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Experimental evolution reveals that males evolving within warmer thermal regimes improve reproductive performance under heatwave conditions in a model insect

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Abstract

Climate change is increasing mean temperatures, and intensifying heatwaves. Natural populations may respond to stress through shorter-term acclimation via plasticity and/or longer-term inter-generational evolution. However, if the pace and/or extent of thermal change is too great, local extinctions occur; one potential cause in ectotherms is identified to be the heat-liability of male reproductive biology. Recent data from several species, including the beetle *Tribolium castaneum*, confirmed that male reproductive biology is vulnerable to heatwaves, which may constrain populations. However, such reproductive-damage may be overestimated, if there is potential to adapt to elevated mean temperatures associated with climate change via evolution and/or acclimation. Here, we tested this to evaluate whether pre-exposures could improve heatwave tolerance (adaptation or acclimation), by experimentally evolving *Tribolium castaneum* populations to divergent thermal regimes (30 °C vs. 38 °C). Findings across assays revealed that relative to 30 °C-regime males, males from the 38 °C regime, maintained constantly at 8 °C warmer for 25 generations, displayed an increase; (i) in post heatwave (42 °C) reproductive fitness by 55%, (ii) survival by 33%, and (iii) 32% larger testes volumes. Unexpectedly, in the acclimation assay, warm-adapted males' post-heatwave survival and reproduction were best if they experienced cool developmental acclimation beforehand, suggesting a cost to adapting to 38 °C. These results help progress knowledge of the potential for survival and reproduction to adapt to climate change; trait specific adaptation to divergent thermal regimes can occur over relatively few generations, but this capacity depended on the interaction of evolutionary and thermal acclimatory processes.

Keywords: adaptation, acclimation, climate change, testes, experimental, fertility, heatwaves, insect, reproduction

Introduction

The Earth's climate is warming, and by 2,100 mean global temperatures will likely rise beyond 2 °C relative to pre-industrial conditions (IPCC, 2021; Raftery et al., 2017). As mean temperature increases, so too does its variability (Sexton & Harris, 2015; Wiel & Bintanja, 2021), with extreme temperature events like heatwaves expected to become longer, more variable, hotter and more frequent (Christidis et al., 2015; Hobday et al., 2016; IPCC, 2021; Meehl & Tebaldi, 2004; Perkins et al., 2012).The definition, regional metrics of heatwave varies (e.g., Perkins-Kirkpatrick & Lewis, 2020), but often incorporates a period of abnormally hot weather relative to a reference threshold, which can last from 2 days to months (IPCC, 2021). Here, we focus on Frich et al.'s (2002) definition of daily temperatures exceeding the long-term average by at least 5 °C for at least 5 days.

Species respond to climate change partly because temperature is a fundamental abiotic variable (Bellard et al., 2012) which influences cells' structural integrity, metabolism and water balance (Frazier et al., 2006; Willmer, 1982). Commonly, life-history traits express a negatively-skewed thermal performance curve; with a gradually accelerating increase in performance from a critical thermal minimum (CT_{min}) to a critical thermal optimum (CT_{opt}), the temperature where a trait performs at its best, then rapid decline to a critical thermal maximum (CT_{max}), beyond which the trait becomes dysfunctional (Angilletta et al., 2010; Terblanche et al., 2007). General patterns across metazoans seems that the ancestral CT_{opt} clusters around 20 °C, CT_{min} varies between 17 °C and −70 °C, whereas the CT_{max} only between 15 °C and 47 °C (Hoffmann et al., 2003). However, lifecycle completion often requires a narrower range such as 10 °C to 30 °C for insects (Angilletta, 2009; Gibbs & Eran, 2009). Natural populations' stress responses, from temperatures encroaching their CT_{max}s, have been extensively recorded over decades (Root et al., 2003; Ummenhofer & Meehl, 2017; van de Pol et al., 2017); examples include range shifts (Chen et al., 2011), phenological changes (Cohen et al., 2018), metabolic adjustments (Bickford et al., 2010), and physiological alterations (Sheridan & Bickford, 2011; Zeuss et al., 2014).

Climate-induced extinctions are a phenomena which are widely reported (Fox et al., 2014; Hallmann et al., 2017; Harvey et al., 2023; Lister & Garcia, 2018; Parmesan & Yohe, 2003). However, there has been "*disturbingly*" little

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knowledge and uncertainty around the biological mechanisms underlying such losses (Cahill et al., 2013, 2014; van de Pol et al., 2017). For example, Urban (2015) reviewed 131 studies, and calculated that on average 7.9% of species are threatened, however, predictions varied between 0% and 54%.

Common research frameworks on extinction often incorporate: "exposure," "vulnerability" and 'adaptive capacity' (Chen et al., 2011; Gienapp et al., 2008; Palmer et al., 2015; Willis et al., 2015). In the context of global warming, exposure equates to the rate of temperature increase and intensity of extreme values, vulnerability is a population's current conservation status, while adaptive capacity is the potential for a population to reduce its future vulnerability. Exposure and vulnerability have been relatively well explored using climate modelling approaches (Oliver et al., 2015; Palmer et al., 2015). However, there is uncertainty for long-term predictions and conservation efforts, because the adaptive capacities of various biological traits remains relatively unknown (Angilletta, 2009; Gienapp et al., 2008; Palmer et al., 2015; Sgrò et al., 2016). For example, extinction rates may be underestimated if just CT_{max} and CT_{opt} metrics are assumed, as this might not to be homogenous across populations/individuals. It might be overestimated if populations are assumed to have no adaptive capacity (Deutsch et al., 2008; Harte et al., 2004). Consequently, laboratory experiments have been recommended to address such knowledge gaps on the drivers of insect extinctions (Cahill et al., 2013; Hoffmann et al., 2003; Hufbauer et al., 2015; Maino et al., 2016).

Two broad mechanisms exist for phenotypes adapt to climate change. The first being evolution by natural selection applying a filter on existing allelic diversity, so that beneficial genes are selected for, and increase in frequency over generations (Bellard et al., 2012; Bijlsma & Loeschcke, 1997). The second adaptive mechanism operates through plasticity, where the phenotype expressed by an individual changes depending on the environmental stimuli previously encountered, without altering deoxyribonucleic acid (DNA)'s primary structure (Loeschcke & Sorensen, 2005; Sgrò et al., 2016). Beneficial plasticity can be divided into short acute hardening or more gradual acclimation; moreover, acclimation can be divided into conditioning individuals during their development, conditioning as adults, or preparing offspring intergenerationally through parental conditioning (Hoffmann et al., 2003; Terblanche et al., 2011). Generally, some researchers view plasticity as a fast time-buying mechanism until more persistent evolutionary selection can occur (Guillaume et al., 2016; Martinez et al., 2016). However, others researcher caution that plasticity can be a stress response with neutral (Woods & Harrison, 2002), or even a maladaptive effects (Franke et al., 2014).

The adaptive potential for increasing the CT_{max} of survival has been explored using a range of biology and diverse methodologies (Hoffmann et al., 2003; Terblanche et al., 2011). Often findings seem mixed, but show that increasing CT_{max} is relatively difficult (Bauerfeind & Fischer, 2014; Hoffmann et al., 2013). However, study of CT_{rng} for fertility seems overlooked (Dolgin et al., 2006; *fig. 2e* Seebacher et al., 2015), despite male reproduction being established as vulnerable trait to temperature, directly linked to fitness, and a constraint on population viability (David et al., 2005; Porcelli et al., 2017; Walsh et al., 2019). The thermal liability of male fertility was recognised in endotherms decades before ectotherms (Setchell, 2006), despite the latter's ecological importance (Dicks et al., 2016), abundance (Stork et al., 2015) and dependence on external temperatures (Paaijmans et al., 2013).

Initially, extensive research in vinegar flies (Drosophilidae) revealed that the critical thermal range (CT_{rng}) of male fertility defined population viability, by being least 1–2 °C narrower than for survival and female fertility (David et al., 2005; Rohmer et al., 2004). Subsequent research has highlighted that heat-sensitivity of male fertility seems widespread across ectothermic taxa (Dougherty et al., 2024; Ørsted et al., 2024; Parratt et al., 2021; Rodrigues et al., 2022; van Heerwaarden & Sgrò, 2021; Walsh et al., 2019; Weaving et al., 2024; Zwoinska et al., 2020), with examples across: Hymenoptera (Dávila & Aron, 2017; Stürup et al., 2013), Lepidoptera (Liang et al., 2014; Zhang et al., 2013), Diptera (Araripe et al., 2004; David et al., 2005), Coleoptera (Saxena et al., 1992; Vasudeva et al., 2014), Nematoda (Harvey & Viney, 2007; Prasad et al., 2011), and Annelida (Guillaume et al., 2016).

Here, the adaptive potential of male biology to increasing mean temperatures associated with climate change, and whether it could mitigate against detrimental impacts of heatwave extremes was assessed. This knowledge gap was tested using the powerful combination of experimental evolution (*reviewed in* Garland & Rose, 2009; Hoffmann & Sgrò, 2011) and the red flour beetle (*Tribolium castaneum*) model insect. *T. castaneum* has several advantages as a amenable research model to investigate thermal adaptation (Mahroof, Subramanyam et al., 2005; Sokoloff, 1974). First, inter-species local thermal adaptation is apparent in natural populations of *Tribolium*, for example, *T. confusum*'s distribution is poleward of *T. castaneum*'s, and the former's CT_{opt} is lower for survival and developmental rate (Park & Frank 1948; Raros & Chiang, 1970; *fig. 10.1,* Sokoloff 1974). Second, *T. castaneum* has intra-specific genetic variation in traits linked to temperature like $CT_{max/min}$ (*fig. 11.2,* Sokoloff 1974), heat-sensitive mutants (Morgan, 1965), and morphology (Noh et al., 2016). Third, *T. castaneum* shows intra-population plasticity with temperature for behaviour (Amos et al., 1968; Park & Frank, 1948) and heat shock protein (HSP) expression (Mahroof, Yan Zhu et al., 2005; Mahroof, Zhu et al., 2005; Schinko et al., 2012). Previously, ecologically relevant 5-day heatwave simulations (Frich et al., 2002; Mherrera, 2016), 5–7 °C higher than *T. castaneum*'s general CT_{opt} (Howe, 1956; Sokoloff, 1974), halved male reproductive fitness and damaged several aspects of fertility such as offspring production, sperm count, sperm storage and sperm viability (Sales et al., 2018, 2024).

Here, by experimentally evolving replicate populations from a common stock in divergent thermal regimes 8 °C apart (30 °C and 38 °C regimes) through non-overlapping generations, we tested the prediction that (1) males from warmer populations (38 °C regime) would have a relatively higher CT_{opt} and better performance near their CT_{max} than males from cooler populations (30 °C regime and progenitor populations). Particularly, that at warm-regime males would have (a) relatively larger testes at 38 °C and that their, (b) post-42 °C heatwave survival, and (c) post-heatwave reproductive fitness would be comparatively greater than cool-regime males. Conversely, thermal adaptation would not be apparent if 30 °C and 38 °C thermal regime males' traits performed similarly. Additionally, we tested (2) whether any apparent adaptation warm-regime males' (a) survival, (b) reproductive fitness, or (c) testes size to higher temperatures was specifically due to long-term inter-generational genetic evolution, or via short-term intra-generational acclimatory modifications during divergent developmental temperatures, using transplant experiments (*following* Dolgin et al., 2006; Hoffmann et al., 2013). Evolution would prevail if the higher inter-generational regime temperature treatment was more effective for the success of traits at higher temperatures, acclimation would be more influential if the immediate generation's exposure to higher temperature during development was more effective for trait success at high temperatures.

Materials and methods

General methods

General maintenance, *T. castaneum* biology, fitness assays, dissections, equipment, and heatwave simulation rationale have previously been described in full detail (Sales, 2019; Sales et al., 2018, 2021, 2024; Vasudeva et al., 2019, 2021). Maintenance of all populations involved in this research were derived from the genetically diverse progenitor stock of 'Kraków Super Strain' (KSS), maintained as non-overlapping generations on a 16L:8D photoperiod, at 30 ± 1 °C and 60 ± 5%RH, with ad libitum fodder under these standard conditions every four weeks. Fodder was nine parts organic flour to one part yeast, and covered with oats to provide traction to adults.

Establishment of experimental evolution lines; thermal regimes

Experimentally evolved populations were created in 2010 from the KSS, an outbred 30 °C progenitor stock (Dickinson, 2018). Each was the product of 7 days of mating and oviposition between 50 males and 50 females, which were randomly selected and sexually mature. Following adult removal, the egg-laden fodder was then either transferred to 300 ml containers to develop at their ancestral temperature of 30 ± 1 °C ($n = 20$) or in simulated warming conditions of 38 ± 1 °C and 60 ± 5%RH ($n = 20$), adhering to standard sampling recommendations (Bretman et al., 2024). Both thermal regimes were maintained using A.B. Newlife 75 Mk4 egg incubators (A.B. Incubators, Suffolk, UK). The long-term 38 °C treatment was not considered a heatwave as (i) 35 °C to 38 °C is the CT_{opt} for several life history traits reported in *T. castaneum* (reviewed in Sales 2019), and (ii) kept constantly since this regime was initiated ~14 years ago. From generation one, these independent replicate populations were continually maintained in their divergent thermal regimes; adults were separated from offspring through non-overlapping generations. At each generation, a random selection of 100 adults (~50:50 sex ratios) were paired for 7-days after which the parents were removed, eggs oviposited during this 7-day period, developed to seed the subsequent generation that took a period of ~30–35 under respective standard rearing conditions.

Experiment 1: General potential for heatwave tolerance (adaptation protocol)

To test whether male reproductive fitness could adapt to local thermal regimes, male pupae were isolated from randomly selected populations grown at control 30 °C (n = 8 populations), or warm 38 °C (n = 8 populations), conditions for 25 generations. 20 male pupae from each population were selected and stored in population-specific Petri dishes, to sexually mature over 12 days. Storage temperatures matched that of the males' original regime. Adult males were allocated to 30 °C or 38 °C treatments, or a 42 °C 5-day heatwave simulation (Section "Heatwave simulations and survival assessment"), in a fully factorial design (Figure 1A) (*following* Hoffmann & Sgrò, 2011). Males were rested for one day at 30 °C before their reproductive fitness was assessed (Section "Mating and males' reproductive fitness") (n ≈ 5 per population per treatment). See Figure 1A for sample sizes, predictions, and interpretation.

Heatwave simulations and survival assessment

Previous studies investigating heat-stress vary widely with exposure times and intensities (*reviewed in* Hoffmann et al., 2003; Walsh et al., 2019). Here, 12 ± 2-day post-eclosion virgin males were exposed to heatwave simulations using Octagon 20 Eco incubators (Brinsea Incubation Specialists, Somerset, UK) (in Experiment 1 and 2). These heatwaves elevated air temperatures to 42 °C for 5 days (also see, Sales et al., 2018, 2021). This treatment corresponds with a common heatwave event definition (Frich et al., 2002). Moreover, 42 °C exceeds the CT_{opt} for several of *T. castaneum*'s life history traits by 5–7 °C (Sales et al., 2018; Sokoloff, 1974). Furthermore, 42 °C is an ecologically relevant temperature, with over 90 countries recently recording heatwave air temperatures above 41 °C (Mherrera, 2016; *table 2.3* Sales 2019). Once heatwave simulations and their controls were accomplished, survival was assessed as the number of adults with, or without, co-ordinated locomotion (in Experiment 2).

Mating and males' reproductive fitness

When assessing males' reproductive fitness following experimental treatments (in Experiment 1 and 2), virgin males were randomly paired with virgin females in 4 ml vials containing 0.5 grams [g] of fodder for a 2-day mating opportunity. Using KSS females from the ancestral stock mitigated possible co-evolutionary thermal and compatibility confounds (*following* Dolgin et al., 2006). After these monogamous pairings, females were transferred to Petri dishes containing 7 g of fodder to oviposit across two independent 10-day blocks. After female removal (terminating oviposition), offspring developed, over 35 days into mature adults. Male reproductive fitness was quantified by the sum of adult offspring produced over the sum of two independent 10-day blocks (block 1: days 1–10 and block 2: days 11–20). Matings, oviposition and offspring development were all maintained at 30 °C.

Testes dissections

Tribolium have paired testes, each pair contains six ellipsoid follicles and the volume of each follicle was calculated from its circumference by drawing an outline using a segmented line tool within ImageJ (Schneider et al. 2012; for method see, Sales et al., 2021). When adult males were dissected to measure testes volume (in Experiment 2), dissections were in saline buffer (1% NaCl solution) under a Zeiss Discovery V.12 stereomicroscope at ×20 magnification using a 0.10*0.06 mm-fine-tipped forceps on a subset of the sample (for further details, see Sales et al., 2021). Each image was acquired by translocating both testes to 30 µl of buffer to a cavity slide, sealing with a coverslip, and capturing images with a phase-contrast microscope. Dissected males' elytra (right side) were also measured to control for body size (see, Vasudeva et al., 2019).

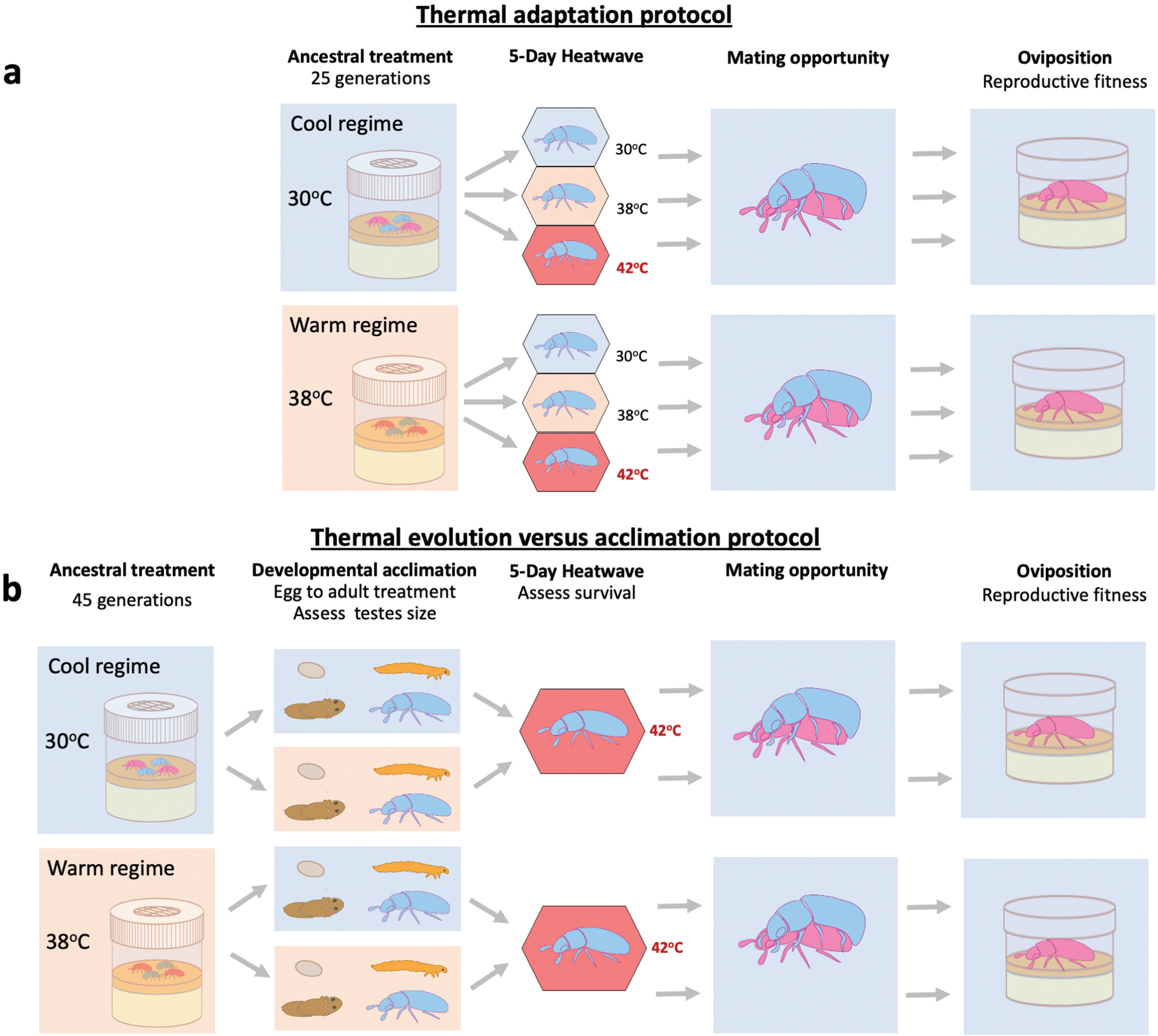

Figure 1. Protocols for assessing exposures to ancestral vs. novel temperatures on male reproductive biology. (A) Experiment 1: Adaptation protocol in replicate populations (*n* = 8 independent populations per regime) cultured for 25 generations in thermal regimes 8 °C apart. Males' (blue) reproductive fitness was assessed following differential temperature exposures for 5-days (*n* = 5 per population per treatment). Any local thermal adaptation present could be due to genetic evolution across generations and/or developmental acclimation via plasticity within the immediate generation. (B) Experiment 2: Acclimation protocol at generation 45 to test the 30 °C and 38 °C regimes. The immediate generation of each thermal regime was either maintained at their historic temperature (30 °C) for a single generation or reciprocally transplanted at the egg stage (sampled within 3 days, before egg hatching into larvae). Once developed into adults males, testes were measured (n = 4 per population per treatment), and post heatwave survival (n = 20 per population per treatment) and reproductive fitness assessed (n = 4 per population per treatment). Evolutionary adaptation would be apparent if males from the warm 38 °C regime (orange ancestry) performed relatively better following exposure to heatwaves. Conversely, acclimatory adaptation would be evident if males from the warm developmental temperatures (orange development) performed relatively better following heatwaves.

Experiment 2: Elucidating acclimation vs. evolved response

Further assays aimed to determine whether evolution and/or acclimation underpinned any potential thermal adaptation (Section "Experiment 1: General potential for heatwave tolerance (adaptation protocol)"). After 45 generations of experimental evolution, the egg laden fodder of each population, produced from a 7-day-long mating opportunity between 100 adults per population, was split in half the amount (egg clutches take ~3–4 days to hatch, see detailed assays within Vasudeva et al., 2019). Half the amount of fodder (laden with fertilised eggs) from each population remained at its regime temperature, the other half was transplanted to the reciprocal regime temperature. As transplants occurred immediately after adult removal cohorts were mostly unhatched, freshly oviposited egg clutches, which maximised the potential for developmental acclimation to novel temperatures (Zhang et al., 2015). Thus, four thermal ancestry-development combinations were produced (30 °C–30 °C, 30 °C–38 °C, 38 °C–30 °C, 38 °C–38 °C) (Figure 1B). 20 male pupae from each ancestry-development temperature combination were selected and stored for 12 days in population-specific Petri dishes to sexually mature. Storage temperatures matched that of the males' development. Testes volumes were measured on a subset of the sample prior to heatwave exposure (Section "Testes dissections"). After exposure to a 42 °C heatwave treatment (Section "Heatwave simulations and survival assessment"), male survival (Section"Heatwave simulations and survival assessment"), and reproductive fitness (Section "Mating and males' reproductive fitness") were assessed ($n = 4$ per population per treatment). See Figure 1B for predictions and interpretation.

Statistical analyses

Data was analysed in RStudio (version 2022.07.2 + 576) with R (version 4.2.1.) (The RStudio Team, 2016). Figures consist of boxplots and error-bars. Boxplots display a mean dot, median line, Inter-quartile rage boxes, 1.5*IQR whiskers and replicate data-points. Error-bars show means ± standard error (*SE*). Significance thresholds are indicated by asterisks (***$p < 0.001$; **$p < 0.010$; *$p < 0.050$), with letters identifying differences between treatments. All test statistics are reported to one decimal place (d.p.), all parameter estimates to one d.p., and all p values to three d.p. Percentage changes are relative to 30 °C treatments. Generalised linear models (GLMs) were implemented with "glm{stats}" (The R Core Team, 2017b), and Generalised Linear Mixed Models (GLMMs) with "glmer{lme4}" (Bates et al., 2016). The most appropriate structures were identified with diagnostic residual plots (Barnard et al., 2011; Bolker et al., 2008; Field, 2009) using "plot{graphics}" (The R Core Team, 2017a) and 'mcp.fnc{LMERConvenienceFunctions}' (Tremblay 2015). R^2 metrics were derived from a pseudo-R^2 function (Thomas et al., 2015; p.110), and from "r.squaredGLMM{MuMIn}" (Barton, 2016) for GLMs and GLMMs, respectively. The overall statistical significance of a factor was calculated by analysis of deviance using "drop1{stats}" (Thomas, et al., 2015). Post-hoc p values were calculated by "summary{model}" (Crawley, 2013) for differences between factor levels and the intercept and by "lsmeans{lsmeans}" (Lenth, 2017) was for pairwise Tukey-adjusted comparisons between all treatments.

The post-heatwave 20-day reproductive fitness of males from the 30 °C and 38 °C regimes in Experiment 1 (Figure 1A), produced count data, which were fitted with a log-linked Poisson GLMM. Fixed factors included the long-term thermal selection regime (30 °C or 38 °C), the 5-day male heatwave treatment (30 °C, 38 °C, or 42 °C), and the interaction between them. Both thermal regimes consisted of eight independently-evolved populations, which were represented by replication (5.7 ± 2.0 *SD* males). Therefore, to account for the population structure of repeatedly sampling from independently evolving population within thermal regimes (Grueber et al., 2011; Crawley 2013, p. 712), a random factor of population code (1–16) was included. Overdispersion was present, where variance of the data was greater than expected (usually due to zero-inflation, excessive variance and/or outliers), which risked unreliable parameter estimates. Therefore, a negative-binomial GLMM was fitted to the data with "glm.nb{MASS}" (Ripley et al., 2016; Thomas et al., 2015), but despite including 'glm.nb(optimiser="bobyqa," optCtrl=list(maxfun=100000))' (Bolker et al., 2008; Powell, 2009) model convergence failed. Consequently, overdispersion was alleviated by adding a "random observer level effect" to the original Poisson GLMM, where each individual replicate was coded as a different number (Bolker et al., 2008; Harrison 2014).

For the post-heatwave mortality analysis across ancestral and developmental treatments in experiment 2 (Figure 1B), data-points were represented by the survival of males grouped into Petri dishes (19.8 ± 0.4 *SD* males). The proportion surviving was analysed with a logit-linked binomial GLM (Crawley, 2013; Thomas et al., 2015), where the response was a paired "cbind(success, fail){base}" frequency variable, with success as survivors and fail as deaths (Thomas et al., 2015). The long-term selection regime (30 °C or 38 °C), developmental temperature (30 °C or 38 °C), and their interaction were inputted as fixed factors. Survival for each population was assessed by measuring a single group of individuals; therefore, no population-level pseudo-replication was present and enabling a GLM. A second logit-linked binomial GLM compared the evolved treatments to a baseline of progenitor KSS stock's survival at 30 °C ($n = 17$ groups), the KSS being genetically diverse and 30 °C-adapted.

The post-heatwave reproductive fitness of males across ancestral and developmental treatments in experiment 2 (Figure 1B), was analysed with a log-linked Poisson GLMM, with the long-term selection regime (30 °C or 38 °C), developmental temperature (30 °C or 38 °C), and their interaction entered as fixed factors. A random factor of population code (1-15) accounted for replicated representation (4.3 ± 1.2 *SD* males) of the independently evolving populations. Both the 30 °C and 38 °C regimes contained eight populations, however, one 30 °C population was omitted because no adults survived the 38 °C-developmental-42 °C-heatwave treatment. The initial Poisson GLMM was overdispersed. Overdispersion was not improved by fitting a negative-binomial GLMM, but was by adding a random observer level effect.

Male testes volume across ancestral and developmental treatments, in experiment 2, was analysed with an identity-linked Gaussian GLMM, with the long-term regime (30 °C or 38 °C), developmental temperature (30 °C or 38 °C), and their interaction entered as fixed factors. Elytra length was included as a co-variate. A random factor of population code (1–16) accounted for replicated representation (4.0 ± 0.0 *SD* males) of the independently evolving populations.

Results

General model structures and results are summarised in Table 1, while Supplementary Table 1 provides further detail with descriptive statistics, beta estimates, associated *z*/*t* scores and *p* values (Table S1).

Experiment 1: Reproductive fitness of males from warm-regime populations was more heatwave-tolerant

The mean 20-day reproductive fitness of males pooled from both the 30 °C and 38 °C selection regime differed following 30 °C, 38 °C, and 42 °C 5-day heatwaves ($\chi^2_{(2,273)} = 22.6$, $p < 0.001$; Figure 2). In particular, the average (pooled) offspring counts were 213.6 ± 16.2, 233.2 ± 13.7, and 159.1 ± 26.5 for 30 °C, 38 °C, and 42 °C, respectively. However, the mean male reproductive fitness from each thermal regime across the three 5-day heatwave temperatures did not differ ($\chi^2_{(1,274)} = 0.7$, $p = .388$); 30 °C-regime males produced 196.7 ± 24.0 offspring, while 38 °C-regime males sired 205.4 ± 21.1.

Table 1. Model summaries for experiments testing whether potential adaptation to increasing mean temperatures improves males' tolerance to heatwave extremes in the red flour beetle (*T. castaneum*). Descriptive statistics, model parameters, and R packages are reported in Supplementary Table 1.

Response variable	Experiment number	Fixed factor	DF	χ^2	p	Model, error distribution and link function	Random effects	R^2
Male survival after 42°C heatwave	2	Ancestral regime temperature	1	83.1	**<0.001**	GLM Binomial (logit)	NA, only one replicate per population (percentage survival in a group)	43%
		Developmental acclimation temperature	1	23.6	**<0.001**			
		Regime*development	1	15.4	**<0.001**			
		Residual	28					
Male reproductive fitness	1	Ancestral regime temperature	1	0.7	0.388	GLMM Poisson (log)	Hierarchical population nesting to prevent pseudo-replication Random observer effect to control for overdispersion	m14% c99%
		Heatwave temperature	2	22.6	**<0.001**			
		Regime*heatwave	2	14.1	**<0.001**			
		Residual	271					
Male reproductive fitness after 42 °C heatwave	2	Ancestral regime temperature	1	4	**0.047**	GLMM Poisson (log)	Hierarchical population nesting to prevent pseudo-replication Random observer effect to control for overdispersion	m12% c99%
		Developmental acclimation temperature	1	7.5	**0.006**			
		Regime*development	1	0.7	0.393			
		Residual	125					
Male testes volume (mm^3)	2	Ancestral regime temperature	1	0.7	0.401	GLMM Gaussian (identity)	Hierarchical population nesting to prevent pseudo-replication Elytra length was a co-variate to control for body-size	m27% c27%
		Developmental acclimation temperature	1	0.8	0.373			
		Regime*development	1	40.7	**<0.001**			
		Elytra length	1	0.0	0.868			
		Residual	123					

Values in bold indicate statistical significance ($P<0.05$).

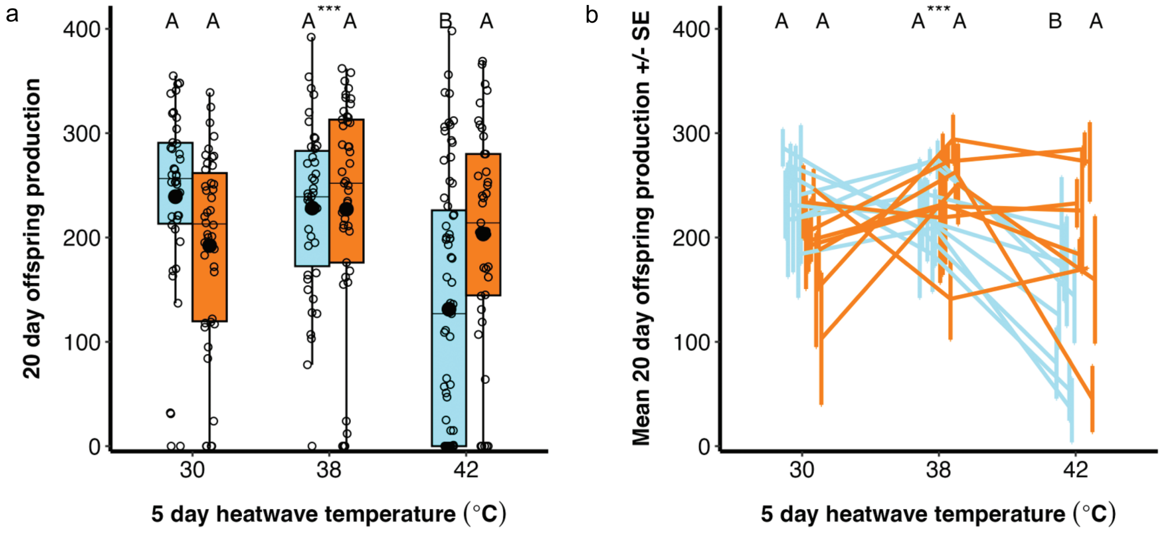

Figure 2. Comparison of male reproductive fitness from independent populations under different thermal regimes after exposure to a range of 5-day heatwave temperatures. (A) Contrast between relative warm 38 °C regime males (orange fill) ($n_{population}$ = 8) and 30 °C regime males (blue fill) ($n_{population}$ = 8), where each population was replicated (5.7 ± 2.0 *SD* males). (B) Population-specific patterns across heatwave temperatures, sample sizes and colour scheme match (A).

Nonetheless, there was a significant interaction as the regimes responded differently across heatwave temperatures ($\chi^2_{(2,273)} = 14.1$, $p < 0.001$). The reproductive fitness of males from both thermal regimes were comparable following 30 °C ($z = 1.0$, $p = 0.923$) and 38 °C heatwaves ($z = 1.1$, $p = 0.899$). However, the average reproductive fitness of 38 °C-regime males was 55% greater following 42 °C heatwaves relative to 30 °C-regime males ($z = 3.5$, $p = 0.005$), indicating adaption to extreme temperatures. The reproductive fitness of males from the 30 °C regime, after a 42 °C heatwave, was also significantly reduced compared to 30 °C ($z = -5.1$, $p < 0.001$) and 38 °C ($z = -5.3$, $p < 0.001$) exposures. Whereas, the reproductive fitness of 38 °C-regime males was relatively constant across heatwave-exposures.

Experiment 2: Fitness outcome was modulated by interaction of inter-generational evolution and developmental acclimation temperatures

Survival of adult males after a 42 °C heatwave event was affected by the ancestral temperature of populations; relative to 30 °C-regime males, 38 °C males were 77% more resistant to heatwave mortality ($\chi^2_{(1,30)} = 83.1$, $p < 0.001$; Figure 3). Moreover, the survival of 38 °C-regime males in heatwaves was comparable to males from the 30 °C-adapted progenitor KSS stock in standard 30 °C conditions ($\chi^2_{(2,46)} = 155.4$, $p < 0.001$; $z = 1.1$, $p = 0.502$). The developmental temperature of the immediate generation was also important for survival, with populations developing at 38 °C being 26% worse at surviving heatwaves than those developing at 30 °C ($\chi^2_{(1,30)} = 23.6$, $p < 0.001$). Additionally, there was an interaction between ancestral temperature and developmental temperature on heatwave survival ($\chi^2_{(1,30)} = 15.4$, $p < 0.001$). 30 °C-regime male heatwave survival was lower than 38 °C males at 30 °C ($z = -7.6$, $p < 0.001$), and at 38 °C ($z = -4.3$, $p < 0.001$), but was relatively consistent across developmental temperature ($z = 1.4$, $p = 0.519$). During this assay, one of the populations from 30 °C failed to survive at 38 °C, so was lost within this set-up (therefore, $N = 7$ as opposed to $N = 8$ populations). Contrarily, 38 °C-regime survival was greatest when the immediate generation grew at 30 °C ($z = 5.5$, $p < 0.001$).

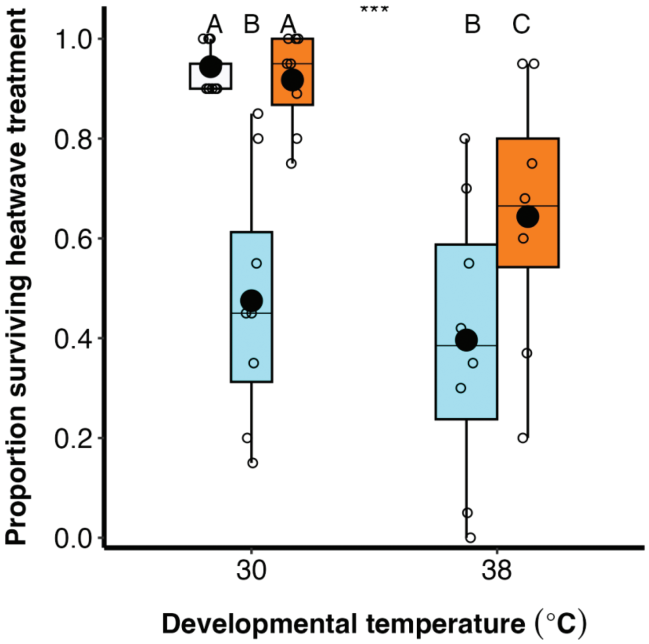

Figure 3. Comparison of male post-heatwave survival between populations maintained at 30 °C or 38 °C. Contrast between relatively warm 38 °C regime males (orange fill) ($n_{\text{population}} = 8$) and 30 °C males (blue fill) ($n_{\text{population}} = 8$) survival following a 42 °C 5-day heatwave. Comparison to males from 30 °C-adapted progenitor KSS stock, maintained at 30 °C, and not exposed to heatwaves (white fill) ($n = 17$). Each data-point was a group (19.8 ± 0.4 SD males) maintained in single-sex groups.

The 20-day reproductive fitness of adult males after a 42 °C heatwave was affected by the ancestral temperature of populations. Relative to the 30 °C regime, 38 °C populations were 41% more productive ($\chi^2_{(1,126)} = 4.0$, $p = 0.047$; Figure 4), indicating local thermal adaptation. Additionally, the immediate generation's developmental temperature altered reproductive fitness. Males from populations developing at 38 °C sired 29% fewer offspring than those at 30 °C ($\chi^2_{(1,126)} = 7.5$, $p = 0.006$). There was no interaction between developmental and ancestral temperature on reproductive fitness ($\chi^2_{(1,126)} = 0.7$, $p = 0.393$). There was also no difference in reproductive fitness across developmental differences within the 30°C-regime ($z = 2.4$, $p = 0.076$) and 38 °C-regime males ($z = 1.6$, $p = 0.391$). Likewise, reproductive fitness was similar between the ancestral regimes within the 30 °C ($z = -1.2$, $p = 0.639$) and 38 °C ($z = -2.2$, $p = 0.133$) developmental temperatures. However, the reproductive fitness of 30 °C-regime males developing at 38 °C was significantly lower than the 38 °C regime developing at 30° C ($z = -3.4$, $p = 0.003$).

Overall, adult male testes volume was not affected by their evolutionary thermal regime ($\chi^2_{(1,126)} = 0.7$, $p = 0.401$), their immediate developmental temperature ($\chi^2_{(1,126)} = 0.8$, $p = 0.373$), or their body size ($\chi^2_{(1,126)} = 0.0$, $p = 0.868$). However, there was an antagonistic interaction of evolutionary regime and developmental temperature on testes volume ($\chi^2_{(1,126)} = 40.4$, $p < 0.001$, Figure 5), which was relatively smaller in populations developing in temperatures discordant from their ancestry. Specifically, males from the cool 30 °C regime had relatively smaller testes if they developed 38 °C ($z = -3.8$, $p = 0.001$), whereas males from the warm 38 °C regime had relatively smaller testes if they developed 30 °C ($z = -5.5$, $p = 0.001$).

Discussion

Local thermal adaptation and heatwave resistance of male fertility

Heatwaves reduced the reproductive fitness of males from experimentally-evolved 30 °C-regime populations by approximately half, which conformed to previous experiments (Sales et al., 2018; Vasudeva et al., 2021). Elevating populations by 8 °C for 25 generations raised the CT_{opt}/CT_{max} for male fertility, resulting in a 55% relative increase in post-heatwave reproductive fitness. The consistent improvement across independent population replicates suggested adaptation rather than genetic drift (Garland & Rose, 2009). Moreover, this warm adaptation may have been a conservative estimate because post-heatwave recovery, oviposition, and offspring development occurred at 30 °C; because of logistical constraints and to preserve experimental consistency between treatments (Dolgin et al., 2006), which was likely advantageous for developing offspring from 30 °C-regime males.

The 38 °C-regime males were predicted to perform relatively worse at 30 °C because adaptation to increasing temperature seems physiologically difficult (Li et al., 2015), and can trade-off against cold resistance (David et al., 2005). Surprisingly, here in *T. castaneum* this was not apparent. Previous work investigating thermal adaptation of *Tribolium* species is limited, especially for male fertility. However, *T. castaneum*, which is more tropical in its distribution than *T.*

confusum and *T. madens,* has higher CT_{opt}/CT_{rng} for survival and population fitness in culture comparison experiments (Freeman, 1962; Howe, 1956; Park & Frank, 1948; Raros & Chiang, 1970). Here, thermal selection on 38 °C-regime males has elevated their fertility CT_{opt} beyond their ancestral CT_{opt} for fertility and other life-history traits including: larval survival (Sokoloff 1974), oviposition behaviour (Waterhouse et al., 1971), population fitness (Howe 1956; Sokoloff 1974), and female fertility (Sales et al., 2018).

There is a greater amount of published research outside *Tribolium* for exploring local thermal adaptation using *Drosophila*, focussing mainly on survival traits ($n = 18$ studies, 6 species) (*table 4* Hoffmann et al., 2003). However, some studies have shown local thermal adaptation of male fertility, like here in *T. castaneum,* by using: (a) inter-specific comparisons, (b) intra-specific contrasts, or (c) experimental evolution. Generally, the inter-species CT_{rng} for male reproductive fitness, was between 6 and 32 °C ($n = 9$ species) (*table 1*, David et al., 2005), and correlated with latitude. For example, heat-tolerant tropical species *Zaprionus indianus* (Araripe et al., 2004) was fertile at 30 °C, while the European *D. subobscura* was unculturable beyond 25 °C (Moreteau et al., 1997). Similarly, CT_{max} for male fertility seemed to correlate with latitude intra-specifically, such as with *D. melanogaster* ($n = 24$ populations) (Rohmer et al., 2004). CT_{max} for *D. melanogaster* male fertility has been artificially selected from 29 °C to 32 °C, but no higher (Chakir et al., 2002; Zatsepina et al., 2001). Beyond *Tribolium* and *Drosophila*, research demonstrating local thermal reproductive adaptation seems infrequent but includes: nematodes (*Caenorhabditis* spp.) (Harvey & Viney,

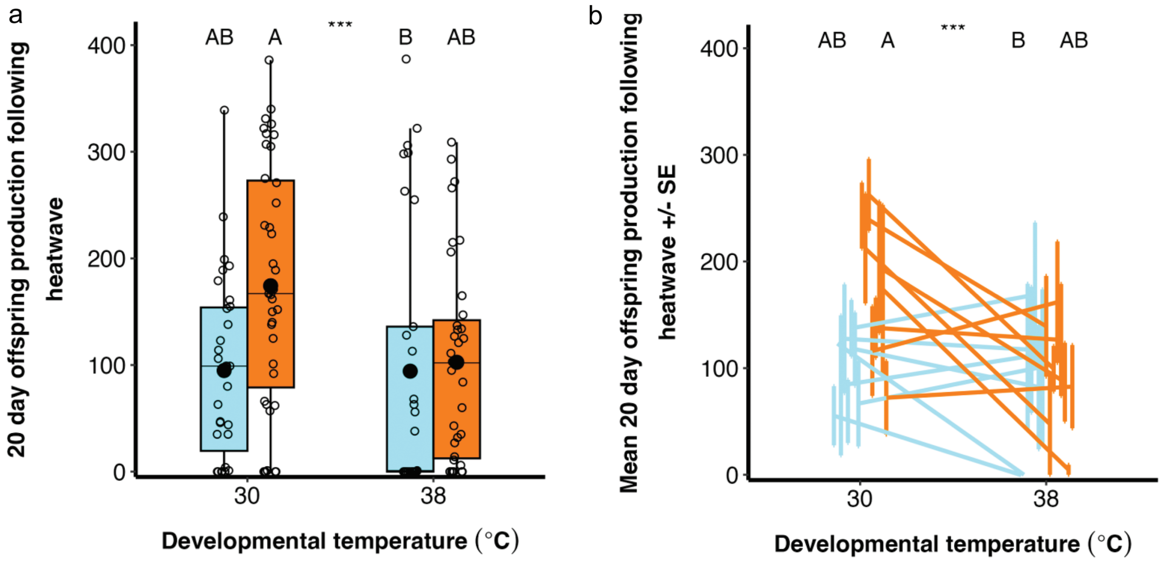

Figure 4. Comparison of ancestral or developmental temperature impacts on male reproductive fitness after exposure to a 5-day heatwave. (A) Contrast between relatively warm 38 °C-regime males (orange fill) ($n_{population} = 8$) and 30 °C-regime males (blue fill) ($n_{population} = 7$), where each population was replicated (4.3 ± 1.2 *SD* males). (B) Population-specific patterns across developmental temperatures, sample sizes and colour scheme match (A).

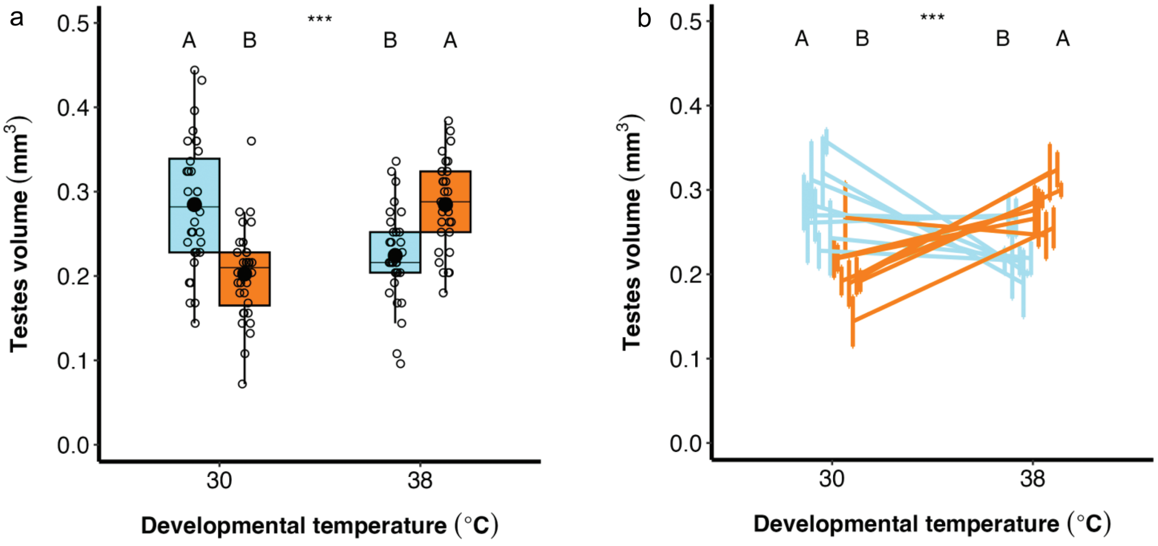

Figure 5. Comparison of ancestral or developmental temperature impacts on testes volume. (A) Contrast between relatively warm 38 °C regime males (orange) ($n_{population} = 8$) and 30 °C males (blue) ($n_{population} = 8$), where each population was replicated (4 ± 0 *SD* males). (B) Population-specific patterns across developmental temperatures, sample sizes and colour scheme match (A).

2007; Prasad et al., 2011), cattle (*Bos* spp.) (Rahman et al., 2018; Skinner & Louw, 1966), butterflies (Karlsson & Wiklund, 2005), and aphids (*f*ig. 2, Buckley & Kingsolver 2012).

Potential physiological mechanisms for thermal adaptation of male fertility

The underlying resistance of warm-adapted male fertility to heatwaves may be due to CT_{max} increasing for spermatogenic traits. Post-heatwave sperm from 38 °C-regime males could have been relatively more viable, motile and/or less prone to breaks. Sperm motility and viability was shown to underlie CT_{max} patterns for reproductive fitness in *D. melanogaster* and *D. simulans* (*f*ig. 4, Chakir et al., 2002), the more tropical *D. melanogaster*'s motility and fertility was 1.5 °C higher comparatively. Other research has linked reduced sperm length in *Drosophila* species to increased heat resistance, because of the decreased likelihood of breaks (Araripe et al., 2004; David et al., 2005). Likewise, warm-adapted males' spermatogenesis may more consistently produce morphologically-normal sperm at higher temperatures (Rohmer et al., 2004).

The 38 °C-regime males may also have more general adaptations to mitigate heatwave damage to general body condition. Behaviourally, they may have more sensitive thermotaxis to limit heat-exposure (Amos et al., 1968; Kim et al., 2015). Morphologically, they may have developed better circulation and insulation to thermoregulate testes (Skinner & Louw 1966), and/or less permeable cuticles to limit water stress (Noh et al., 2016). Metabolically, they may have developed more heat-stable and efficient proteins like phosphoglucose isomerase (PGI) (Dahlhoff et al., 2008). They may have better antioxidant defences in the testes to limit DNA damage (Kodama et al., 1997), and/or produce sperm membranes rich heat-tolerant polyunsaturated fatty acids (van Dooremalen et al., 2011). Local thermal adaptation, as seen here in *T. castaneum,* is likely linked to more efficient and effective HSP expression under extreme temperatures, which has been frequently reported in *Drosophila* ($n = 20$ studies) (*table 5,* Hoffmann et al., 2003). First, several HSPs have been characterised in *T. castaneum*, and have been associated with traits like heat-shock survival (Mahroof, Yan Zhu et al., 2005; Mahroof, Zhu et al., 2005; Schinko et al., 2012). Second, specific HSPs like *HSP70* and *HSP90*, are thought to protect and regulate spermatogenesis, as they are expressed comparatively strongly in the testes during stress in other species (Han et al., 2016; Long et al., 2015), and spermatogenic failures occur when the HSPs are interrupted (Dix et al., 1996).

Potential genetic mechanisms for thermal adaptation of male fertility

The 38 °C-regime males' heatwave tolerance could be due to thermal selection on phenotypes suggested above, which have genetically-evolved through existing allelic diversity and mutations (Bijlsma & Loeschcke, 1997). Variability in genetic diversity for heat tolerance should exist, with CT_{max} differing between reproductively isolated populations (*reviewed in* Allen et al., 2006; Hoffmann et al., 2013). Other *T. castaneum* strains maintained in the same environment show some variability in thermotolerance, for example, 42 °C caused a 50% decline in reproductive fitness of KSS, 100% in GA-1, and 100% mortality in GFP (Dickinson, 2018; Sales et al., 2024).

In *Drosophila,* the variability of traits' thermotolerance has been related to specific genetic components including: genome size, chromosome number, inversion frequencies, single nucleotide polymorphism (SNP) mutations, and/or gene duplications (Balanya et al., 2006; Bundgaard & Barker, 2017; Gienapp et al., 2008; Vollmer et al., 2004). Generally, larger genomes and higher ploidy are thought to be better for adaptation, as there is more raw material for selection (Meirmans et al., 2006). However, with the relatively few generations of *T. castaneum* experimental evolution, genetic changes are likely smaller scale; SNPs and allele frequencies. Fertility-linked changes may focus on the Y-chromosome, which across *D. melanogaster* populations influenced 50% of the variation in CT_{max} for male fertility (Rohmer et al., 2004). Selection on 38 °C-regime male genomes may have been purifying to remove deleterious alleles and/or positive for beneficial ones. Examples of thermal selection on alleles for fertility-linked genes in other species include: *fer-15* (Kenyon, 2011), *alg-3/4* (Plesnar-Bielak et al., 2017; Prasad et al., 2011), and the *dynein* gene family (Carvalho et al., 2001). Examples of thermal selection on alleles for traits linked to broader condition include: HSPs (*table 5* Hoffmann et al., 2003; Ramon et al., 2014), PGI (Dahlhoff et al., 2008), DNA repair (Paul et al., 2008), and mtDNA (Camus et al., 2017; Ellers et al., 2008).

Thermal adaptation by acclimation or evolution?

Experimental evolution to increased mean temperature proved adaptive for 42 °C-heatwave resistance in *T. castaneum*; the survival and reproduction of 38 °C-regime males, as the mean pooled across 30 °C and 38 °C acclimation temperatures, was greater than males from 30 °C populations. However, unexpectedly, the post-heatwave survival and reproduction of 38 °C-regime males was greatest when the developmental temperature of the immediate generation was 30 °C rather than 38 °C. Likewise, there was an antagonistic interaction between ancestral and developmental temperature for testes volume. These findings indicate that acclimation temperature is context specific for certain fitness traits.

Several species, including *D. melanogaster*, have shown that acclimating the parental generation, the immediate generation during development, or the immediate generation as adults over prolonged periods to warmer conditions can improve subsequent metabolism and survival (Gilchrist & Huey, 2001; *table 2* Hoffmann et al., 2003; Seebacher et al., 2015). Less frequently demonstrated is high temperature acclimation being beneficial for reproductive traits (Araripe et al., 2004; Fenkes et al., 2017; Harvey & Viney, 2007; Porcelli et al., 2017; Prasad et al., 2011; Purchase et al., 2010). Conversely, other research parallels the *T. castaneum* findings here, that acclimation to elevated temperature seems neutral, or even detrimental to fitness outcomes, in subsequent heat-stress. For example, studies of butterflies (Bauerfeind & Fischer, 2014), parasitoid wasps (Scott et al., 1997), and *Drosophila* spp. (Vollmer et al., 2004) found that increasing developmental temperature exerted life-history costs, without improving subsequent adult performance in heat-stress. Other studies have found that elevated acclimatory temperatures impacted negatively on sperm traits (Adriaenssens et al., 2012; Ojima et al., 2015; Zeh et al., 2014).

Overall, there is uncertainty across literature as to whether inter-generational evolution or intra-generational acclimation is more important for adaptation, and whether acclimation is beneficial or detrimental ($n = 64$ articles) (*table 6.2,* Sales, 2019). Furthermore, mixed fitness outcomes have been found for: (a) different traits while keeping the species and acclimation method constant (Janowitz & Fischer, 2011; Porcelli et al., 2017; Stillwell & Fox, 2004; Woestmann & Saastamoinen, 2016) and (b) different acclimation methods while holding the species and traits constant (Kellermann et al., 2017; Porcelli et al., 2017; Richter et al., 2010; Scott et al., 1997; Zeh et al., 2014). The varied effectiveness of acclimation between studies, organisms, acclimation types and traits to could be due to several interacting causes (Boggs, 2016; Ellers et al., 2008; Hoffmann et al., 2003; Sgrò et al., 2016). In particular, fitness outcomes from acclimatory periods likely depend on how close the temperature is to a trait's CT_{max}, the timing of its onset, its duration, and the timing between acclimation and stress-testing.

Acclimation is thought to alter gene expression to create a more heat resistant phenotype through a variety of pathways including: metabolism (Ellers et al., 2008), nutrient transport (Veilleux et al., 2015); antioxidants (Kodama et al., 1997), solvent modifiers (Salvucci, 2000), lipid membranes (van Dooremalen et al., 2011) and HSPs (Mahroof, Yan Zhu et al., 2005). The failure of the 38 °C-developmental acclimation to benefit 38 °C-regime males' heatwave resistance may have been because 38 °C was too low to stimulate the correct triggers for an effective induced acclimatory response (*f*ig. 1, Angilletta 2009; Tomanek 2008). Alternatively, the potential adaptive mechanisms of 38 °C-regime males may be genetically fixed with little scope for plasticity, or that the phenotypic changes required above CT_{opt} were too costly (Overgaard et al., 2011). Conversely, the acclimatory period may have been too protracted and/or hot so the costs of stress may have outweighed the benefits of altered gene expression (Stillwell & Fox, 2004; Terblanche et al., 2005). For example, 42 °C heat-shocks on butterfly (*Junonia orithya*) pupae produced morphologically adaptive colouration associated with summer morphs, however, higher temperatures produced maladaptive colouration and mortality (Mahdi et al., 2011). Similarly, if the 38 °C-acclimation of the 38 °C-regime proved stressful, and despite their evolutionary genetic adaptation of heatwave tolerance, their CT_{opt} may still be closer to 30 °C. Thus, their metabolism could have benefitted from a relaxation at 30 °C, prior to heatwave challenge. The drain on reproductive performance with super-optimal development has also been demonstrated in other insects (Franke et al., 2014; Vollmer et al., 2004). There could have even been a hormetic effect, with the active reallocation of resources from reproduction to soma maintenance, if acclimation proved stressful (Chirault et al., 2015; Sheridan & Bickford, 2011).

Limitations and extensions

The baseline control temperature selected in the experiments was 30 °C, which is the ancestral temperature, but marginally suboptimal for several life history traits (approximately 35 °C), and it is likely that 38 °C regime experienced the upper boundaries of their CT_{opt}. Therefore, testing in a common garden intermediate temperature (e.g., sub-optimal for both regimes in opposing directions) might yield further insights and help better understand how individuals/populations respond to different thermal averages, which are likely under continued global climate change.

Across both experiments post-heatwave recovery, oviposition, and offspring development occurred at 30 °C, which may have benefitted 30 °C regime males and reduced effect sizes. Similarly, the adaptive reproductive fitness of 38 °C regime males to higher temperatures may have been hindered by all female mates being from 30 °C-KSS stock as opposed to the 38 °C regime. For fuller understanding of potential local thermal adaptation at different levels of life history, differential temperature treatments could be applied on mating behaviour, fertilisation, and maternal effects. Moreover, experiment two only assessed survival and reproductive fitness after 42 °C as this was the treatment most of interest, but equivalent controls at 30 °C and 38 °C would have been comprehensive.

Future work should also elucidate proximate mechanisms likely to promote adaptation and the role of gametes in this with signatures for adaptive plasticity. Here, the response of *T. castaneum* has been shown with constant temperature changes, however, 'Jensen's Inequality' predicts that traits respond differently with fluctuating conditions, including their magnitude and mean relative to CT_{opt} (Franke et al., 2014; Rolandi & Schilman, 2018; Zeh et al., 2014). Because of mixed findings in previous research, and the need for relevance to natural environments, further research into effects of temperature fluctuations has been recommended; so too has incorporating interactions between multiple realistic stressors like inbreeding (Pedersen et al., 2011) and dietary stress.

Conclusions

Here, experimental evolution with warming could rapidly improve the heatwave resistance of male reproductive biology, but fitness outcomes seemed dependent on the interaction of evolution and acclimation. Finding that male fertility could adapt to long-term warming is broadly positive, especially in the context of previous findings that spermatogenesis can recover from heatwave damage (Sales et al., 2018, 2021, 2024). However, our work exploring thermal plasticity via hardening (Sales et al., 2018, 2021) or acclimation (here; Vasudeva et al., 2019), have shown that plasticity is only adaptive under certain specific thermal contexts (e.g., developmental exposure of gametes at lower temperatures, 30 °C vs. 38°C). In general, this makes studying fertility impacts to global climate change using ectothermic models exciting with the technological evolution of genomics, epigenomics and metabolomics (Kokko et al., 2017). Although the experimental designs here were balanced, several other contrasts could extend this framework, in addition to investigating many other response variables and treatment combinations available. Now that the experimental evolution has exceeded over 100 generations, divergence across cellular, morphological, and/or genomes potentially underlying male fertility seems possible and urgent.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Data availability

The datasets associated with this manuscript can be found here: https://doi.org/10.5061/dryad.m0cfxppd4.

Author contributions

Kris Sales (Conceptualization [Equal], Data curation [Equal], Formal analysis [Equal], Investigation [Equal], Methodology [Equal], Project administration [Equal], Resources [Equal], Software [Equal], Supervision [Equal], Validation [Equal], Visualization [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), Ramakrishnan Vasudeva (Conceptualization [Equal], Data curation [Equal], Investigation [Equal], Methodology [Equal], Project administration [Equal], Resources [Equal], Software [Equal], Supervision [Equal], Validation [Equal], Writing—review & editing [Equal]), and Matthew Gage (Conceptualization [Equal], Data curation [Equal], Funding acquisition [Equal], Methodology [Equal], Project administration [Equal], Resources [Equal], Supervision [Equal], Writing—original draft [Equal], Writing—review & editing [Equal])

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Conflicts of interest

None declared.

Ethical statement

This study was approved by, and followed strict guidelines to, the University of East Anglia's Animal Welfare and Ethical Review Board.

References

Adriaenssens, B., van Damme, R., Seebacher, F., & Wilson, R. (2012). Sex cells in changing environments: Can organisms adjust the physiological function of gametes to different temperatures? *Global Change Biology*, *18*, 1797–1803. https://doi.org/10.3389/fphys.2022.754830

Allen, A. P., Gillooly, J. F., Savage, V. M., & Brown, J. H. (2006). Kinetic effects of temperature on rates of genetic divergence and speciation. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(24), 9130–9135. https://doi.org/10.1073/pnas.0603587103

Amos, T. G., Waterhouse, F. L., & Chetham, N. A. (1968). Temporal distribution of Tribolium castaneum Herbst and *Cadra cautella* (Walker) on temperature gradients. *Experientia*, *24*(1), 86–87. https://doi.org/10.1007/BF02136812

Angilletta, M. J. (2009). Looking for answers to questions about heat stress: Researchers are getting warmer. *Functional Ecology*, *23*, 231–232. https://doi.org/10.1111/j.1365-2435.2009.01548.x

Angilletta, M. J., Huey, R. B., & Frazier, M. R. (2010). Thermodynamic effects on organismal performance: Is hotter better? *Physiological and Biochemical Zoology*, *83*(2), 197–206. https://doi.org/10.1086/648567

Araripe, L. O., Klaczko, L. B., Moreteau, B., & David, J. R. (2004). Male sterility thresholds in a tropical cosmopolitan Drosophilid, *Zaprionus indianus*. *Journal of Thermal Biology*, *29*(2), 73–80. https://doi.org/10.1016/j.jtherbio.2003.11.006

Balanya, J., Oller, J. M., Huey, R. B., … Serra, L. (2006). Global genetic change tracks global climate warming in *Drosophila subobscura*. *Science*, *313*(5794), 1773–1775. https://doi.org/10.1126/science.1131002

Barnard, C., Gilbert, F., & McGregor, P. (2011). *Asking questions in biology: A guide to hypothesis testing, experimental design and presentation in practical work and research projects* (4th ed.). Pearson Education.

Barton, K. (2016). *MuMIn: Multi-model inference*. https://cran.r-project.org/web/packages/MuMIn/MuMIn.pdf

Bates, D., Maechler, M., Bolker, B., … Green, P. (2016). *Lme4: Linear mixed-effects models using "Eigen" and S4*. https://cran.r-project.org/web/packages/lme4/lme4.pdf

Bauerfeind, S. S., & Fischer, K. (2014). Simulating climate change: Temperature extremes but not means diminish performance in a widespread butterfly. *Population Ecology*, *56*(1), 239–250. https://doi.org/10.1007/s10144-013-0409-y

Bellard, C., Bertelsmeier, C., Leadley, P., … Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, *15*(4), 365–377. https://doi.org/10.1111/j.1461-0248.2011.01736.x

Bickford, D., Howard, S. D., Ng, D. J. J., & Sheridan, J. A. (2010). Impacts of climate change on the amphibians and reptiles of Southeast Asia. *Biodiversity and Conservation*, *19*(4), 1043–1062. https://doi.org/10.1007/s10531-010-9782-4

Bijlsma, R., & Loeschcke, V. (1997). *Environmental stress, adaptation, and evolution* (1st ed.). Springer.

Boggs, C. L. (2016). The fingerprints of global climate change on insect populations. *Current Opinion in Insect Science*, *17*, 69–73. https://doi.org/10.1016/j.cois.2016.07.004

Bolker, B. M., Brooks, M. E., Clark, C. J., … White, J. S. S. (2008). Generalized linear mixed models: A practical guide for ecology and evolution. *TREE*, *24*(3), 127–135. https://doi.org/10.1016/j.tree.2008.10.008

Bretman, A., Fricke, C., Baur, J., … Snook, R. R. (2024). Systematic approaches to assessing high-temperature limits to fertility in animals. *Journal of Evolutionary Biology*, *37*(4), 471–485. https://doi.org/10.1093/jeb/voae021

Buckley, L. B., & Kingsolver, J. G. (2012). Functional and phylogenetic approaches to forecasting species' responses to climate change. *Annual Review of Ecology, Evolution, and Systematics*, *43*(1), 205–226. https://doi.org/10.1146/annurev-ecolsys-110411-160516

Bundgaard, J., & Barker, J. S. F. (2017). Genetic variation for resistance to high temperature stress of mature sperm – A study in *Drosophila*. *PLoS One*, *12*(3), e0173990. https://doi.org/10.1371/journal.pone.0173990

Cahill, A. E., Aiello-Lammens, M. E., Fisher-Reid, C. M., … Wiens, J. J. (2013). How does climate change cause extinction? *Proceedings of the Royal Society of London. Series B*, *280*, 20121890. https://doi.org/10.1098/rspb.2012.1890

Cahill, A. E., LammensM. E.Aiello-Lammens, M. E., ReidM. C.Fisher-Reid, M. C., … Wiens, J. J. (2014). Causes of warm-edge range limits: Systematic review, proximate factors and implications for climate change. *Journal of Biogeography*, *41*, 429–442.

Camus, M. F., Wolff, J. N., Sgrò, C. M., & Dowling, D. K. (2017). Experimental support that natural selection has shaped the latitudinal distribution of mitochondrial haplotypes in Australian *Drosophila melanogaster*. *Molecular Biology and Evolution*, *34*(10), 2600–2612. https://doi.org/10.1093/molbev/msx184

Carvalho, A. B., Dobo, B. A., Vibranovski, M. D., & Clark, A. G. (2001). Identification of five new genes on the Y chromosome of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(23), 13225–13230. https://doi.org/10.1073/pnas.231484998

Chakir, M., Chafik, A., Moreteau, B., … David, J. R. (2002). Male sterility thermal thresholds in *Drosophila*: *D. simulans* appears more cold-adapted than its sibling *D. melanogaster*. *Genetica*, *114*(2), 195–205. https://doi.org/10.1023/a:1015154329762

Chen, I., Hill, J. K., Ohlemüller, R., … Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024–1026. https://doi.org/10.1126/science.1206432

Chirault, M., Lucas, C., Goubault, M., … Lécureuil, C. (2015). A combined approach to heat stress effect on male fertility in *Nasonia vitripennis*: From the physiological consequences on spermatogenesis to the reproductive adjustment of females mated with stressed males. *PLoS One*, *10*(3), e0120656. https://doi.org/10.1371/journal.pone.0120656

Christidis, N., Jones, G. S., & Stott, P. A. (2015). Dramatically increasing chance of extremely hot summers since the 2003 European heatwave. *Nature Climate Change*, *5*(1), 46–50. https://doi.org/10.1038/nclimate2468

Cohen, J. M., Lajeunesse, M. J., & Rohr, J. R. (2018). A global synthesis of phenological responses to climate change. *Nature Climate Change*, *8*, 224–228. https://doi.org/10.1101/164806

Crawley, M. J. (2013). *The R book* (2nd ed.). John Wiley and Sons Ltd. https://doi.org/10.1037/023990

Dahlhoff, E. P., Fearnley, S. L., Bruce, D. A., … Rank, N. E. (2008). Effects of temperature on physiology and reproductive success of a montane leaf beetle: Implications for persistence of native populations enduring climate change. *Physiological and Biochemical Zoology*, *81*(6), 718–732. https://doi.org/10.1086/590165

David, J. R., Araripe, L. O., Chakir, M., … Moreteau, B. (2005). Male sterility at extreme temperatures: A significant but neglected phenomenon for understanding *Drosophila* climatic adaptations. *Journal of Evolutionary Biology*, *18*(4), 838–846. https://doi.org/10.1111/j.1420-9101.2005.00914.x

Dávila, F., & Aron, S. (2017). Protein restriction affects sperm number but not sperm viability in male ants. *Journal of Insect Physiology*, *100*, 71–76. https://doi.org/10.1016/j.jinsphys.2017.05.012

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., … Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(18), 6668–6672. https://doi.org/10.1073/pnas.0709472105

Dickinson, M. (2018). *The impacts of heat-wave conditions on reproduction in a model insect, Tribolium castaneum* [PhD thesis]. University of East Anglia (UEA).

Dicks, L. V., Viana, B., Bommarco, R., … Potts, S. G. (2016). Ten policies for pollinators: What governments can do to safeguard pollination services. *Science*, *356*, 975–976. https://doi.org/10.1126/science.aai9226

Dix, D. J., Allen, J. W., Collins, B. W., … Eddy, E. M. (1996). Targeted gene disruption of Hsp70-2 results in failed meiosis, germ cell apoptosis, and male infertility. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(8), 3264–3268. https://doi.org/10.1073/pnas.93.8.3264

Dolgin, E. S., Whitlock, M. C., & Agrawal, A. F. (2006). Male *Drosophila melanogaster* have higher mating success when adapted to their thermal environment. *Journal of Evolutionary Biology*, *19*(6), 1894–1900. https://doi.org/10.1111/j.1420-9101.2006.01168.x

Dougherty, L. R., Frost, F., Maenpaa, M. I., … Price, T. A. R. (2024). A systematic map of studies testing the relationship between temperature and animal reproduction. *Ecological Solutions and Evidence*, *5*(1), e12303.

Ellers, J., Marien, J., Driessen, G., & Van Straalen, N. M. (2008). Temperature-induced gene expression associated with different thermal reaction norms for growth rate. *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution*, *310*(2), 137–147. https://doi.org/10.1002/jez.b.21194

Fenkes, M., Fitzpatrick, J. L., Ozolina, K., … Nudds, R. L. (2017). Sperm in hot water: Direct and indirect thermal challenges interact to impact on brown trout sperm quality. *Journal of Experimental Biology*, *220*(Pt 14), 2513–2520. https://doi.org/10.1242/jeb.156018

Field, A. (2009). *Discovering statistics using SPSS* (3rd ed.). SAGE Publications.

Fox, R., Oliver, T. H., Harrower, C., … Roy, D. B. (2014). Long-term changes to the frequency of occurrence of British moths are consistent with opposing and synergistic effects of climate and land-use changes. *Journal of Applied Ecology*, *51*(4), 949–957. https://doi.org/10.1111/1365-2664.12256

Franke, K., Heitmann, N., Tobner, A., & Fischer, K. (2014). Fitness costs associated with different frequencies and magnitudes of temperature change in the butterfly *Bicyclus anynana*. *Journal of Thermal Biology*, *41*, 88–94. https://doi.org/10.1016/j.jtherbio.2014.02.006

Frazier, M. R., Huey, R. B., & Berrigan, D. (2006). Thermodynamics constrains the evolution of insect population growth rates: "warmer is better.". *American Naturalist*, *168*(4), 512–520. https://doi.org/10.1086/506977

Freeman, J. A. (1962). The influence of climate on insect populations of flour mills. *Transactions 11th Congress of Entomology*, *2*, 301–308.

Frich, P., Alexander, L. V., Della-Marta, P., … Peterson, T. (2002). Observed coherent changes in climatic extremes during the second half of the twentieth century. *Climate Research*, *19*, 193–212. https://doi.org/10.3354/cr019193

Garland, T., & Rose, M. R. (2009). *Experimental evolution: Concepts, methods, and application of selection experiments*. University of California Press.

Gibbs, A. G., & Eran, G. (2009). Physiological adaptation in laboratory environments. In M. R. Rose, & T. Garland (Eds.), *Experimental evolution: Concepts, methods, and applications of selection experiments* (pp. 523–550). University of California Press.

Gienapp, P., Teplitsky, C., Alho, J. S., … Merilä, J. (2008). Climate change and evolution: Disentangling environmental and genetic responses. *Molecular Ecology*, *17*(1), 167–178. https://doi.org/10.1111/j.1365-294X.2007.03413.x

Gilchrist, G. W., & Huey, R. B. (2001). Parental and developmental temperature effects on the thermal dependence of fitness in *Drosophila melanogaster*. *Evolution*, *55*(1), 209–214. https://doi.org/10.1111/j.0014-3820.2001.tb01287.x

Grueber, C. E., Nakagawa, S., Laws, R. J., … Bates, D. (2011). Multimodel inference in ecology and evolution: Challenges and solutions. *Journal of Evolutionary Biology*, *24*, 699–711. https://doi.org/10.1111/j.1420-9101.2010.02210.x

Guillaume, A. S., Monro, K., Marshall, D. J., & Pfrender, M. (2016). Transgenerational plasticity and environmental stress: do paternal effects act as a conduit or a buffer? *Functional Ecology*, *30*, 1175–1184. https://doi.org/10.1111/1365-2435.12604

Hallmann, C. A., Sorg, M., Jongejans, E., … De Kroon, H. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One*, *12*(10), e0185809. https://doi.org/10.1371/journal.pone.0185809

Han, Y., Yang, W., Long, L., … Zhu, J. (2016). Molecular cloning, expression pattern, and chemical analysis of heat shock protein 70 (HSP70) in the mudskipper *Boleophthalmus pectinirostris*: Evidence for its role in regulating spermatogenesis. *Gene*, *575*(2 Pt 1), 331–338. https://doi.org/10.1016/j.gene.2015.09.010

Harrison, X. A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, *2*, e616. https://doi.org/10.7717/peerj.616

Harte, J., Ostling, A., Green, J. L., & Kinzig, A. (2004). Biodiversity conservation: Climate change and extinction risk. *Nature*, *430*(6995), 34–34. https://doi.org/10.1038/nature02718

Harvey, J. A., Tougeron, K., Gols, R., … Chown, S. L. (2023). Scientists' warning on climate change and insects. *Ecological Monographs*, *93*, 1–37. https://doi.org/10.1002/ecm.1553

Harvey, S. C., & Viney, M. E. (2007). Thermal variation reveals natural variation between isolates of *Caenorhabditis elegans*. *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution*, *308*(4), 409–416. https://doi.org/10.1002/jez.b.21161

Hobday, A., Alexander, L., Perkins, S., … Wernberg, T. (2016). A hierarchical approach to defining marine heatwaves. *Progress in Oceanography*, *141*, 227–238. https://doi.org/10.1016/j.pocean.2015.12.014

Hoffmann, A. A., Chown, S. L., & Clusella-Trullas, S. (2013). Upper thermal limits in terrestrial ectotherms: How constrained are they? *Functional Ecology*, *27*, 934–949. https://doi.org/10.1111/j.1365-2435.2012.02036.x

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479–485. https://doi.org/10.1038/nature09670

Hoffmann, A. A., Sørensen, J. G., & Loeschcke, V. (2003). Adaptation of *Drosophila* to temperature extremes: Bringing together quantitative and molecular approaches. *Journal of Thermal Biology*, *28*(3), 175–216. https://doi.org/10.1016/s0306-4565(02)00057-8

Howe, R. W. (1956). The effect of temperature and humidity on the rate of development and mortality of *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). *Annals of Applied Biology*, *44*(2), 356–368. https://doi.org/10.1111/j.1744-7348.1956.tb02128.x

Hufbauer, R. A., Szűcs, M., Kasyon, E., … Melbourne, B. A. (2015). Reply to Wootton and Pfister: The search for general context should include synthesis with laboratory model systems. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(44), E5904. https://doi.org/10.1073/pnas.1517210112

IPCC. (2021). *Climate change 2021: The physical science basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press. https://doi.org/10.1017/9781009157896

Janowitz, S. A., & Fischer, K. (2011). Opposing effects of heat stress on male versus female reproductive success in *Bicyclus anynana* butterflies. *Journal of Thermal Biology*, *36*(5), 283–287. https://doi.org/10.1016/j.jtherbio.2011.04.001

Karlsson, B., & Wiklund, C. (2005). Butterfly life history and temperature adaptations; dry open habitats select for increased fecundity and longevity. *Journal of Animal Ecology*, *74*(1), 99–104. https://doi.org/10.1111/j.1365-2656.2004.00902.x

Kellermann, V., van Heerwaarden, B., & Sgrò, C. M. (2017). How important is thermal history? Evidence for lasting effects of developmental temperature on upper thermal limits in *Drosophila melanogaster*. *Proceedings of the Royal Society of London. Series B*, *284*(1855), 20170447. https://doi.org/10.1098/rspb.2017.0447

Kenyon, C. (2011). The first long-lived mutants: Discovery of the insulin/IGF-1 pathway for ageing. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, *366*(1561), 9–16. https://doi.org/10.1098/rstb.2010.0276

Kim, H. G., Margolies, D., & Park, Y. (2015). The roles of thermal transient receptor potential channels in thermotactic behavior and in thermal acclimation in the red flour beetle, *Tribolium castaneum*. *Journal of Insect Physiology*, *76*, 47–55. https://doi.org/10.1016/j.jinsphys.2015.03.008

Kodama, H., Kasai, H., Yamaguchi, R., … Fukuda, J. (1997). Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertility and Sterility*, *68*, 519–524. https://doi.org/10.1016/S0015-0282(97)00236-7

Kokko, H., Chaturvedi, A., Croll, D., … Stapley, J. (2017). Can evolution supply what ecology demands? *Trends in Ecology and Evolution*, *32*(3), 187–197. https://doi.org/10.1016/j.tree.2016.12.005

Lenth, R. (2017). *Lsmeans: Least-squares means*. https://www.rdocumentation.org/packages/lsmeans/versions/0.9/topics/lsmeans

Li, A. J., Leung, P. T. Y., Bao, V. W. W., … Leung, K. M. Y. (2015). Temperature-dependent physiological and biochemical responses of the marine medaka Oryzias melastigma with consideration of both low and high thermal extremes. *Journal of Thermal Biology*, *54*, 98–105. https://doi.org/10.1016/j.jtherbio.2014.09.011

Liang, L. -N., Zhang, W., Ma, G., … Ma, C. -S. (2014). A single hot event stimulates adult performance but reduces egg survival in the oriental fruit moth, *Grapholitha molesta*. *PLoS One*, *9*(12), e116339. https://doi.org/10.1371/journal.pone.0116339

Lister, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(44), E10397–E10406. https://doi.org/10.1073/pnas.1722477115

Loeschcke, V., & Sorensen, J. G. (2005). Acclimation, heat shock and hardening – A response from evolutionary biology. *Journal of Thermal Biology*, *30*(3), 255–257. https://doi.org/10.1016/j.jtherbio.2004.12.005

Long, L. -L., Han, Y. -L., Sheng, Z., … Zhu, J. -Q. (2015). Expression analysis of HSP70 in the testis of *Octopus tankahkeei* under thermal stress. *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology*, *187*, 150–159. https://doi.org/10.1016/j.cbpa.2015.05.022

Mahdi, S. H., Yamasaki, H., & Otaki, J. M. (2011). Heat-shock-induced color-pattern changes of the blue pansy butterfly *Junonia orithya*: Physiological and evolutionary implications. *Journal of Thermal Biology*, *36*(6), 312–321. https://doi.org/10.1016/j.jtherbio.2011.06.002

Mahroof, R., Subramanyam, B., & Flinn, P. (2005). Reproductive performance of *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to the minimum heat treatment temperature as pupae and adults. *Journal of Economic Entomology*, *98*(2), 626–633. https://doi.org/10.1093/jee/98.2.626

Mahroof, R., Yan Zhu, K., Neven, L., … Bai, J. (2005). Expression patterns of three heat shock protein 70 genes among developmental stages of the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Comparative Biochemistry and Physiology A*, *141*, 247–256. https://doi.org/10.1016/j.cbpb.2005.05.044

Mahroof, R., Zhu, K. Y., Subramanyam, J. E., & Subramanyam, B. (2005). Changes in expression of heat shock proteins in *Tribolium castaneum* (Coleoptera: Tenebrionidae) in relation to developmental stage, exposure time, and temperature. *Annals of the Entomological Society of America*, *98*, 100–107.

Maino, J. L., Kong, J. D., Hoffmann, A. A., … Kearney, M. R. (2016). Mechanistic models for predicting insect responses to climate change. *Current Opinion in Insect Science*, *17*, 81–86. https://doi.org/10.1016/j.cois.2016.07.006

Martinez, E., Porreca, A. P., Colombo, R. E., & Menze, M. A. (2016). Tradeoffs of warm adaptation in aquatic ectotherms: Live fast, die young? *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *191*, 209–215. https://doi.org/10.1016/j.cbpa.2015.07.014

Meehl, G. A., & Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. *Science*, *305*(5686), 994–997. https://doi.org/10.1126/science.1098704

Meirmans, S., Skorping, A., Løyning, M. K., & Kirkendall, L. R. (2006). On the track of the Red Queen: Bark beetles, their nematodes, local climate and geographic parthenogenesis. *Journal of Evolutionary Biology*, *19*(6), 1939–1947. https://doi.org/10.1111/j.1420-9101.2006.01153.x

Mherrera. (2016). *Extreme temperatures around the world*. http://www.mherrera.org/temp.html

Moreteau, B., Morin, J. P., Gibert, P., … David, J. R. (1997). Evolutionary changes of nonlinear reaction norms according to thermal adaptation: A comparison of two *Drosophila* species. *Comptes rendus de l'Academie des sciences. Serie III, Sciences de la vie*, *320*(10), 833–841. https://doi.org/10.1016/s0764-4469(97)85020-2

Morgan, M. T. (1965). *Ecological variations of laboratory populations of three mutants of Tribolium castaneum (Herbst) and three mutants of Trioblium confusum (Duval)*. Catholic University of America.

Noh, M. Y., Muthukrishnan, S., Kramer, K. J., & Arakane, Y. (2016). Cuticle formation and pigmentation in beetles. *Current Opinion in Insect Science*, *17*, 1–9. https://doi.org/10.1016/j.cois.2016.05.004

Ojima, N., Ishiguro, S., An, Y., … Suzuki, K. (2015). Male reproductive maturity and diapause termination in the leaf beetle *Gastrophysa atrocyanea*. *Physiological Entomology*, *40*(4), 277–283. https://doi.org/10.1111/phen.12112

Oliver, T. H., Marshall, H. H., Morecroft, M. D., … Huntingford, C. (2015). Interacting effects of climate change and habitat fragmentation on drought-sensitive butterflies. *Nature Climate Change*, *5*(10), 941–945. https://doi.org/10.1038/nclimate2746

Ørsted, M., Willot, Q., Olsen, A. K., … Overgaard, J. (2024). Thermal limits of survival and reproduction depend on stress duration: A case study of *Drosophila suzukii*. *Ecology Letters*, *27*(3), e14421. https://doi.org/10.1111/ele.14421

Overgaard, J., Kristensen, T. N., Mitchell, K. A., & Hoffmann, A. A. (2011). Thermal tolerance in widespread and tropical *Drosophila* species: Does phenotypic plasticity increase with latitude? *American Naturalist*, *178*(Suppl 1), S80–S96. https://doi.org/10.1086/661780

Paaijmans, K. P., Heinig, R. L., Seliga, R. A., … Thomas, M. B. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, *19*(8), 2373–2380. https://doi.org/10.1111/gcb.12240

Palmer, G., Hill, J. K., Brereton, T. M., … Thomas, C. D. (2015). Individualistic sensitivities and exposure to climate change explain variation in species' distribution and abundance changes. *Science Advances*, *1*(9), e1400220. https://doi.org/10.1126/sciadv.1400220

Park, T., & Frank, M. B. (1948). The fecundity and development of the flour beetles, *Tribolium confusum* and *Tribolium castaneum*, at three constant temperatures. *Ecology*, *29*(3), 368–374. https://doi.org/10.2307/1930996

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*(6918), 37–42. https://doi.org/10.1038/nature01286

Parratt, S. R., Walsh, B. S., Metelmann, S., … Price, T. A. R. (2021). Temperatures that sterilize males better match global species distributions than lethal temperatures. *Nature Climate Change*, *11*, 481–484. https://doi.org/10.1038/s41558-021-01047-0

Paul, C., Melton, D. W., & Saunders, P. T. K. (2008). Do heat stress and deficits in DNA repair pathways have a negative impact on male fertility? *Molecular Human Reproduction*, *14*(1), 1–8. https://doi.org/10.1093/molehr/gam089

Pedersen, L. D., Pedersen, A. R., Bijlsma, R., & Bundgaard, J. (2011). The effects of inbreeding and heat stress on male sterility in *Drosophila melanogaster*. *Biological Journal of the Linnean Society*, *104*, 432–442. https://doi.org/10.1111/j.1095-8312.2011.01725.x

Perkins, S. E., Alexander, L. V., & Nairn, J. R. (2012). Increasing frequency, intensity and duration of observed global heatwaves and warm spells. *Geophysical Research Letters*, *39*(20), L20714. https://doi.org/10.1029/2012GL053361

Perkins-Kirkpatrick, S. E., & Lewis, S. C. (2020). Increasing trends in regional heatwaves. *Nature Communications*, *11*(1), 3357. https://doi.org/10.1038/s41467-020-16970-7

Plesnar-Bielak, A., Labocha, M. K., Kosztyła, P., … Prokop, Z. M. (2017). Fitness effects of thermal stress differ between outcrossing and selfing populations in *Caenorhabditis elegans*. *Evolutionary Biology*, *44*(3), 356–364. https://doi.org/10.1007/s11692-017-9413-z

Porcelli, D., Gaston, K. J., Butlin, R. K., & Snook, R. R. (2017). Local adaptation of reproductive performance during thermal stress. *Journal of Evolutionary Biology*, *30*(2), 422–429. https://doi.org/10.1111/jeb.13018

Powell, M. J. (2009). *The BOBYQA algorithm for bound constrained optimization without derivatives*. https://www.damtp.cam.ac.uk/user/na/NA_papers/NA2009_06.pdf

Prasad, A., Croydon-Sugarman, M. J. F., Murray, R. L., & Cutter, A. D. (2011). Temperature-dependent fecundity associates with latitude in *Caenorhabditis briggsae*. *Evolution*, *65*(1), 52–63. https://doi.org/10.1111/j.1558-5646.2010.01110.x

Purchase, C., Butts, I., Alonso-Fern´andez, A., & Trippel, E. (2010). Thermal reaction norms in sperm performance of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, *67*, 498–510. https://doi.org/10.1139/F10-001

The R Core Team. (2017a). *Graphics: The R graphics package*. https://rdrr.io/r/graphics/graphics-package.html

The R Core Team. (2017b). *Stats: The R stats package*. https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/stats-package

Raftery, A. E., Zimmer, A., Frierson, D. M. W., … Liu, P. (2017). Less than 2°C warming by 2100 unlikely. *Nature Climate Change*, *7*, 637–641. https://doi.org/10.1038/nclimate3352

Rahman, M. B., Schellander, K., Luceño, N. L., & Van Soom, A. (2018). Heat stress responses in spermatozoa: Mechanisms and consequences for cattle fertility. *Theriogenology*, *113*, 102–112. https://doi.org/10.1016/j.theriogenology.2018.02.012

Ramon, M., Salces-Ortiz, J., Gonzalez, C., … Garde, J. (2014). Influence of the temperature and the genotype of the HSP90AA1 gene over sperm chromatin stability in Manchega rams. *PLoS One*, *9*, e86107. https://doi.org/10.1371/journal.pone.0086107

Raros, R. S., & Chiang, H. C. (1970). The effects of exposure to low temperature on survival and fecundity in the flour beetles *Tribolium confusum* and *T. castaneum*. *Tribolium Information Bulletin*, *13*, 76–77. https://doi.org/10.1016/j.jtherbio.2020.102774

Richter, K., Haslbeck, M., & Buchner, J. (2010). The heat shock response: Life on the verge of death. *Molecular Cell*, *40*(2), 253–266. https://doi.org/10.1016/j.molcel.2010.10.006

Ripley, B., Venables, B., Bates, D. M., … Firth, D. (2016). *MASS: Support functions and datasets for Venables and Ripley's MASS*. https://cran.r-project.org/web/packages/MASS/MASS.pdf

Rodrigues, L. R., McDermott, H. A., Villanueva, I., … Snook, R. R. (2022). Fluctuating heat stress during development exposes reproductive costs and putative benefits. *Journal of Animal Ecology*, *91*(2), 391–403. https://doi.org/10.1111/1365-2656.13636

Rohmer, C., David, J. R., Moreteau, B., & Joly, D. (2004). Heat induced male sterility in Drosophila melanogaster: Adaptive genetic variations among geographic populations and role of the Y chromosome. *Journal of Experimental Biology*, *207*(Pt 16), 2735–2743. https://doi.org/10.1242/jeb.01087

Rolandi, C., & Schilman, P. E. (2018). The costs of living in a thermal fluctuating environment for the tropical haematophagous bug, *Rhodnius prolixus*. *Journal of Thermal Biology*, *74*, 92–99. https://doi.org/10.1016/j.jtherbio.2018.03.022

Root, T., Price, J., Hall, K., & Schneider, S. (2003). Fingerprints of global warming on wild animals and plants. *Nature*, *421*, 57–60. https://doi.org/10.1038/nature01333

The RStudio Team. (2016). *RStudio: Integrated development for R*. https://www.r-project.org/conferences/useR-2011/abstracts/180111-allairejj.pdf

Sales, K. (2019). *Impacts of heatwave conditions and temperature variation on reproduction in the insect model Tribolium castaneum* [PhD thesis]. UEA.

Sales, K., Thomas, P., Gage, M. J., & Vasudeva, R. (2024). Experimental heatwaves reduce the effectiveness of ejaculates at occupying female reproductive tracts in a model insect. *Royal Society Open Science*, *11*(5), 231949. https://doi.org/10.1098/rsos.231949

Sales, K., Vasudeva, R., Dickinson, M., … Gage, M. J. G. (2018). Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nature Communications*, *9*, 4771. https://doi.org/10.1038/s41467-018-07273-z

Sales, K., Vasudeva, R., & Gage, M. J. G. (2021). Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. *Royal Society Open Science*, *8*(3), 201717. https://doi.org/10.1098/rsos.201717

Salvucci, M. E. (2000). Sorbitol accumulation in whiteflies: evidence for a role in protecting proteins during heat stress. *Journal of Thermal Biology*, *25*(5), 353–361. https://doi.org/10.1016/s0306-4565(99)00107-2

Saxena, B. P., Sharma, P. R., Thappa, R. K., & Tikku, K. (1992). Temperature induced sterilization for control of three stored grain beetles. *Journal of Stored Products Research*, *28*(1), 67–70. https://doi.org/10.1016/0022-474x(92)90031-k

Schinko, J. B., Hillebrand, K., & Bucher, G. (2012). Heat shock-mediated misexpression of genes in the beetle *Tribolium castaneum*. *Development Genes and Evolution*, *222*(5), 287–298. https://doi.org/10.1007/s00427-012-0412-x

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*, 671–675.

Scott, M., Berrigan, D., & Hoffmann, A. A. (1997). Costs and benefits of acclimation to elevated temperature in *Trichogramma carverae*.

Entomologia Experimentalis et Applicata, *85*(3), 211–219. https://doi.org/10.1046/j.1570-7458.1997.00251.x

Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, *5*(1), 61–66. https://doi.org/10.1038/nclimate2457

Setchell, B. (2006). The effects of heat on the testes of mammals. *Animal Reproduction*, *3*, 81–91. https://doi.org/10.1098/rstb.2009.0131

Sexton, D. M. H., & Harris, G. R. (2015). The importance of including variability in climate change projections used for adaptation. *Nature Climate Change*, *5*(10), 931–936. https://doi.org/10.1038/nclimate2705

Sgrò, C. M., Terblanche, J. S., & Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annual Review of Entomology*, *61*, 433–451. https://doi.org/10.1146/annurev-ento-010715-023859

Sheridan, J. A., & Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nature Climate Change*, *1*(8), 401–406. https://doi.org/10.1038/nclimate1259

Skinner, J. D., & Louw, G. N. (1966). Heat stress and spermatogenesis in *Bos indicus* and *Bos taurus* cattle. *Journal of Applied Physiology*, *21*(6), 1784–1790. https://doi.org/10.1152/jappl.1966.21.6.1784

Sokoloff, A. (1974). *The biology of Tribolium with special emphasis on genetic aspects*. (Vol. 2). Clarendon Press.

Stillwell, R. C., & Fox, C. W. (2004). Complex patterns of phenotypic plasticity: Interactive effects of temperature during rearing. *Evolution in Health and Disease*, *86*(4), 924–934. https://doi.org/10.1890/04-0547

Stork, N. E., McBroom, J., Gely, C., & Hamilton, A. (2015). New approaches narrow global species estimates for beetles, insects, and terrestrial arthropods. *PNAS*, *112*, 7519–7523. https://doi.org/10.1073/pnas.1502408112

Stürup, M., Baer-Imhoof, B., Boomsma, D. R., & Nash, J. J. (2013). When every sperm counts: Factors affecting male fertility in the honeybee *Apis mellifera*. *Behavioral Ecology*, *24*, 1192–1198. https://doi.org/10.1093/beheco/art049

Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., … Chown, S. L. (2007). Critical thermal limits depend on methodological context. *Proceedings Biological Sciences*, *274*(1628), 2935–2942. https://doi.org/10.1098/rspb.2007.0985

Terblanche, J. S., Hoffmann, A. A., Mitchell, K. A., … Chown, S. L. (2011). Ecologically relevant measures of tolerance to potentially lethal temperatures. *Journal of Experimental Biology*, *214*(Pt 22), 3713–3725. https://doi.org/10.1242/jeb.061283

Terblanche, J. S., Sinclair, B. J., Klok, C. J., … Chown, S. L. (2005). The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalcoptera* (Coleoptera: Chrysomelidae). *Journal of Insect Physiology*, *51*, 1013–1023. https://doi.org/10.1016/j.jinsphys.2005.04.016

Thomas, R., Lello, J., Medeiros, R., … Vaughan, I. (2015). *Data analysis with R statistical software*. Eco-explore.

Tomanek, L. (2008). The importance of physiological limits in determining biogeographical range shifts due to global climate change: The heat-shock response. *Physiological and Biochemical Zoology*, *81*(6), 709–717. https://doi.org/10.1086/590163

Tremblay, A. (2015). *LMERConvenienceFunctions: Model selection and post-hoc analysis for (G)LMER models*. https://cran.r-project.org/web/packages/LMERConvenienceFunctions/index.html

Ummenhofer, C. C., & Meehl, G. A. (2017). Extreme weather and climate events with ecological relevance: A review. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, *372*(1723), 20160135. https://doi.org/10.1098/rstb.2016.0135

Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science*, *348*(6234), 571–573. https://doi.org/10.1126/science.aaa4984

van de Pol, M., Jenouvrier, S., Cornelissen, J. H. C., & Visser, M. E. (2017). Behavioural, ecological and evolutionary responses to extreme climatic events: Challenges and directions. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, *372*(1723), 20160134. https://doi.org/10.1098/rstb.2016.0134

van Dooremalen, C., Suring, W., & Ellers, J. (2011). Fatty acid composition and extreme temperature tolerance following exposure to fluctuating temperatures in a soil arthropod. *Journal of Insect Physiology*, *57*(9), 1267–1273. https://doi.org/10.1016/j.jinsphys.2011.05.017

van Heerwaarden, B., & Sgrò, C. M. (2021). Male fertility thermal limits predict vulnerability to climate warming. *Nature Communications*, *12*(1), 2214. https://doi.org/10.1038/s41467-021-22546-w

Vasudeva, R., Deeming, D. C., & Eady, P. E. (2014). Developmental temperature affects the expression of ejaculatory traits and the outcome of sperm competition in Callosobruchus maculatus. *Journal of Evolutionary Biology*, *27*(9), 1811–1818. https://doi.org/10.1111/jeb.12431

Vasudeva, R., Dickinson, M., Sutter, A., … Gage, M. J. G. (2021). Facultative polyandry protects females from compromised male fertility caused by heatwave conditions. *Animal Behaviour*, *178*, 37–48. https://doi.org/10.1016/j.anbehav.2021.05.016

Vasudeva, R., Sutter, A., Sales, K., … Gage, M. J. (2019). Adaptive thermal plasticity enhances sperm and egg performance in a model insect. *Elife*, *8*, e49452. https://doi.org/10.7554/eLife.49452

Veilleux, H. D., Ryu, T., Donelson, J. M., … Munday, P. L. (2015). Molecular processes of transgenerational acclimation to a warming ocean. *Nature Climate Change*, *5*, 1074–1078. https://doi.org/10.1038/NCLIMATE2724

Vollmer, J. H., Sarup, P., Kaersgaard, C. W., … Loeschcke, V. (2004). Heat and cold-induced male sterility in *Drosophila buzzatii*: Genetic variation among populations for the duration of sterility. *Heredity (Edinb)*, *92*(3), 257–262. https://doi.org/10.1038/sj.hdy.6800405

Walsh, B. S., Parratt, S. R., Hoffmann, A. A., … Price, T. A. R. (2019). The impact of climate change on fertility. *Trends in Ecology and Evolution*, *34*(3), 249-259. https://doi.org/10.1016/j.tree.2018.12.002

Waterhouse, F. L., Onyearu, A. K., & Amos, T. G. (1971). Oviposition of *Tribolium* in static environments incorporating controlled temperature and humidity gradients. *Oikos*, *22*(1), 131–135. https://doi.org/10.2307/3543374

Weaving, H., Terblanche, J. S., & English, S. (2024). Heatwaves are detrimental to fertility in the viviparous tsetse fly. *Proceedings Biological Sciences*, *291*(2018), 20232710. https://doi.org/10.1098/rspb.2023.2710

Wiel, K., & Bintanja, R. (2021). Contribution of climatic changes in mean and variability to monthly temperature and precipitation extremes. *Communications Earth and Environment*, *2*(1), 1-11. https://doi.org/10.1038/s43247-020-00077-4

Willis, S. G., Foden, W., Baker, D. J., … Butchart, S. H. M. (2015). Integrating climate change vulnerability assessments from species distribution models and trait-based approaches. *Biological Conservation*, *190*, 167–178. https://doi.org/10.1016/j.biocon.2015.05.001

Willmer, P. G. (1982). Microclimate and the environmental physiology of insects. *Advances in Insect Physiology*, *16*, 1–57. https://doi.org/10.1016/S0065-2806(08)60151-4

Woestmann, L., & Saastamoinen, M. (2016). The importance of trans-generational effects in Lepidoptera. *Current Zoology*, *62*(5), 489–499. https://doi.org/10.1093/cz/zow029

Woods, H. A., & Harrison, J. F. (2002). Interpreting rejections of the beneficial acclimation hypothesis: When is physio-logical plasticity adaptive? *Evolution*, *56*(9), 1863–1866. https://doi.org/10.1111/j.0014-3820.2002.tb00201.x. https://www.jstor.org/stable/3094746

Zatsepina, O. G., Velikodvorskaia, V. V., Molodtsov, V. B., … Evgenev, M. B. (2001). A *Drosophila melanogaster* strain from sub-equatorial Africa has exceptional thermotolerance but decreased Hsp70 expression. *Journal of Experimental Biology*, *204*(Pt 11), 1869–1881. https://doi.org/10.1242/jeb.204.11.1869

Zeh, J. A., Bonilla, M. M., Su, E. J., … Zeh, D. W. (2014). Constant diurnal temperature regime alters the impact of simulated climate warming on a tropical pseudoscorpion. *Scientific Reports*, *4*, 3706. https://doi.org/10.1038/srep03706

Zeuss, D., Brandl, R., Brändle, M., … Brunzel, S. (2014). Global warming favours light-coloured insects in Europe. *Nature Communications*, *5*, 3874. https://doi.org/10.1038/ncomms4874

Zhang, W., Chang, X. -Q., Hoffmann, A., … Ma, C. -S. (2015). Impact of hot events at different developmental stages of a moth: The closer to adult stage, the less reproductive output. *Scientific Reports*, *5*, 10436. https://doi.org/10.1038/srep10436

Zhang, W., Zhao, F., Hoffmann, A. A., & Ma, C. S. (2013). A single hot event that does not affect survival but decreases reproduction in the diamondback moth, *Plutella xylostella*. *PLoS One*, *8*(10), e75923–e75928. https://doi.org/10.1371/journal.pone.0075923

Zwoinska, M. K., Rodrigues, L. R., Slate, J., & Snook, R. R. (2020). Phenotypic responses to and genetic architecture of sterility following exposure to sub-lethal temperature during development. *Frontiers in Genetics*, *11*, 540354. https://doi.org/10.3389/fgene.2020.00573