulation, or physiological harm mediated via toxic ejaculate compounds

118   (Arnqvist & Rowe, 2005; Dougherty et al., 2017; Parker, 2006), could increase female TSF, if

119   TSF is dependent on the condition of the individual and costly thermal buffering

120   mechanisms. On the other hand, some ejaculate compounds typically have beneficial effects

121   in females (Arnqvist & Nilsson, 2000; Karlsson et al., 1997; Oku et al., 2019; Reinhardt et al.,

122   2009; Savalli & Fox, 1999), and remating could potentially improve male fertility via gamete

123   renewal, suggesting that multiple mating also may have positive effects on TSF. This

124   suggests that sex differences in TSF are bound to vary dynamically with mating system

125   parameters which could have important consequences for evolutionary demography in

126   sexually reproducing species.

127   Here we explore the role of the mating system in shaping sex differences in TSF in

128   populations of the polyandrous seed beetle *Callosobruchus maculatus*. We first quantified

129   how natural and sexual selection on artificially induced mutations affected short-term

130   evolutionary responses in male and female TSF. This approach was motivated by i) theory

131   often assuming that sexual selection is a more potent force of purifying selection against

132   deleterious genetic variation compared to natural selection (Rowe & Houle 1996, Tomkins

133   et al. 2004, Whitlock & Agrawal 2009) and that purging of deleterious genetic variation is

134   much more efficient via sexual selection in males in *C. maculatus* (Grieshop et al., 2016,

135   2020), ii) that compensatory physiological responses to temperature stress are costly (Feder

136   et al., 2000) suggesting that TSF may be dependent on the condition and overall genetic

137   quality of the individual, and iii) that elevated temperature can increase the effects of

138   deleterious genetic variation in ectotherms (Berger et al., 2021).

139   To this end, we induced an appreciable genetic load via mutagenesis in replicate

140   populations that were subsequently propagated for two generations under three alternative

141   experimental evolution regimes: polygamy (imposing natural and sexual selection), enforced

142   monogamy (natural selection only), and relaxed selection (natural and sexual selection

143   removed). Comparisons to the ancestral (non-irradiated) populations allowed us to assess

144   the relative impact of the induced mutations and the extent to which the two mating

145   systems (polygamy and monogamy) had purged the mutations relative to the relaxed

146   selection treatment. Thus, in addition to providing information on sex differences in TSF in

147   *C. maculatus*, this panel of populations allowed us to assess not only sex-specific effects of

148   *de novo* mutations on TSF but also how natural and sexual selection on *de novo* mutations

149   influence sex differences in TSF.

150   We conducted the experiment using two different types of heat stress. We applied a short-
151   term but high intensity heat shock on adult beetles, reflecting extreme daily maximal
152   temperatures, which are predicted to increase in frequency due to climate change (Johnson
153   et al., 2018). In a parallel experiment using the same populations, we applied long-term heat
154   stress throughout the entire larval development, as may result from increasing variation in
155   average monthly temperatures (Bathiany et al., 2018; Varela et al., 2020). We find no
156   evidence that the genetic load of a population is related to its average TSF. Strikingly,
157   however, experimental evolution under sexual selection increased sex differences in TSF in
158   only two generations. To elucidate the mechanism behind this result we measured TSFs
159   while manipulating male-female (re)mating interactions. Our results show that the mating
160   system can be a key driver of realized TSF in males and females.

161

162   **Methods**

163   **Study population**

164   *Callosobruchus maculatus* is a common pest on fabaceous seeds in tropical and subtropical
165   regions. Females cement eggs onto host seeds and the larvae burrow into the seed where
166   they complete their development within 3 weeks under standard laboratory conditions (29°
167   C, 12L:12D light cycle, 55% rel. humidity), on their preferred host, *Vigna unguiculata* (Fox,
168   1993). Unless otherwise stated, these conditions were also used in the experiments
169   described below. Egg-to-adult survival is above 90% in the populations used here. Adults are
170   facultatively aphageous and start reproducing within hours after emergence. Under
171   laboratory conditions without food or water adult beetles live just over one week with most
172   of the reproduction taking place within the first few days (Fox, 1993). *C. maculatus* has a
173   polyandrous mating system with documented sexual conflict over (re)mating and high
174   remating rates, leading to both pre- and post-copulatory sexual selection on males (Berger
175   et al., 2016; Crudgington & Siva-Jothy, 2000; Eady, 1995; Gay et al., 2009; Hotzy & Arnqvist,
176   2009). Once a male manages to successfully initiate copulation, spines on its genitalia help
177   to prevent it from being dislodged but at the same time harm the female (Bagchi et al.,
178   2021; Edvardsson & Tregenza, 2005; Rönn et al., 2007; Rönn & Hotzy, 2012). The effects of
179   the genital spines and the harm imposed on the females have been found to correlate with

180 a male's sperm competitiveness (Hotzy & Arnqvist, 2009) and, in a congener that exhibits

181 similar genital structures, also increase female oviposition rate as a response to genital

182 scarring (Haren et al., 2017). Female reproductive behaviour is further modulated by

183 lifespan-extending nutrients and water (Rönn et al., 2006) and likely also other functional

184 compounds in the male ejaculate (Bayram et al., 2019), suggesting that the male ejaculate

185 can have both positive and negative effects on female fertility (Arnqvist et al., 2004; Yamane

186 et al., 2015).

187 The stock used for the experimental populations originates from 41 iso-female lines

188 sampled in Lomé, Togo (06°10#N 01°13#E) (see Berger et al., 2014) that were mixed and

189 maintained at large population size (N>300) for roughly 50 generations under standard

190 conditions prior to the start of this experiment.

191

192 **Mutagenesis**

193 The stock population was split into three replicate founder populations (Fig. 1a). We first

194 introduced a genetic load in the three founders by exposing male beetles to 25 Gy of γ-

195 radiation over 32 minutes (dose rate: ~0.79 Gy/min). This dose is known to reduce

196 laboratory fertility (i.e., number of emerging adults) of the parental generation by roughly

197 70% and that of F1 offspring by roughly 40% (Baur & Berger, 2020; Grieshop et al., 2016). All

198 irradiated males (N = 150 per founder) were virgin and eclosed between 0 and 24 hours

199 prior to irradiation. All males were then mated to a randomly assigned female (enforced

200 monogamy) which was allowed to lay eggs for 48 hours. All egg-laden beans were mixed

201 and distributed into three aliquots marking the starting point for the three different

202 experimental evolution regimes (see below). At this point, all offspring are expected to carry

203 a random set of mutations induced via their fathers. Another 150 control (non-irradiated)

204 males were used to seed the control population for each founder. These control populations

205 were propagated according to standard laboratory protocol.

206

207 **Experimental Evolution regimes**

208   The selection regime protocols (Fig. 1a), outlined below, have previously been described

209   and used in several, more long-term (up to 60 generations), experimental evolution studies

210   in *C. maculatus* and have been shown to result in pronounced sex-specific adaptations

211   (Bagchi et al., 2021; Baur et al., 2019; Baur & Berger, 2020; Martinossi-Allibert et al., 2019).

212   *Monogamy*: This regime removes sexual selection but applies natural (fecundity) selection

213   on females and males. Within 72 hours after eclosion, 100 virgins of each sex were picked

214   and randomly paired and allowed to mate for 5 hours. During this period, the male and

215   female could freely interact and mate repeatedly. After 5 hours the males were removed,

216   and all females were collected and placed together in a 1liter jar containing host seeds *ad*

217   *libitum*. After 48 hours of egg laying the females were removed. To ensure minimal larval

218   competition and viability selection, all populations used in this experiment were provided

219   with beans *ad libitum* for egg deposition (~4800 black-eyed beans).

220   *Polygamy:* This regime simulates the natural mating system, including sexual and natural

221   selection on males and females. 100 virgin males and 100 virgin females were picked within

222   72 hours after eclosion and collected in a 1-liter jar with beans *ad libitum*. The beetles could

223   freely interact, compete, mate and lay eggs for 48 hours, after which all beetles were

224   removed. This mating scheme was also used to propagate the non-irradiated control

225   populations and corresponds to the standard laboratory protocol.

226   *Relaxed selection:* This regime removes both natural and sexual selection to retain the

227   induced (non-lethal) deleterious mutations in the populations. Within 72 hours after

228   eclosion, 100 virgin females and 100 virgin males were assigned to form random

229   monogamous couples (avoiding inbreeding) as in the monogamous mating regime.

230   Thereafter, males were removed, and each female was provided with beans *ad libitum*.

231   Females laid eggs for 48 hours in isolation, after which all females were removed from the

232   beans. In the next generation offspring were picked so that each parental couple

233   contributed exactly one female and one male to the next generation.

234   After two generations of propagation under the respective selection regimes, we applied

235   one generation of common garden relaxed selection to all 12 populations to both

236   counteract potential differences in parental effects brought about by the different evolution

237   regimes, and to prevent further selection against deleterious mutations (Fig. 1a). We then

8

238   established 30 mating couples per population (total n_{family} = 360). After mating we allowed

239   the female to lay eggs for 48 hours. We then removed the female and evenly split the beans

240   from each female into one half that was subjected to the **Juvenile heat stress** treatment

241   (outlined below), and one half that was kept developing at benign 29°C. The beetles

242   developing at 29°C were assigned to undergo the **Adult heat-shock** treatment (outlined

243   below) or to remain untreated and serve as control for both heat treatments (Fig. 1a).

244

245   **1. Juvenile heat stress**

246   *1.1.* This experiment was designed to resemble a longer period of elevated temperature as

247   for example a heat wave, which can occur in the months of March and April in Lomé, Togo.

248   Current projections for Lomé predict an increase of the average daily maximal temperature

249   in the months from February to April from 32°C in the late 20th century, up to a maximum of

250   37.2°C by the end of the 21st century (Varela et al., 2020). Beetles assigned to this

251   treatment developed at an elevated temperature of 35°C throughout their entire larval and

252   pupal stage (ca. 21 days in total). After eclosion, we crossed two treated male and two

253   treated female individuals per family with untreated individuals from other families within

254   the same population, allowing us to estimate the sex-specific fertility loss due to

255   development at 35°C for each of the 12 populations. This resulted in a total of 818

256   untreated couples, 458 couples with a treated female and 443 couples with a treated male,

257   or roughly 40 couples per treated sex and population. Each couple was provided with beans

258   *ad-libitum* in a 60 mm petri dish and allowed to mate and lay eggs for the rest of their lives.

259   Emerging adult offspring were later counted to obtain the fertility of the couple.

260   *1.2. Re-mating and male harassment:* We hypothesized that one explanation for our results

261   from the first experiment could be that females developing at stressful temperature might

262   be worse at coping with the harm inflicted by males during mating, but may on the other

263   hand benefit from nutrients in ejaculates. Using the stock population from which the

264   experimental evolution populations were derived, we ran an experiment to tease apart

265   potential effects of remating on female TSF mediated via harmful physical mating

266   interactions and ejaculatory compounds. We exposed 24 hours old virgin heat-treated

267   (developed at 35°C) and untreated (developed at 29°C) females to three male treatments

268   (all males developed at 29° C). The first treatment underwent a single observed mating,

269   after which we removed the male and allowed the female to deposit eggs for the rest of her

270   lifetime. Females of the second treatment were mated once per day to same male for three

271   consecutive days. After the matings the male was removed. In the third treatment we co-

272   reared the male and female for their entire life, allowing them to interact freely as was the

273   case in the original experiment.

274   *1.3. Interaction of male and female heat stress*: We also tested whether the cumulative

275   effects of female and male juvenile heat stress act in an additive manner on a couple's

276   fertility. Beetles of both sexes, originating from the stock population, were reared at benign

277   29°C and at stressful 35°C, as in the main experiment. Within 24 hours after emergence, we

278   paired a male and female beetle of which either the male, the female, both sexes, or none

279   of the sexes, had developed at elevated temperature. Males and females were co-reared for

280   their entire life, and we counted the couple's reproductive output.

281

282   2. **Adult heat shock**

283   *2.1.* This experiment was designed to simulate a short-term heat extreme as occurring in the

284   form of extreme daily maxima. We chose an exposure intensity of 50° C for 20 minutes

285   because a pilot experiment showed that ca. 50% of the beetles are knocked-out in the

286   process (no more perceptible movement) but at the same time it remains in the range that

287   we consider ecologically relevant as such temperatures are likely to be reached in sun

288   exposed microclimates within the range of this species (Deutscher Wetterdienst, n.d.). From

289   the beetles that developed at benign temperature we randomly picked three adult female

290   and three adult male beetles per family, resulting in 818 untreated couples, 500 couples

291   with a treated female and 540 couples with a treated male. We put beetles in a perforated

292   0.5 ml Eppendorf tube placed in a closed 200 mm petri dish on a heating plate set to 50°C.

293   The air temperature inside the upper part of the petri dish was also monitored and

294   remained constant at 43°C for the duration of the treatment. We paired all heat-exposed

295   beetles with an untreated individual of opposite sex from a different family, but of the same

296   population. Each couple was provided with *ad-libitum* beans in a 60 mm petri dish and

297   lifetime reproductive output was recorded.

298   *2.2. Male recovery:* Using the stock population, we investigated if recovery of the male after

299   heat shock may have shaped the observed sex differences in TSF in the main experiment

300   outlined above. We allowed one group of males to mate 2 hours after the heat shock

301   treatment. Beetles of this group were then allowed to mate again 26 hours after heat shock.

302   The second group of male beetles were only allowed to mate once, after 26 hours. Beetles

303   assigned to the untreated control group were subdivided into the same two groups to

304   control for a possible decline in fertility due to repeated mating, although this has been

305   shown to be minimal in *C. maculatus* (Rönn et al., 2008). This allowed us to independently

306   estimate effects of recovery over time and recovery through mating causing ejaculate

307   replacement on male TSF.

308

309   **Statistical analysis:**

310   All analyses were executed in R (R Core Team, 2020). We fitted generalized linear mixed

311   models assuming a Poisson distributed response using the lme4-package (Bates et al., 2015,

312   p. 4) unless stated otherwise. The R package ggplot2 (Wickham, 2016) was used for

313   graphical illustration. P-values were calculated using the package car (Fox & Weisberg, 2019)

314   using type-II sums of squares. Planned post-hoc comparisons, applying Tukey correction,

315   were conducted using the package emmeans (Lenth, 2020).

316   *1.1 & 2.1 Sex-specific TSF in evolution regimes.* Offspring number was used as the response

317   while evolution regime and treatment (male stress, female stress or untreated), as well as

318   their interaction, were added as fixed effects. We also added experimental block and the

319   identity of the experimenter counting offspring as additional terms. Population replicate

320   crossed with treatment, as well as dam and sire effects, were included as random effects.

321   Additionally, an observation level random effect (OLRE) was included to control for over-

322   dispersion.

323   *1.2. Re-mating and male harassment*: We analysed main effects of female development

324   treatment (29°C/35°C), mating (single/multiple) and cohabitation (isolated/cohabiting), as

325   well as two-way interactions between development treatment and mating and

326   cohabitation, respectively. Experimental date was added as an additional main effect. We

327   assumed quasi-Poisson distributed errors in both models.

11

328 *1.3. Interaction of male and female heat stress*: We analysed effects of female development

329 treatment (29°C/35°C), male development treatment (29°C/35°C) and their interaction as

330 fixed factors.

331 *2.2 Male recovery*: We analysed effects of a recovery treatment (mated 2 or 26 hours after

332 the heat shock) with and without remating separately. To test for the effect of remating

333 after heat shock, we ran a generalized linear mixed model with a Poisson response including

334 treatment (untreated, heat shocked), mating number (one (2h) or two (26h)) and their

335 interaction as fixed effects, and male identity as well as an OLRE as a random effect. We

336 used the same model type and structure but without the male ID and the interaction term

337 because males only mated once. In this model treatment included untreated, heat shock

338 and 2 hours recovery, and heat shock and 26 hours recovery.

339

340 **Results**

341 There were pronounced fertility differences between irradiated and control populations

342 when assayed only in the benign environment (i.e., untreated beetles), illustrating that

343 mutagenesis had induced a sizeable genetic load (X^2= 60.88, df = 3, p < 0.001) (Fig. 1c,

344 Supplementary table 1). Pairwise post-hoc comparisons revealed that the regime under

345 relaxed selection carried a larger genetic load than the monogamy and the polygamy

346 regime, demonstrating efficient purging of deleterious mutation during experimental

347 evolution (Tukey$_{relax-poly}$: z = -4.52, p < 0.001; Tukey$_{relax-mono}$: z = -5.16, p = p < 0.001). There

348 was no difference in fertility between the monogamy and polygamy regime (Tukey$_{poly-mono}$: z

349 = -0.68, p = 0.91). All pairwise contrasts in supplementary table S1.

350

351 **Juvenile heat stress**

352 *1.1.* Elevated temperature during juvenile development strongly reduced the reproductive

353 output of adults (X^2 = 179.07, df = 2, p < 0.001). In the control populations, a female

354 developing at 35°C showed an average reduction in fertility of 32% (31 fewer offspring)

355 compared to an untreated female, while male fertility was reduced by 22% (21 fewer

356 offspring). To investigate if the induced mutations affected TSF, we first compared the effect

12

357 of heat stress in the control regime and the regime evolving under relaxed selection

358 (containing the largest genetic load; Fig. 1c). We found no evidence that genetic load

359 affected TSF (X^2 = 3.75, df = 2, p = 0.15). Strikingly, however, sex differences in TSF

360 depended on selection regime (sex:regime; X^2= 14.04, df = 6, p = 0.029) (Fig. 2a, b). There

361 were clear reductions in fertility via heat stress in both sexes in all but the polygamy regime,

362 where exposed males showed no statistically significant fertility loss (Fig. 2a, Supplementary

363 table S2;). To directly assess the effect of sexual selection on TSF we compared the

364 monogamy (natural selection) and polygamy (natural + sexual selection) regimes. This

365 analysis confirmed the results of the global model (sex:regime; X^2 = 6.96, df = 2, p = 0.030,

366 Fig 2b). Heat-treated females from the polygamy regime produced significantly fewer

367 offspring than heat-treated females from the monogamy regime (Tukey$_{poly\_female35-}$

368 $_{mono\_female35}$: z = -2.08, p = 0.04), while heat-treated polygamy males instead tended to

369 produce more offspring than heat-treated monogamy males, although this effect was not

370 statistically significant (Tukey$_{poly\_male35-mono\_male35}$: z = 1.41, p = 0.16). Full model specification

371 and output in supplementary material S3.

372 *1.2.* To gain more insights into the underlying mechanisms responsible for the evolved sex

373 difference in TSF, we ran an additional experiment on the stock population to investigate

374 the role of repeated mating and male harassment on female TSF. Females exposed to

375 juvenile heat stress suffered more under cohabitation with a male than females developing

376 at benign temperature (temperature:cohabitation; X^2 = 4.53, df = 1, p = 0.033, Fig 2c), which

377 could in part explain the evolved increase in female-bias of TSF observed in the polygamy

378 regime, if polygamy males were more persistent during mating. Interestingly, there was also

379 a positive effect of re-mating, and this effect was more beneficial in females developing at

380 elevated temperature (temperature:mating; X^2= 4.99, df = 1, p = 0.025). Crucially, however,

381 the beneficial effect of re-mating depended strongly on the exclusion of the male between

382 matings (Tukey$_{single\_35\ vs.\ cohabitation\_35}$: z = -1.62, p = 0.24, Tukey$_{single\_35\ vs.\ remated\_35}$: z = -4.345, p

383 < 0.0001, Fig. 2c); and strikingly, re-mating with experimental exclusion of the male between

384 matings sufficed to completely thwart the negative effect of developmental temperature

385 stress in females (Tukey$_{remated\_29\ vs.\ remated\_35}$: z = -0.40, p = 0.69). Hence, changes in the

386 relative costs and benefits of multiple mating between the polygamy and monogamy regime

387 is likely to have driven the evolved sex difference in TSF.

13

388   *1.3.* We also explored whether the effect of males on female TSF was dependent on

389   whether males had also been exposed to heat. As expected, we found strong effects of both

390   male and female developmental heat stress (Female: X^2= 11.98, df = 1, p < 0.001; Male: X^2 =

391   10.75, df = 1, p = 0.001) (Fig. 2d). The interaction between female and male heat stress,

392   however, was non-significant, suggesting that the effects of female and male juvenile heat

393   stress on TSF are mostly additive. This result might be explained by heat stress reducing the

394   underlying male components with antagonistic effects on female TSF (level of male harm

395   and beneficial ejaculate compounds) to similar extent.

396

Effects of adult heat shock

398   *2.1.* The adult heat shock treatment led to an overall loss of fertility (X^2= 17.17, df = 2, p <

399   0.001) even though its impact was much weaker compared to the impact of juvenile heat

400   stress (average fertility loss for females was 10.2% and for males 4.2%). The effect of heat

401   shock was significantly stronger in females (Tukey$_{female - male}$: z = -2.38, p = 0.045) and, in fact,

402   not statistically detectable in males (Tukey$_{untreated - male}$: z = 2.08, p = 0.09) (Fig. 3a,

403   Supplementary table S2). The effect of heat shock was generally too weak to be detected

404   when analysing subsets of the data (results not shown), resulting in neither the induced

405   genetic load (regime$_{control vs. relaxed}$:treated sex: X^2 = 0.44, df = 2, p = 0.88) nor sexual selection

406   (regime$_{polygamy vs. monogamy}$:treated sex: X^2 = 0.43, df = 2, p = 0.81) having a statistically

407   significant effect on TSF. Full model specification and output in supplementary material S4.

408   *2.2.* To elucidate underlying mechanisms explaining sex differences in TSF under adult heat

409   shock, we analysed effects of male recovery, in terms of both time and remating (inducing

410   ejaculate renewal) after heat shock. Our data show that male beetles can recover almost

411   completely from the applied heat shock treatment within a 26-hour recovery period. Males

412   showed strong TSF, signified by an 18% reduction in fertility compared to the untreated

413   control group, when mating within two hours after heat shock (Tukey$_{untreated - 2 hours}$: z = 3.55,

414   p = 0.001, Fig. 3b). If males were given 26 hours of recovery in isolation, however, no

415   significant effect on fertility could be found (Tukey$_{untreated - 26 hours}$: z = 1.14, p = 0.49) (Fig. 3b).

416   Similarly, there was no reduction in fertility in beetles that mated a second time 26 hours

417   after the treatment (Tukey$_{untreated 2 hours - 2 hours}$: z = 0.64, p = 0.003; Tukey$_{untreated 26 hours - 26}$

418 $_{\text{hours}}$: z = 0.006, p = 1) (Fig. 3c). Recovery with or without remating showed similar effects on

419 TSF (Tukey$_{\text{heat 26 hours mate – heat 26 hours isolated}}$: z = 0.33, p = 0.74), suggesting that timing is crucial

420 when assessing TSF, and that realized sex differences in TSFs in natural populations are

421 state-dependent properties of mating system and ecology.

422

423 **Comparing intra- and interspecific variation in sex differences in TSF**

424 To put our results into perspective, we performed a (non-exhaustive) literature search for

425 studies on other insects that had estimated effects of heat stress on both male and female

426 fertility (summarized in Supplement S5 and S6). This allowed us to calculate and compare

427 standardized estimates of sex differences in TSF (Fig. 4). The variability in this estimate

428 obtained by manipulating mating system parameters and the timing of heat stress relative

429 to (re)mating in our study roughly corresponds to that reported between species in

430 previously published studies, demonstrating that the mating system can be a main

431 determinate of sex differences in TSF. Moreover, in contrast to occasional claims of male

432 biased TSF, there is no such consistent bias in the reviewed studies on insects estimating

433 male and female TSF under the same experimental conditions.

434

435 **Discussion**

436 In this study we have demonstrated that the mating system can affect sex differences in TSF

437 using the seed beetle *C. maculatus*. Strikingly, sexual selection on induced mutations over

438 only two generations of experimental evolution led to increased female-bias in TSF in

439 polygamous populations experiencing developmental heat stress. Male harassment

440 aggravated the negative effects of heat stress on females, suggesting that increased male

441 harassment might explain the increased female-bias in TSF in polygamous experimental

442 populations. In *C. maculatus,* sexual selection in males is more than three times as effective

443 at purging deleterious alleles compared to fecundity selection on females under semi-

444 natural laboratory setting, as used here (Grieshop et al. 2016, Grieshop et al. 2021). One

445 plausible mechanism behind the result is therefore that sexual selection in the polygamous

446 mating regime led to more efficient purging of alleles with deleterious effects on male

447 mating success, relative to purging of alleles with deleterious effects on female viability and

15

448   fertility, potentially shifting the balance between male persistence and female resistance

449   during (re)mating interactions. Male-biased selection on deleterious alleles can also improve

450   population fitness by sparing females the cost of adaptation (Manning 1984, Agrawal 2001,

451   Siller 2001, Agrawal & Whitock 2009), if some of the deleterious alleles in males also have

452   deleterious effects in females (Andersson 1994, Rowe & Houle 1996, Chippindale et al.

453   2001, Tomkins 2004, Bonduriansky & Chenoweth 2009), for which there is evidence in *C.*

454   *maculatus* (Grieshop et al. 2021). However, once males and females in the polygamous

455   populations engaged in mating interactions, the heightened genetic quality of male

456   genotypes evolving under strong sexual selection may have resulted in increased male

457   harassment of females, and the negative effects of this sexual conflict may have been

458   exposed under female heat stress. In nature, the relative extent of this negative effect

459   should strongly depend on ecological settings and population densities modulating the

460   degrees of conflict (Arbuthnott et al., 2014; Gomez-Llano et al., 2018; MacPherson et al.,

461   2018; Yun et al., 2017). We also note that our comparison of monogamous and polygamous

462   populations evolving from inflated levels of mutational variation does not describe a natural

463   scenario of long-term evolution in populations under mutation-selection balance. Instead,

464   our approach was designed to reveal how (sex-specific) natural and sexual selection can act

465   on genetic variation to shape TSF. Hence, our study provides a proof-of-principle for a direct

466   link between the mating system and sex differences in TSF.

467   At present, the scant literature available seems to suggest that male reproduction is more

468   sensitive to heat stress than female reproduction (David et al., 2005; Porcelli et al., 2017;

469   Sales et al., 2018), and male fertility has also been demonstrated to be very temperature-

470   sensitive in *C. maculatus*. Our data show that *C. maculatus* females can in fact be more

471   strongly affected by heat stress, and that the realized TSF in males and females can be

472   highly contingent on the experimental design (see also: Terblanche et al., 2007) and mating

473   system parameters such as the extent of sexual conflict and remating rates. For example,

474   the adult heat shock treatment resulted in relatively weak effects on fertility, but with

475   significant female-bias in TSF. However, our additional experiment showed that males fully

476   recovered from heat shock within only 26 hours, implying that the sex-bias in TSF in adults is

477   likely to change throughout life following heat exposure. In the case of juvenile heat stress,

478   male harassment aggravated effects of heat stress on female fertility, while repeated

479   mating instead had positive effects on both male and female TSF. However, the size of the

480   nuptial gift provided by male *C. maculatus* has been found to decrease with temperature,

481   suggesting that this male compensatory effect may diminish when also males are heat

482   stressed (Fox et al., 2006). Other studies on fruit flies (García-Roa et al., 2019, 2020) and *C.*

483   *maculatus* (Martinossi-Allibert et al., 2019), conclude that heat stress generally reduces the

484   female fertility cost of male cohabitation. In both these studies the impact of sexual conflict

485   was assessed directly in the stressful environment when both sexes were stressed, while in

486   the present study the effects of mating interactions on fertility were measured in a benign

487   environment after heat stress had been applied to one, or both sexes. Collectively, this

488   limited set of studies suggest that there is a multitude of ways that temperature can

489   modulate the consequences of sexual selection and conflict (Garcia-Roa et al. 2020), and

490   conversely, that sexual selection and conflict can shape sensitivity to temperature

491   (Martinossi-Allibert et al. 2019). Depending on population density, mating system, and heat

492   stress characteristics, laboratory experiments may thus lead to erroneous estimates of TSFs,

493   and in extension, misjudgements of the threat on population growth imposed by climate

494   warming, even when efforts are made to measure TSF sex-specifically. Indeed, our

495   comparison of variation in sex differences in TSF generated by mating system parameters in

496   our study, to that reported between species (Fig. 4), suggests that TSF is a highly dynamic

497   property that responds to population structure and ecological changes.

498   Directly comparing the effects of our two heat stress treatments is difficult as they were

499   applied with different intensities over different time frames and life stages. Nevertheless,

500   juvenile heat stress is known to affect the development of reproductive organs and result in

501   reduced sperm numbers in insects (Chirault et al., 2015; Kirk Green et al., 2019; Nguyen et

502   al., 2013; Vasudeva et al., 2014). Vasudeva, Deeming and Eady (2014), found not only a

503   decrease in sperm number but also a reduction in relative testis size by almost 25% in *C.*

504   *maculatus* males exposed to similar juvenile heat stress as in our experiment. In a later

505   study, the same authors determined the first 20% of larval development to be the most

506   temperature sensitive period of testis development (Vasudeva et al., 2021). A recent study

507   using the flour beetle *Tribolium castaneum* found that the most sensitive phase of testis

508   development is likely during the pupal stage and that testis size can be almost complete

509   recovered in males exposed to heat stress at an immature adult stage (Sales et al., 2021).

510   Together, these studies suggest that there are several time points with heightened

511   temperature sensitivity throughout male reproductive development. Heat shock treatments

512   applied on adults have also been found to decrease numbers of transferred sperm and

513   reduce fertility (Chevrier et al., 2019; Sales et al., 2018, 2021), but considering the data

514   presented here and in Sales et al. (2021), such effects may be reversible in most cases.

515   Similar changes in the morphology of female reproductive organs (i.e. smaller ovaries)

516   combined with a strong reduction in egg number have also been reported for flies of the

517   species *Drosophila Suzuki* developing at elevated temperature (Kirk Green et al., 2019).

518   However, little is known about the ability of female reproduction to recover from heat

519   stress. Data from an experiment exposing newly emerged cotton bollworm females,

520   *Helicoverpa armigera*, to a range of heat shock treatments shows a postponement of peak

521   reproduction correlated to the treatment intensity, suggesting some recovery processes

522   taking place between the heat shock event and the onset of reproduction (Mironidis &

523   Savopoulou-Soultani, 2010). In summary, this suggest that, at least in holometabolous

524   insects, heat stress during development can cause an impairment of reproductive organs

525   which is only reversible given a considerable amount of recovery time (if at all), while heat

526   shock experienced at the adult life stage might be reversible on shorter time scales.

527   Importantly, however, we also show that strategies such as remating or postponement of

528   reproduction may mitigate the impact of heat stress experienced both early and late in life.

529   Inducible compensatory responses that buffer the effects of heat stress are costly and may

530   therefore depend on the genetic quality of the organism. Moreover, it has recently been

531   shown that elevated temperatures can aggravate the deleterious effect of mutations

532   (Berger et al., 2021). We therefore predicted that populations with larger genetic loads

533   might show increased TSF but found no support for this in our data. The model applied by

534   Berger et al. (2021) predicts that temperature-dependent increases in mutational effects

535   stem from reversable misfolding of proteins at high temperature. It is possible that such

536   effects were no longer apparent following heat stress in our experiment as individuals were

537   shifted back to benign temperature (i.e., temperature-sensitive mutants either died during

538   development, or survived and got "rescued" by being placed at benign temperature).

539   Indeed, individuals surviving short term heat stress may even elicit compensatory stress

540   responses that mitigate deleterious effects of mutations (Casanueva et al., 2012).

18

541   Additionally, as we here studied temperature effects in the adult stage, where realized TSFs

542   are consequences of mating interactions, it is possible that a weakening of sexually

543   antagonistic interactions in populations with large genetic loads (and low-condition

544   individuals) may have contributed to mitigating the detrimental effects of temperature.

545   Indeed, a general tenet highlighted throughout this study for how frequency-dependent

546   processes in general (Bolnick et al., 2011; Brady et al., 2019; Dall et al., 2012; Svensson &

547   Connallon, 2019), and sexual selection in particular (Chenoweth et al., 2015; García-Roa et

548   al., 2020; MacPherson et al., 2018; Martinossi-Allibert et al., 2019; Martinossi-Allibert et al.,

549   2019; Rankin et al., 2011; Yun et al., 2017) may affect population vulnerability and

550   adaptation to abiotic factors.

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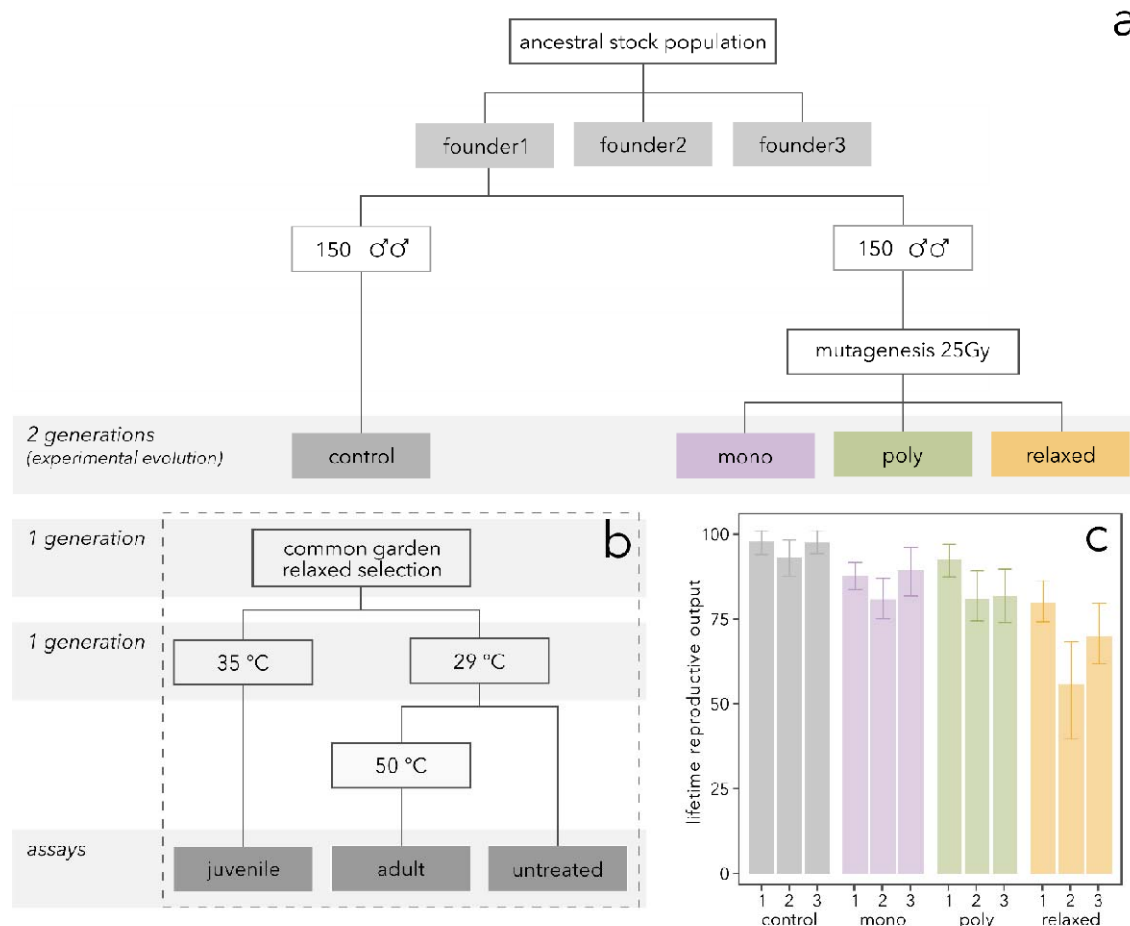
908

909   **Figure 1**

910

911

912   **Fig. 1:** Experimental design used to obtain the two main data sets. a) Founding populations,

913   mutagenesis and experimental evolution. b) Juvenile heat stress (development at 35°C) and

914   adult heat shock (20 min heat shock at 50°C) applied to all 12 populations. c) Lifetime

915   reproductive output of all 12 populations in the benign environment (untreated). Shown are

916   means ± 95% confidence intervals.

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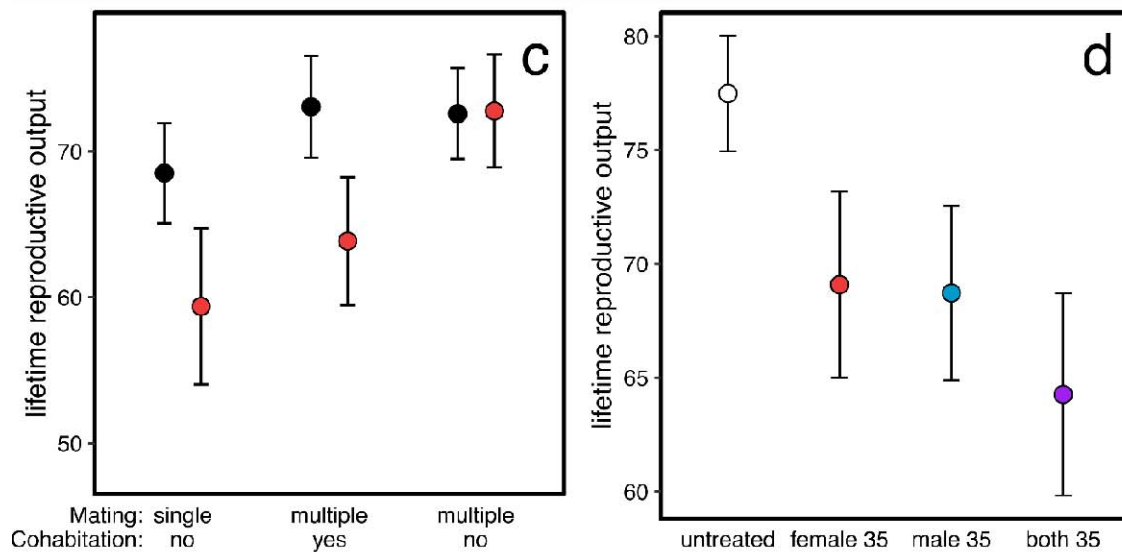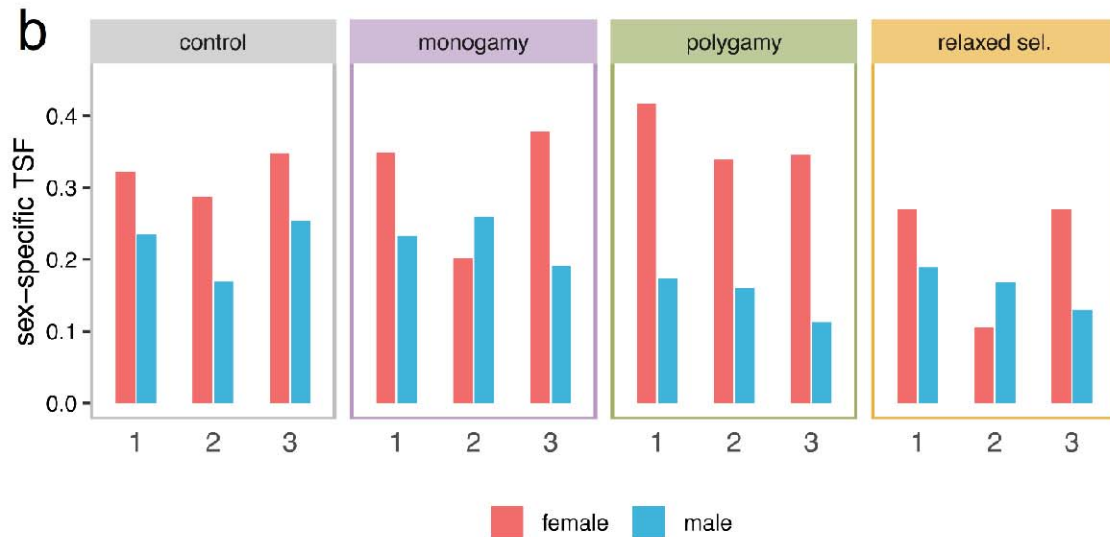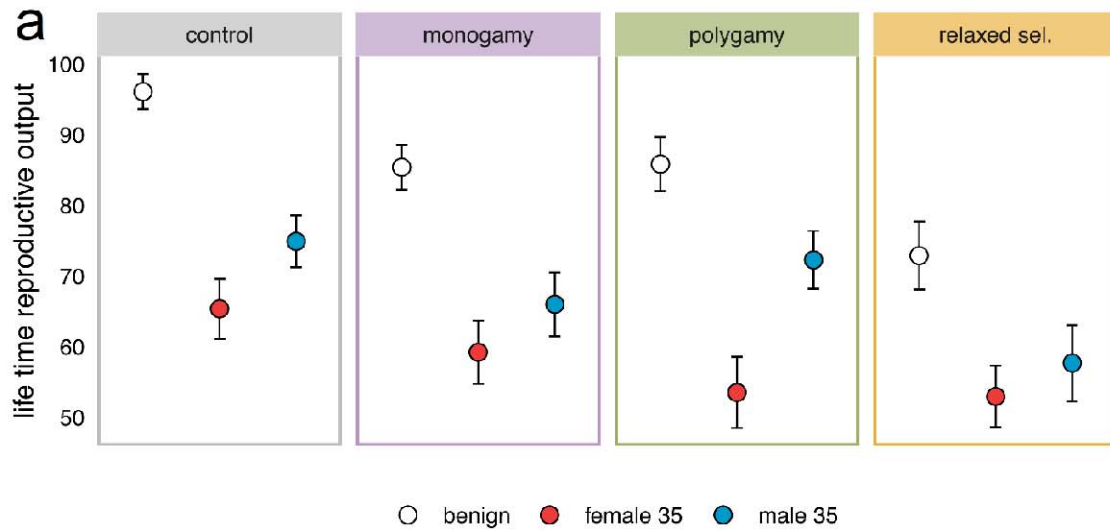922   **Figure 2**

923

924 **Fig. 2**: Sex differences in TSF under juvenile heat stress (experiments *1.1-1.3*) a) Lifetime
925 reproductive output of couples with either the female (red symbols), the male (blue
926 symbols), or no parent (open symbols) developing at elevated temperature. b) Relative loss
927 in fertility (1-stressed/control) per population. Sex differences in TSF in all three blocks are
928 greater for polygamy populations compared to all other regimes. c) Lifetime reproductive
929 output of female beetles developing at benign (black symbols) or stressful (red symbols)
930 temperature. d) Lifetime reproductive output of pairs in which no parent (white), the female
931 (red), the male (blue), or both parents (purple) were exposed to juvenile heat stress. Panels
932 a, c and d show means ± 95% confidence intervals.

933

934   **Figure 3**
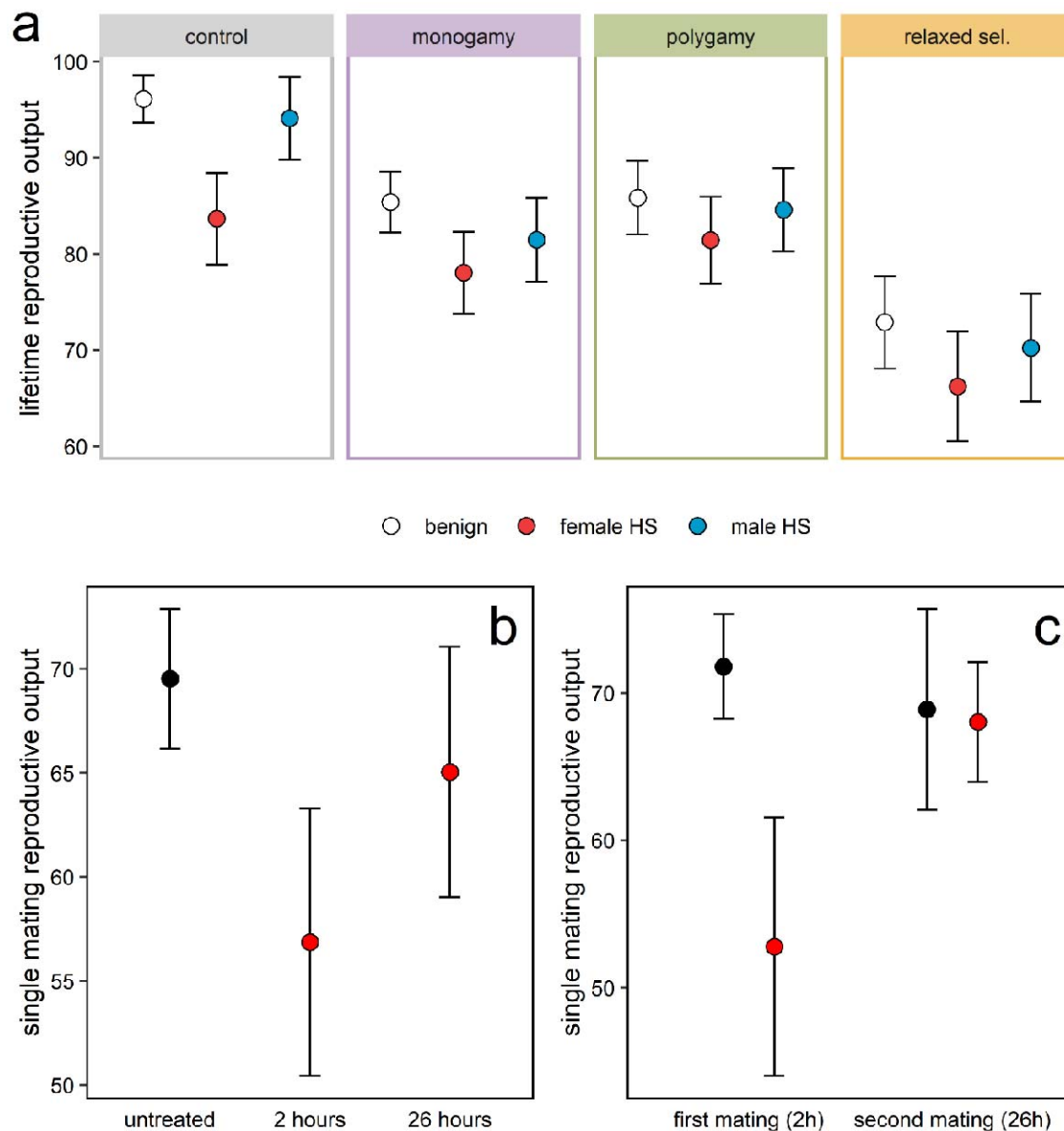
Fig. 3: Sex differences in TSF under adult heat shock (Experiments *2.1 & 2.2*) a) Lifetime reproductive output depending on the sex that underwent adult heat shock (HS). b) Recovery of male fertility after adult heat shock. c) Lifetime reproductive output of untreated and heat-shocked males that were mated to a virgin female 2 hours after the treatment and again, to a second female, 26 hours after the treatment. All panels show means ± 95% confidence intervals.

945   **Figure 4**

946
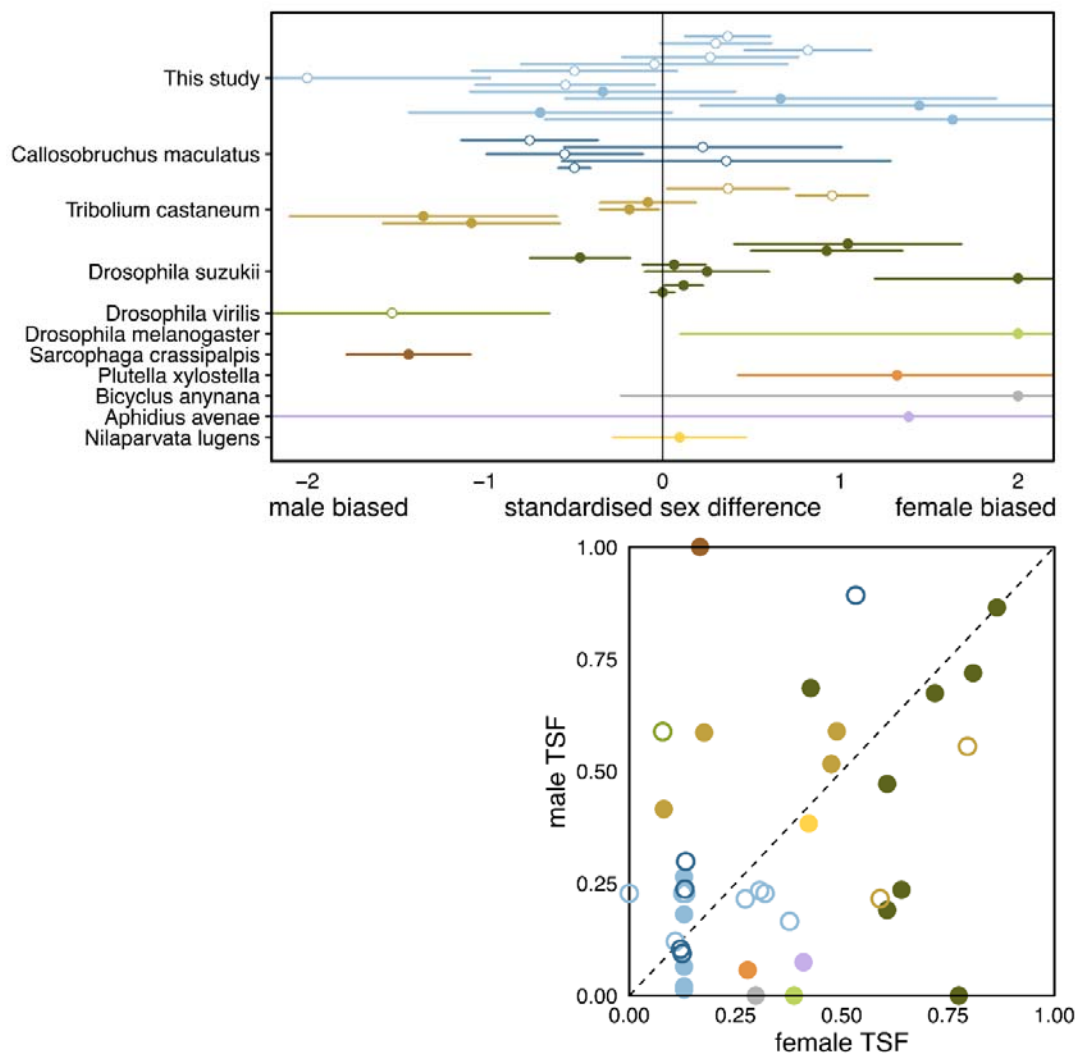
Fig. 4: Intraspecific variation in the sex difference in TSF generated through manipulation of the mating system, timing of heat stress relative to (re)mating in this study, compared to estimates from other studies on insects. Open symbols represent heat treatments applied at a juvenile stage while symbols represent heat treatments applied during the adult stage. a) A standardized measure of the sex difference in TSF was calculated as:

$$ \overline{} $$, where TSF is the relative fertility loss due to heat stress (1-benign/stress). A given SD_{TSF} from this study was derived by first calculating the TSF of one sex in a given experimental condition (e.g. TSF_{female} with remating but no cohabitation from experiment *1.2*) and then always using the TSF observed in one of the two main experiments (*1.1* for juvenile stress and *2.1* for adult stress) for the opposite sex from control populations as comparison (e.g. TSF_{male} from experiment *1.1*). 95% confidence intervals were calculated through propagation of the uncertainty reported for measures of reproductive output within the respective studies. b) Comparison of male and female TSF for the studies presented in panel a. Further details, including inclusion criteria for reviewed studies and a table of all values, are presented in Supplementary material S5 & S6.

962   **Supplementary material**

963

964   **Supplement S1:** Table of all pairwise contrasts between regimes for offspring numbers at

965   benign temperature.

Contrast	Estimate	Std.Error	z	P-value
monogamy - control	-0.122	0.045	-2.713	**0.034**
polygamy - control	-0.154	0.046	-3.369	**0.004**
relaxed - control	-0.392	0.050	-7.778	**<0.001**
polygamy - monogamy	-0.032	0.048	-0.675	0.906
relaxed - monogamy	-0.270	0.052	-5.159	**<0.001**
relaxed - polygamy	-0.238	0.053	-4.523	**<0.001**

966

967   **Supplement S2:** Pairwise comparisons between treatments (untreated, stressed female,

968   stressed male) within selection regimes for both juvenile heat stress and adult heat shock

969   main datasets (experiments 1.1 and 2.2). Marginal means were obtained by averaging over

970   blocks and experimenter. All p-values are Tukey corrected.

971

		juvenile heat stress					adult heat shock			
regime	contrast	estimate	SE	z.ratio	p.value		estimate	SE	z.ratio	p.value
control	untreated - female	0.467	0.063	7.407	**<.0001**		0.239	0.058	4.148	**0.0001**
	untreated - male	0.269	0.063	4.258	**0.0001**		0.077	0.058	1.339	0.373
	female - male	-0.198	0.073	-2.719	**0.018**		-0.162	0.066	-2.447	**0.038**
monogamy	untreated - female	0.441	0.067	6.600	**<.0001**		0.144	0.063	2.278	0.059
	untreated - male	0.304	0.069	4.423	**<.0001**		0.098	0.060	1.635	0.231
	female - male	-0.137	0.078	-1.758	0.184		-0.046	0.070	-0.652	0.791
polygamy	untreated - female	0.568	0.069	8.173	**<.0001**		0.089	0.063	1.413	0.334
	untreated - male	0.151	0.068	2.215	0.069		0.032	0.062	0.521	0.861
	female - male	-0.417	0.078	-5.329	**<.0001**		-0.056	0.071	-0.798	0.704
relaxed	untreated - female	0.295	0.073	4.060	**0.0001**		0.128	0.070	1.836	0.158
	untreated - male	0.247	0.074	3.338	**0.002**		0.054	0.068	0.791	0.709
	female - male	-0.048	0.079	-0.616	0.811		-0.074	0.074	-0.999	0.577

972
973

974 **Supplement S3:** Details global model – juvenile heat stress

975

976 glmer(offspring ~ regime*treatment + block + counter + (1|line) + (1|treatment:line) +

977 (1|familyID_female) + (1|familyID_male) + (1|OLRE_ID), family="poisson", data = data,

978 control=glmerControl(optimizer="bobyqa",optCtrl=list(maxfun=100000)))

979 Sample size: 1719, groups: OLRE_ID, 1719; familyID_male, 344; familyID_female, 341;

980 treatment:line, 36; line, 12

981

982

983 Random effects:

Groups	Variance	Std.Dev.
OLRE_ID	2.94E-01	5.42E-01
familyID_male	1.76E-02	1.33E-01
familyID_female	8.32E-03	9.12E-02
treatment:line	1.32E-15	3.63E-08
line	0.00E+00	0.00E+00

984

985 Fixed effects:

	Estimate	Std.Err.	z	Pr(>|z|)
Intercept (ctrl, benign temp.)	4.5983	0.04607	99.81	**<0.001**
regimemono	-0.12952	0.05794	-2.235	**0.02539**
regimepoly	-0.16538	0.05886	-2.81	**0.00496**
regimerelax	-0.41747	0.06406	-6.517	**<0.001**
treatedFemale	-0.46662	0.06299	-7.407	**<0.001**
treatedMale	-0.2685	0.06305	-4.258	**<0.001**
block2	-0.18958	0.03962	-4.785	**<0.001**
block3	-0.08568	0.04038	-2.122	**0.03383**
counter2	0.01375	0.02828	0.486	0.62678
regimemono:treatedFemale	0.02598	0.09176	0.283	0.77705
regimepoly:treatedFemale	-0.10092	0.09367	-1.077	0.28127
regimerelax:treatedFemale	0.17153	0.09606	1.786	0.07416
regimemono:treatedMale	-0.03515	0.09322	-0.377	0.70617
regimepoly:treatedMale	0.11752	0.09282	1.266	0.20548
regimerelax:treatedMale	0.02184	0.097	0.225	0.82189

986

987   Anova table (type II SS):

	X2	Df	Pr(>X2)
regime	60.5142	3	**<0.001**
treatment	179.0658	2	**<0.001**
block	22.9029	2	**<0.001**
counter	0.2365	1	0.62678
regime:treatment	14.0398	6	**0.02919**

988

989

990   **Supplement S4:** Details global model – adult heat shock

991   glmer(offspring ~ regime*treatment + block*treatment + counter + (1|line) +

992   (1|treatment:line) + (1|familyID_female) + (1|familyID_male) + (1|OLRE_ID),

993   family="poisson", data = data,

994   control=glmerControl(optimizer="bobyqa",optCtrl=list(maxfun=100000)))

995   Sample size: 1858, groups: OLRE_ID, 1858; familyID_male, 343; familyID_female, 343;

996   treatment:line, 36; line, 12

997

998   Random effects:

Groups	Variance	Std.Dev.
OLRE_ID	2.67E-01	5.17E-01
familyID_male	2.29E-02	1.51E-01
familyID_female	8.62E-03	9.28E-02
treatment:line	0.00E+00	0.00E+00
line	0.00E+00	0.00E+00

999

1000   Fixed effects:

	Estimate	Std.Err.	z	Pr(>|z|)
Intercept (ctrl, benign temp.)	4.59271	0.04831	95.067	**<0.001**
regimemono	-0.12807	0.05713	-2.242	**0.02498**
regimepoly	-0.16222	0.05798	-2.798	**0.00515**
regimerelax	-0.40836	0.06316	-6.465	**<0.001**
treatedFemale	-0.14945	0.0695	-2.15	**0.03152**
treatedMale	-0.05093	0.06956	-0.732	0.46409
block2	-0.20795	0.05093	-4.083	**<0.001**
block3	-0.07442	0.05336	-1.395	0.16309
counter2	0.03837	0.02666	1.439	0.15014
regimemono:treatedFemale	0.09522	0.08499	1.12	0.26255
regimepoly:treatedFemale	0.15054	0.08471	1.777	0.07554
regimerelax:treatedFemale	0.11093	0.09022	1.229	0.21889

regimemono:treatedMale	-0.02127	0.08308	-0.256	0.79788
regimepoly:treatedMale	0.04498	0.0842	0.534	0.59318
regimerelax:treatedMale	0.02312	0.08897	0.26	0.795
treatedFemale:block2	0.05539	0.07281	0.761	0.44682
treatedMale:block2	0.07261	0.07233	1.004	0.31539
treatedFemale:block3	-0.32439	0.07928	-4.091	**<0.001**
treatedMale:block3	-0.15126	0.07624	-1.984	**0.04724**

1001

1002   Anova table (type II SS):

	X^2	Df	Pr(>X2)
regime	68.2803	3	**<0.001**
treatment	17.1739	2	**<0.001**
block	31.7977	2	**<0.001**
counter	2.0708	1	0.1501429
regime:treatment	4.0144	6	0.6747337
treatment:block	25.3058	4	**<0.001**

1003

1004

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1006

1007 **Supplement S5:** Literature review

1008 To put the variation in the sex-specificity of TSF obtained across the various data sets

1009 presented in our study into perspective, we performed a non-exhaustive literature search

1010 for studies reporting sex-specific effects of heat stress on fertility measured for both sexes

1011 within the same experiment applying the same heat stress treatment. The literature search

1012 was executed between the 3.5.2021 and the 22.5.2021 using Google Scholar. We

1013 considered the results of a search for titles using the key words "fertility", "heat",

1014 "reproduction", "sex", "stress", and "temperature", as well as literature cited by papers

1015 matching the initial search. Note that the literature search was non-exhaustive, but that we

1016 included all studies we could find that had measured fertility for both sexes in terms of

1017 reproductive output. Hence, we excluded studies that had measured traits related to

1018 fertility, such as testis size in males or egg maturation rate in females. This resulted in 12

1019 studies for further analysis (shown in Table S6 below)

1020 We extracted values, and the corresponding standard errors, of male and female

1021 reproductive output under benign and stressful temperature (including development at

1022 elevated temperature, heat shock and ramping temperatures). Figure 4 only includes pairs

1023 of values where the stress treatment led to a statistically significant decrease in fertility in at

1024 least one sex. Supplementary table S6 contains all treatments of the included studies, also

1025 such without significant effects in either sex. We calculated the TSF as the proportional loss

1026 in fertility due to temperature stress:

$$TSF = 1 - \frac{f_{stress}}{f_{benign}}$$

1027

1028 We then proceeded to calculate a standardised estimate of sexual dimorphism in TSF:

1029

$$SD_{TSF} = \frac{(TSF_{female} - TSF_{male})}{(TSF_{female} + TSF_{male})/2}$$

1030

1031   A given SD_{TSF} from this study was derived by first calculating the TSF of one sex in a given

1032   experimental condition (e.g. TSF_{female} with remating but no cohabitation from experiment

1033   *1.2*) and then always using the TSF observed in one of the two main experiments (*1.1* for

1034   juvenile stress and *2.1* for adult stress) for the opposite sex from control populations as

1035   comparison (e.g. TSF_{male} from experiment *1.1*). To assess whether the obtained values of

1036 SD_{TSF} were different from zero we propagated the uncertainty in male and female

1037   reproductive output to calculate 95% confidence intervals. We performed no formal

1038   statistical analysis on this dataset as it only serves to illustrate the comparison of intra- and

1039   interspecific variation in TSF found in this and other studies, and the number of studies is

1040   limited.

1041

1042

1043　**Supplement S6:** Values extracted from studies meeting the criteria outlined in supplement
1044　S5. Only values obtained for stress conditions under which at least one sex showed a
1045　significant fitness reduction were included in the figures presented in the main manuscript.
1046　(2012)

Study	Heat stress	Stress stage	Significant fertility loss	Species	Female load	Male load	TSF dimorphism
This study	developmental	juvenile	yes	C. maculatus	0.319 ± 0.024	0.221 ± 0.022	0.366 ± 0.123
This study	developmental	juvenile	yes	C. maculatus	0.307 ± 0.03	0.227 ± 0.031	0.298 ± 0.161
This study	developmental	juvenile	yes	C. maculatus	0.377 ± 0.033	0.158 ± 0.031	0.817 ± 0.183
This study	developmental	juvenile	yes	C. maculatus	0.273 ± 0.039	0.209 ± 0.046	0.268 ± 0.253
This study	developmental	juvenile	yes	C. maculatus	0.108 ± 0.031	0.114 ± 0.029	-0.047 ± 0.384
This study	developmental	juvenile	yes	C. maculatus	0.133 ± 0.046	0.221 ± 0.022	-0.497 ± 0.295
This study	developmental	juvenile	yes	C. maculatus	0 ± 0.035	0.221 ± 0.022	-2 ± 0.528
This study	developmental	juvenile	yes	C. maculatus	0.126 ± 0.037	0.221 ± 0.022	-0.547 ± 0.259
This study	20 min heat shock	adult	yes	C. maculatus	0.129 ± 0.028	0.021 ± 0.026	1.444 ± 0.629
This study	20 min heat shock	adult	yes	C. maculatus	0.129 ± 0.028	0.265 ± 0.065	-0.689 ± 0.38
This study	20 min heat shock	adult	yes	C. maculatus	0.129 ± 0.028	0.013 ± 0.058	1.632 ± 1.171
This study	20 min heat shock	adult	yes	C. maculatus	0.129 ± 0.028	0.181 ± 0.051	-0.337 ± 0.381
This study	20 min heat shock	adult	yes	C. maculatus	0.129 ± 0.028	0.065 ± 0.05	0.663 ± 0.62
This study	20 min heat shock	adult	no	C. maculatus	0.085 ± 0.031	0.046 ± 0.032	0.607 ± 0.702
This study	20 min heat shock	adult	no	C. maculatus	0.052 ± 0.035	0.015 ± 0.034	1.103 ± 1.641
This study	20 min heat shock	adult	no	C. maculatus	0.092 ± 0.05	0.037 ± 0.051	0.851 ± 1.203
Martinossi-Allibert et al., 2019	developmental	juvenile	yes	C. maculatus	0.133 ± 0.025	0.292 ± 0.03	-0.749 ± 0.196
Martinossi-Allibert et al., 2019	developmental	juvenile	yes	C. maculatus	0.121 ± 0.023	0.096 ± 0.036	0.226 ± 0.399
Martinossi-Allibert et al., 2019	developmental	juvenile	yes	C. maculatus	0.131 ± 0.025	0.23 ± 0.03	-0.552 ± 0.225
Martinossi-Allibert et al., 2017	developmental	juvenile	yes	C. maculatus	0.125 ± 0.028	0.087 ± 0.04	0.358 ± 0.473
Martinossi-Allibert et al., 2017	developmental	juvenile	yes	C. maculatus	0.533 ± 0.027	0.885 ± 0.018	-0.497 ± 0.047
Mahroof et al., 2005	1h heat shock	juvenile	yes	T. castaneum	0.796 ± 0.079	0.548 ± 0.084	0.368 ± 0.175
Mahroof et al., 2005	1h heat shock	juvenile	yes	T. castaneum	0.59 ± 0.024	0.209 ± 0.029	0.954 ± 0.104
Mahroof et al., 2005	39min heat shock	adult	yes	T. castaneum	0.475 ± 0.045	0.516 ± 0.051	-0.083 ± 0.138
Mahroof et al., 2005	39min heat shock	adult	yes	T. castaneum	0.488 ± 0.045	0.589 ± 0.012	-0.187 ± 0.086
Sales et al., 2018	5d heat wave	adult	yes	T. castaneum	0.081 ± 0.065	0.416 ± 0.046	-1.346 ± 0.385
Sales et al., 2018	5d heat wave	adult	yes	T. castaneum	0.176 ± 0.063	0.587 ± 0.058	-1.076 ± 0.254
Sales et al., 2018	5d heat wave	adult	no	T. castaneum	0 ± 0.057	0.273 ± 0.047	-2 ± 0.76
Sales et al., 2018	5d heat wave	adult	no	T. castaneum	0.106 ± 0.067	0.25 ± 0.051	-0.806 ± 0.508
Evans et al., 2018	24h heat wave	adult	yes	D. suzukii	0.607 ± 0.051	0.191 ± 0.104	1.042 ± 0.327
Evans et al., 2018	24h heat wave	adult	yes	D. suzukii	0.64 ± 0.044	0.236 ± 0.074	0.923 ± 0.218
Evans et al., 2018	24h heat wave	adult	yes	D. suzukii	0.427 ± 0.067	0.685 ± 0.04	-0.465 ± 0.144
Evans et al., 2018	24h heat wave	adult	yes	D. suzukii	0.719 ± 0.036	0.674 ± 0.052	0.065 ± 0.091
Evans et al., 2018	72h heat wave	adult	yes	D. suzukii	0.607 ± 0.057	0.472 ± 0.077	0.25 ± 0.178
Evans et al., 2018	72h heat wave	adult	yes	D. suzukii	0.775 ± 0.033	0 ± 0.108	2 ± 0.412
Evans et al., 2018	72h heat wave	adult	yes	D. suzukii	0.809 ± 0.027	0.719 ± 0.033	0.118 ± 0.055
Evans et al., 2018	72h heat wave	adult	yes	D. suzukii	0.865 ± 0.019	0.865 ± 0.024	0 ± 0.035
Walsh et al., 2020	4h pupal heat shock	juvenile	yes	D. virilis	0.079 ± 0.079	0.581 ± 0.089	-1.523 ± 0.453
Krebs & Loeschke, 1994	90 min heat shock	adult	yes	D. melanogaster	0.388 ± 0.075	0 ± 0.11	2 ± 0.97
Rinehart et al., 2000	1h heat shock	adult	yes	S. crassipalpis	0.166 ± 0.084	1 ± 0	-1.429 ± 0.178
Zhang et al., 2013	4h heat shock	adult	yes	P. xylostella	0.279 ± 0.048	0.057 ± 0.042	1.319 ± 0.457
Zhang et al., 2013	3h heat shock	adult	no	P. xylostella	0.029 ± 0.043	0 ± 0.044	2 ± 6.102
Zhang et al., 2013	5h heat shock	adult	no	P. xylostella	0.164 ± 0.036	0.1 ± 0.043	0.486 ± 0.436
Janowitz & Fischer, 2011	1h heat shock	adult	yes	B. anynana	0.298 ± 0.073	0 ± 0.096	2 ± 1.141
Roux et al., 2010	1h heat shock	adult	yes	A. avenae	0.41 ± 0.272	0.075 ± 0.311	1.385 ± 2.076
Piyaphongkul et al., 2012	ramping	adult	yes	N. lugens	0.422 ± 0.049	0.384 ± 0.059	0.096 ± 0.191

1047