Evolution under fluctuating conditions and exposure to heatwaves in the seed beetle, *Callosobruchus maculatus*

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Abstract

Heatwaves, temporary periods of elevated temperatures, are increasing both in magnitude and in frequency and have been shown to have devastating negative effects on a wide range of taxa. However, to date, most studies investigating the impacts of heatwaves have either focus on populations that have evolved under constant conditions prior to assaying or, more importantly, only investigated the short-term outcomes of periods of elevated temperatures. Here, using the seed beetle, *Callosobruchus maculatus*, we investigate both the short- and long-term effects of evolution after 43 generations of fluctuating temperature with added heatwave exposure (a +2°C increase in diurnal temperature peaking at 42°C) on two important life history traits, development time and lifetime reproductive success (LRS). We found that when individuals were assayed under fluctuating conditions, those that evolved under heatwave conditions developed at a similar rate but had reduced LRS than those evolved and assayed under the same fluctuating conditions. In contrast, when individuals were assayed under a novel benign temperature of 29°C, both thermal regimes developed slower and had a similar LRS that was significantly greater than the number produced when treatments were assayed in the stressful fluctuating environment. All together, this suggests that long-term evolution under periods of elevated temperatures may lead to increased resilience both in the long-term with exposure to repeated heatwaves, but also in the short-term when individuals are exposure to rapid environmental change. This study further underscores the importance of using natural diel fluctuations to enhance our understanding of organisms' responses to climate change. Additionally, it emphasises the potency of investigating long-term multigenerational exposure to heatwaves.

Introduction

Natural populations are responding to global temperature increases by shifting ranges, declining in abundance, or ultimately, going extinct (Parmesan & Yohe, 2003; Thomas *et al.*, 2004; Chen *et al.*, 2011). Not only are average annual temperatures rising, but extreme climatic events (ECEs) such as heatwaves are becoming far more frequent (Wang *et al.*, 2024). Heatwaves, defined as three or more consecutive days of temperature above the 90th percentile for each calendar day (Perkins & Alexander, 2013), have been shown to negatively affect the persistence and adaptation of natural populations (Stillman, 2019; Ma *et al.*, 2021; Murali *et al.*, 2023), including plant and animal life (Smith, 2011; Smale & Wernberg, 2013), with broad-scale simultaneous effects on marine and terrestrial ecosystems (Ruthrof *et al.*, 2018). These effects are particularly alarming given that heatwaves are predicted to continue to increase across all scales and impact all inhabited regions of the globe (Perkins-Kirkpatrick & Lewis, 2020; Intergovernmental Panel on Climate Change (IPCC), 2023).

Much of the research investigating the effects of heatwaves has been conducted under controlled laboratory conditions. Such studies have been particularly helpful in highlighting the devasting impact that exposure to extreme elevated temperatures can have on organismal function. For instance, exposure to these periods of persistent elevated temperature has been shown to reduce growth and biomass in plants and reduce reproductive success and increase mortality in a wide variety of animals. In the two tree species *Pinus taeda* and *Quercus rubra,* monthly heatwaves of +12 °C significantly reduced total growth as well as leaf, stem and root biomass (Bauweraerts *et al.*, 2012). In male stickleback fish (*Gasterosteus aculeatus)*, short-term heatwaves at 23°C for five days were found to suppress parental care, delay hatching, reduce hatching success, and negatively impact offspring body condition and swimming performance (Barrett & Stein, 2024). In the zebra finch (*Taeniopygia guttata*), exposure of males to 30°C or 40°C temperatures daily for 14 consecutive days led to an increase in cloacal temperature and a reduction in the proportion of sperm with normal morphology (Hurley *et al.*, 2018).

In insects, extreme high temperatures (EHTs) are known to alter fitness-related life history traits such as survival, development, and reproduction (Ma *et al.*, 2021). Among these traits, reproductive potential is predicted to be the most susceptible to impacts from EHTs (Zhang *et al.*, 2015; Walsh *et al.*, 2019). The fruit fly, *Drosophila melanogaster*, is known to display reproductive sensitivity to temperature, with the over half of males (above 50% median) becoming sterile when temperatures exceed 30°C (Rohmer *et al.*, 2004; David *et al.*, 2005). In the flour beetle, *Tribolium castaneum*, experimental heatwave conditions reduce male fertility and sperm competitiveness, resulting in reduced reproduction and a decrease in the lifespan of offspring (Sales *et al.*, 2018).

Most laboratory studies investigating the impact of heatwaves have used populations that have evolved under constant temperature conditions rather than exposing them to realistic daily temperature fluctuations (Hurley *et al.*, 2018; Sales *et al.*, 2018, 2024; Breedveld *et al.*, 2023; Barrett & Stein, 2024; although see Weaving *et al.*, 2024). Fortunately, the need to consider the effects of thermal fluctuation is now well documented (Vasseur *et al.*, 2014; Colinet *et al.*, 2015; Sinclair *et al.*, 2016; Bagni *et al.*, 2024), and a number of studies have incorporated realistic thermal regimes into experimental designs, including changes to both the mean and variability of temperatures (Hokanson *et al.*, 1977; Niehaus *et al.*, 2012; Paaijmans *et al.*, 2013; Vasseur *et al.*, 2014; Bozinovic *et al.*, 2016; Matsubara, 2018; Schaum *et al.*, 2018, 2022; Buckley & Kingsolver, 2021; but see Bagni *et al.*, 2024). For instance, a study by Weaving *et al.*, (2024) investigated the effects of heatwaves on male and female tsetse flies (*Glossina* *pallidipes*) by exposing the flies to fluctuating temperatures peaking at 36°C, 38°C, or 40°C for two hours. At all heatwave temperatures, males and females experienced equivalent fertility loss. At 38°C in particular, the combination of declining mortality and fertility resulted in a 10.8% population decline compared to the control treatment. In contrast, 40°C resulted in 100% mortality of individuals. Despite this, there is still a lack of data on the long-term evolutionary impacts of exposure to heatwaves (Gutschick & BassiriRad, 2003; van de Pol *et al.*, 2017). Some evidence suggests that populations exposed to realistic fluctuating conditions may end up responding better to both short- and long-term changes in environmental conditions (Ivimey-Cook *et al.*, 2024). This underscores the need to study the long-term evolution of population exposed to heatwaves in combination with realistic diel fluctuations.

Here, we investigated the effect of long-term exposure to heatwaves on the lifetime reproductive success (hereafter, LRS) and development time of the seed beetle, *Callosobruchus maculatus*, after multiple generations of evolving under realistic fluctuating conditions in a laboratory setting. In a previous experiment, we examined the impacts of short- and long-term exposure to fluctuating temperatures (with no exposure to heatwaves) on *C. maculatus* life history (Ivimey-Cook *et al.*, 2024). Briefly, we found that evolving within a fluctuating environment leads to increased reproductive performance upon exposure to constant benign conditions. In contrast, we found no difference in LRS between those that evolved and were assayed under fluctuating or constant conditions (i.e. experienced no change in environment). In this follow-up study, we exposed these same populations to periods of elevated extreme temperatures. Although evolving under realistic diel fluctuations can promote a broadening of thermal niche (Ivimey-Cook *et al.*, 2024), we might predict that exposure to short-term extreme heat will ultimately contribute to a reduction in reproductive fitness and impaired development (Sales *et al.*, 2018, 2021;

Weaving *et al.*, 2024). Alternatively, beetle populations exposed to long-term repeated heatwaves might exhibit improved tolerance and resistance to elevated temperatures, resulting in reproductive fitness and rates of development that are similar to populations evolving under purely fluctuating temperatures (French *et al.*, 2019; Ahrens *et al.*, 2021; Xu *et al.*, 2021). Furthermore, as shown in Ivimey-Cook *et al.*, (2024) and Ketola *et al.*, (2013) evolving under fluctuating conditions may have beneficial effects on reproductive performance when individuals are exposed to a constant benign environment. We may therefore expect a similar increase in fitness upon exposure to a constant environment for those that evolved under fluctuating conditions with repeated heatwaves.

Methods

Study system

Callosobruchus maculatus is a globally widespread agricultural pest native to Africa and Asia. Females of this species deposit their eggs on the surface of dried legumes, such as mung beans (*Vigna radiata*) or black-eyed beans (*Vigna unguiculata*). Upon emergence, the larvae burrow into the bean, eclosing as adults 21-27 days later. Adult seed beetles are facultatively aphagous, obtaining all necessary resources during their larval stage within the bean. Adults without food or water can live up to two weeks, while adults with access to nutrients can live three weeks or more (Fox, 1993; Ursprung *et al.*, 2009). As soon as they emerge from their beans, adult females of this species can start mating and laying eggs immediately (Beck & Blumer, 2014).

In this experiment, we used a strain of *C. maculatus* originating from South India (SI). The original SI beetles were collected in Tirunelveli, India, in 1979 (Mitchell, 1991) and were subsequently raised at the University of Kentucky USA (hereafter referred to as SI USA). We obtained this strain from Uppsala University in

Sweden in 2015 and have since maintained it at the American University of Paris. Stock populations have been cultured exclusively on mung beans and kept in climate chambers at a constant 29°C, 50% relative humidity, and 12:12h light:dark cycle.

Prior to the experiment, beetles were kept in one liter jars containing 250 grams of beans, and approximately 250-350 newly hatched beetles were moved to fresh jars with beans every 24 days. Adequate beans were provided to allow each female to lay just one egg per bean, thus preventing competition among multiple larvae within a single bean (Berg & Maklakov, 2012; Berg *et al.*, 2019). This is important as larval competition has been found to significantly reduce fitness (Vamosi & Lesack, 2007) and body mass (Colegrave, 1993; Vamosi, 2005) at emergence. Indeed, within populations with high larval competition, females readily avoid laying additional eggs on seeds that have already been used (Fox & Messina, 2018).

Thermal evolution lines and assay conditions

See Fig. S1 for a detailed diagram. Before establishing treatment groups, we maintained a single ancestral population of SI USA beetles in two distinct climate chambers. These chambers were maintained under identical humidity and light conditions but differed in their long-term thermal regime. One population was maintained at a fluctuating thermal regime (hereafter, “Fluctuating”) for 130 generations whilst another was maintained for 43 generations at the same fluctuating thermal regime but experienced regular heatwaves (hereafter, “Heatwave”). This provided ample time for the populations to adapt to these conditions. For the “Fluctuating” thermal regime, the seed beetles were subjected to a daily temperature cycle consisting of 12 separate 2 hour periods of constant temperature T_i ,

$$T_i = T_{mean} + \Delta \mathrm{T} \sin\left(\frac{i-12}{12}\pi\right),$$

where T_{mean} = 33°C, ΔT = 7°C, and i = 0,1…. 11. This was a stepwise sinusoidal temperature cycle with T_{max} = 40°C and T_{min} = 26°C which mimics typical late spring condition in Southern India, where this species evolved. For the “Heatwave” thermal regime, the beetles were exposed to an increase of 2°C over the entire diurnal cycle, reaching a T_{max} of 42°C, consistent with a heatwave in Southern India.

$$T^{h}{}_{i} = T_i + 2 = \ T_{mean} + \Delta \mathrm{T} \sin\left(\frac{i-12}{12}\pi\right) + 2$$

Here, $T^{h}{}_{i}$ is the temperature of the chamber during the heatwave regime at step “i”, over a period of 7 days starting at 6:00 am (the point of T_{min}). To mimic the random character of the occurrences, the “Heatwave” regime was initiated on different days during the lifecycle of the beetles. Specifically, during each generation the “heatwave” regime was initiated a day earlier relative to the prior one.

Prior to conducting experimental assays, we subdivided beetles from the two different thermal regimes into three replicates each (*n* = 6, 2 thermal regimes x 3 replicates). For both thermal regimes, beetles from each of the three replicates were used to create a final six groups, which were either kept at their ancestral conditions or were acclimatized for two generations without selection to Constant conditions at 29°C (with a sample size of 50 beetle pairs each; *total n of treatments* = 12, 2 thermal regimes x 3 replicates x 2 environmental conditions). The assay conditions were as follows:

1. **Fluctuating–Constant:** Beetles that had evolved under Fluctuating conditions and then kept for two generations at Constant conditions of 29°C prior to assaying. This was done in order to control for any possible parental effects and to separate genetic adaptation from phenotypic plasticity (Lind *et al.*, 2014; Lymbery *et al.*, 2020).
2. **Heatwave–Constant:** Beetles that had evolved under Heatwave conditions and then kept for two generations at Constant conditions of 29°C prior to assaying (see above).
3. **Heatwave–Fluctuating:** Beetles that had evolved under Heatwave conditions and remained in these conditions prior to assaying. Note that, Fluctuating and

Heatwave conditions are the same except when a periodic heatwave is happening. No heatwaves were applied during the assay period (see below).

4. **Fluctuating–Fluctuating:** Beetles that had evolved under Fluctuating conditions and remained in these conditions prior to assaying.

For the Fluctuating-Constant and the Heatwave-Constant assays, the following steps were performed. First, we transferred beans containing fertilized eggs from jars into a 48-well virgin chamber (aerated plastic culture plates with a separate well for each individual) with one bean allocated per well. The virgin chambers were then moved to the Constant climate chamber where they were kept at constant 29°C.

Once at least 50 males and 50 females from each virgin chamber had hatched, we randomly paired one-day-old males and females and placed them in petri dishes with 100 beans and returned these to the Constant chamber. *C. maculatus* females lay a maximum of 100 beans in their lifetime (Berg & Maklakov, 2012) and we ensured that each petri dish contained a sufficient number of beans to allow females to lay only one egg per bean, thereby minimizing larval competition. To further reduce larval competition, we removed adult beetle pairs from the petri dishes after 72 hours (Berg & Maklakov, 2012; Berg *et al.*, 2019). 19 days later, before any offspring eclosed, we transferred up to 48 beans with eggs from each dish into separate, labelled 48-well virgin chambers. One day following eclosion, we randomly selected and paired 50 females and 50 males (taking care not to pair full siblings) and placed them into 50mm petri dishes, each containing 80 beans. After 72 hours, the adult males and females were removed from the petri dishes. We repeated this process for one additional generation: once again, before eclosion, beans containing fertilized eggs from each petri dish were transferred into separate 48-well virgin chambers. We used the offspring that hatched out of these chambers for the development time (time from laying until hatching) and reproductive fitness assays (below).

For the Fluctuating-Fluctuating and Heatwave-Fluctuating treatments, we omitted the acclimatization steps, as controlling for the influence of parental effects was unnecessary here (see above) and directly transferred beans containing fertilized eggs into virgin chambers prior to hatching. These were kept at the same conditions that the beetles had evolved in, however, for the “Heatwave” thermal regime, we avoided applying heatwaves during the assay period to avoid any unintended effects of elevated heat exposure on larval development. This allowed us to test the effects of heatwaves on adult reproductive fitness specifically. For all assays, one-day-old males and females were randomly selected.

Development time and reproductive fitness assays

Development time and fitness assays were conducted simultaneously. We paired 50 one-day-old male and female beetles and placed them into 60-mm Petri dishes containing 70 beans. After 24 hours, each pair was transferred together into a fresh 60-mm petri dish containing 60 beans, while the initial dish was set aside in the same climate chamber. The following day, this procedure was repeated. The beetles were left in the third and last set of dishes, containing 50 beans, until their death. The number of beans in the dishes always exceeded the total number of eggs any female could lay in a single day at that age. The initial dishes for each pair were labeled as "Day 0," the second dishes were labeled as "Day 1," and the final set of dishes were labeled as "Day 2+." 18 days after each dish was set up, before any new beetles could hatch, all beans with fertilized eggs were transferred into virgin chambers. These were then monitored daily to record the date of eclosion and the sex of all offspring. To calculate development time, we counted the number of days between the date that an egg was laid and the date the adult offspring eclosed. LRS was calculated as the total number of offspring that emerged from each pair, across all three reproductive days (and dishes; Days 0, 1, and 2+).

Statistical Analysis

All code was run using R v.4.4.2 (R Core Team, 2021) and is available on GitHub at ElvimeyCook/Heatwave_Beetles. Linear and generalized linear mixed were run initially in {glmmTMB} v.1.1.10 (Brooks *et al.*, 2017; Magnusson *et al.*, 2017) with residual diagnostics including detection of zero-inflation and dispersion using {DHARMa} v.0.4.7 (Hartig, 2017). Bias-corrected estimated marginals means were then extracted from these models using {emmeans} v.1.10.7 (Lenth *et al.*, 2019) corrected for by a multivariate *t* distribution (mvt). Data was imported, cleaned, and tidied using {readr} v.2.1.5, {tidyr} v.1.3.1, {dplyr} v1.1.4 (Wickham *et al.*, 2019), {hablar} v.0.3.2 (Sjoberg, 2020), {janitor} v.2.2.1 (Firke, 2020), and {data.table} v.1.17.0 (Dowle *et al.*, 2019). Data was then visualised using {ggplot2} v.3.5.1 (Wickham, 2011). Heteroscedasticity was detected in the linear model for development time even after adding a predictor into the dispersion formula of the glmmTMB model so was instead run using {robustlmm} v.3.3-1 (Koller, 2016). All other packages used are found in the "renv" folder stored using the {renv} v.1.0.7 (Ushey, 2023) package and contained within the session.info section of the code README within the repository.

For the model of lifetime reproductive success (or total reproduction across Days 0, 1, 2+) we added fixed categorical predictors of thermal regime (fluctuating and heatwave) in a two-way interaction with assay environment (constant and fluctuating). A 16-level random effect of population (see above) was also added. The following model procedure was followed. Three models were initially fitted (a Poisson model, with and without an object-level random effect, and a Conway-Maxwell Poisson model) and tested for overdispersion and zero-inflation. If significant zero-inflation was still detected, we instead compared a zero-inflated Poisson model (with and without an object-level random effect) to a zero-inflated Conway-Maxwell Poisson model and compared these via Akaike Information Criterion. Lastly, if significant dispersion or heterogeneity of residuals were still detected after accounting for zero-inflation, we performed model selection on parameters in the dispersion formula (including adding a 12-level fixed factor of group, as random effects are not allowed within the dispersion formula). Note, no model selection occurred on the main part of the model.

Development time was log-transformed and ran in a Gaussian model with the same random (albeit with an additional random intercept of pair ID nested within the random factor of group to account for non-independence) and fixed effects with interactions as above. Similar model selection took place on the dispersion formula of the model. However, even with this added dispersion parameter, significant heteroscedasticity was identified in residuals. This led to the use of a robust linear mixed effect model (see above). *P* values for the robust linear model were obtained using the {sjPlot} v. 2.8.17 package (Lüdecke, 2013). The qualitative results did not vary between either method.

Results

Lifetime reproductive success

Lifetime reproductive success was significantly influenced by the interaction between thermal regime (Heatwave and Fluctuating) and assay environment (Constant and Fluctuating; Fig 1; $p<0.001$: Table S2). More specifically, when assayed under fluctuating conditions, individuals that evolved under heatwave conditions had reduced LRS compared to those that evolved under fluctuating conditions ($p<0.001$; Table S3). This difference in LRS disappeared when assayed under constant benign conditions ($p = 0.701$; Fig. 1: Table S3). For both thermal regimes, LRS under constant conditions was higher than fluctuating conditions (both $p<0.001$; Fig. 1; Table S3).

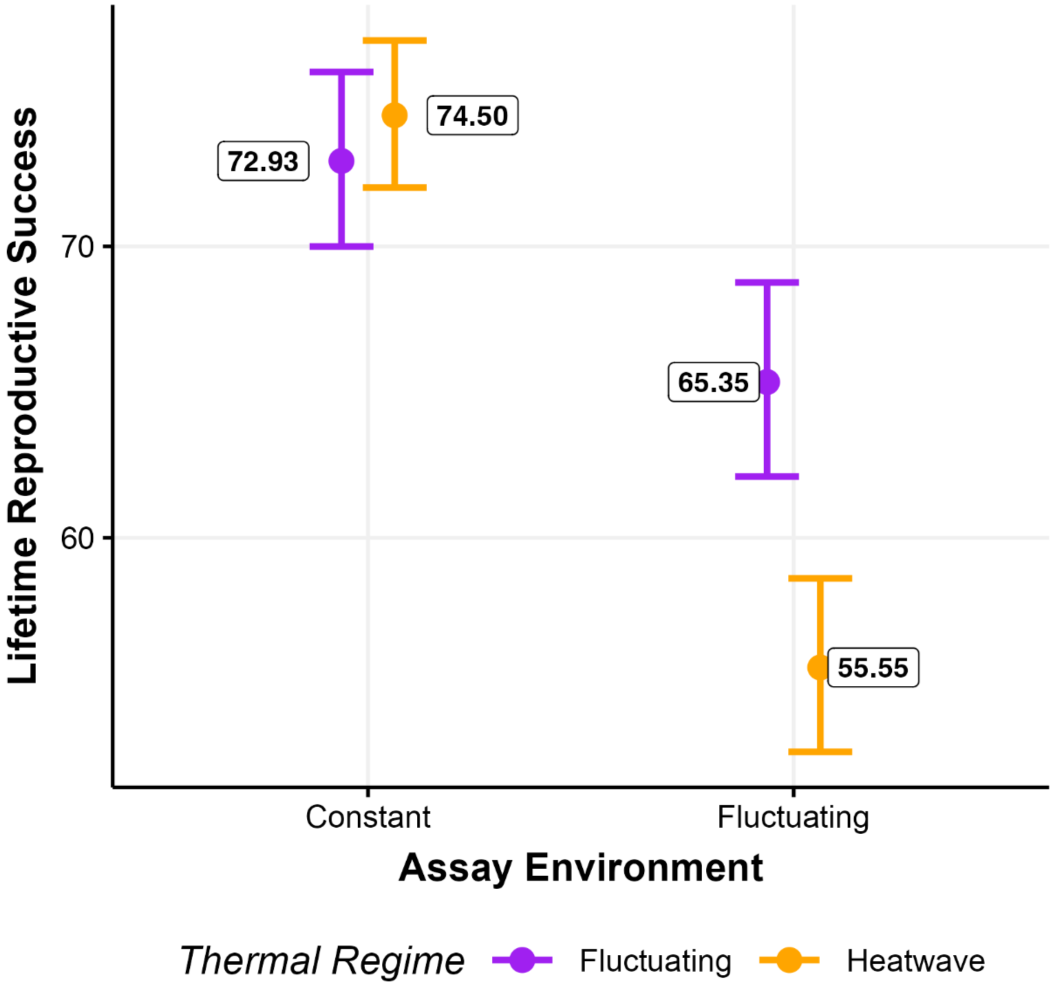

Figure 1. Lifetime reproduction success of individuals from heatwave (orange) and fluctuating (purple) thermal regimes assayed in constant or fluctuating environments. Points with error bars represent mean values with accompanying 95% asymptotic confidence levels taken from the final model of lifetime reproductive success.

Development time

In a similar manner to LRS, the development time of individuals was influenced by the interaction between assay environment and thermal regime (p = 0.001; Fig. 2; Table S4). However, estimated marginal means corrected for by a multivariate t distribution indicated no significant difference between Fluctuating and Heatwave regimes within the Constant or Fluctuating assay environments (p = 0.565 and 0.098, respectively; Fig. 2; Table S5). However, there were differences between environments, with all individuals, regardless of thermal regime, developing slower under the constant conditions (p = 0.050 and <0.001; Table S5), although this effect appeared larger for heatwave individuals (Fig. 2)

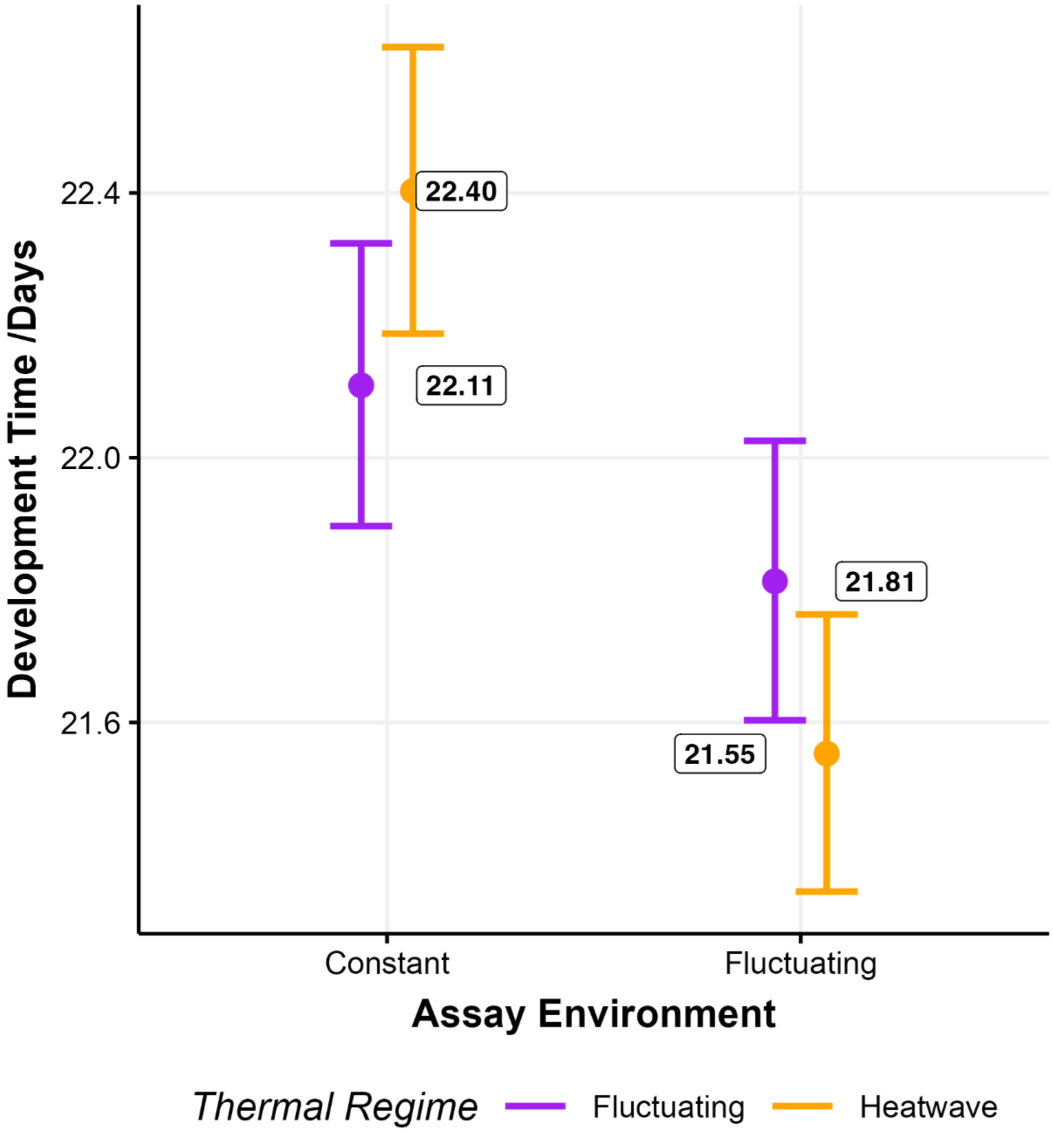

Figure 2. Development time (in days) of individuals from heatwave (orange) and fluctuating (purple) thermal regimes assayed in constant or fluctuating environments. Points with error bars represent mean values with accompanying 95% asymptotic confidence levels taken from the final model of development time.

Discussion

The aim of this study was to investigate the effects of long-term heatwave exposure on seed beetles (*C. maculatus*) that had evolved under natural diel temperature fluctuations. Beetles adapted to fluctuating temperatures were exposed for 43 generations to periodic heatwaves and then assayed in either the ancestral fluctuating conditions or in a novel constant environment. We hypothesized that long-term exposure to heatwaves would result in either 1) decreased fitness and impaired or delayed development relative to beetles that had not evolved with heatwaves (Sales *et al.*, 2018, 2024), or conversely 2) improved resistance and tolerance to elevated temperatures (French *et al.*, 2019; Ahrens *et al.*, 2021; Xu *et al.*, 2021). Additionally, we hypothesized that individuals from both the fluctuating and heatwave conditions would react in a similar manner when exposed to a novel constant (or benign) environment, similar to what Ivimey-Cook *et al* (2024) found in a previous experiment.

In fact, we found that individuals from populations exposed to regular heatwaves had significantly lower fitness than individuals from populations without this exposure. This negative effect of heatwave exposure aligns with previous research across a variety of species across both sexes (Sales *et al.*, 2018; Martinet *et al.*, 2021; Siegle *et al.*, 2022; Ratz *et al.*, 2024; Weaving *et al.*, 2024). This decline is to be expected if there is negative effect of repeated and sustained elevation of heat shock proteins (Feder & Hofmann, 1999; Sørensen *et al.*, 2003; Siegle *et al.*, 2022). Among males, reproductive success might decline if heatwaves lead to reduced sperm count or motility or alter sperm morphology (Sales *et al.*, 2021; Ratz *et al.*, 2024). We may also expect this decrease if females suffered reproductive abnormalities, such as those found in the female tsetse fly after exposure to 30°C heat (Mellanby, 1937;

Weaving *et al.*, 2024). Furthermore, given that the occurrence of heatwaves was random, this difference in LRS could be a result of temporal misalignment between required phenotype and environment, or from the cost of evolving the necessary mechanisms to detect changes within the environment (such as heatwaves) and to produce appropriate phenotypes sufficiently rapidly (Burggren, 2020; Hoffmann & Bridle, 2021). However, it should be noted that the LRS between these two groups, although significant, is not markedly different (i.e, heatwave individuals exposed are still able to reproduce), suggesting either some form of reproductive recovery or repair after prolonged heatwaves exposure (Ma *et al.*, 2018; Sales *et al.*, 2021), or the evolution of improved tolerance and resistance to elevated temperatures (French *et al.*, 2019). Future work involving these long-term evolution lines should therefore aim to compare the physiological differences, such as levels of expression of heat shock proteins or reproductive impairment, after one generation of heatwave exposure versus after multiple generations.

Although long-term exposure to heatwaves appeared to have a negative impact on reproduction when the beetles were assayed at fluctuating conditions, we found that individuals from both fluctuating and heatwave conditions had increased reproductive fitness when assayed in a novel but constant 29°C environment. This is analogous to the results found in Ivimey-Cook *et al.*, (2024), where two different strains of *C. maculatus* (SI USA and Leicester) that had evolved under diel fluctuations had higher LRS when assayed within a constant 29°C environment than when assayed under fluctuating conditions. This suggests that evolution in a fluctuating environment can select for individuals with a broadened thermal niche that are more robust after generations of mutation removal and selective mortality (Rankin & Sponaugle, 2011; Ketola *et al.*, 2013, Ivimey-Cook et al 2024). Also, if an individual has narrowed their thermal niche and postponed critical functions that only occur within a specific temperature window, as in the diel narrowing hypothesis (Kefford *et al.*, 2022), then reproductive performance should increase when individuals are placed within a constant and benign environment (Gilchrist, 1995; Kefford *et al.*, 2022). This is an important result, which further highlights how evolution under fluctuating environments can lead to an improved response to short-term changes in environment, regardless of heatwave exposure.

Lastly, we found no negative effect of heatwave exposure on individual development time. Beetles from both thermal backgrounds developed more quickly (and equally quickly) in fluctuating environments, where the average mean temperature was 4°C hotter than constant conditions. This is similar to the result found in the previous experiment involving the same thermal conditions (Ivimey-Cook *et al.*, 2024), with the fastest development occurring within a fluctuating environment. The lack of a detrimental effect of long-term heatwave exposure on development time is counter to what has been found previously. Some studies have shown a positive effect of elevated temperature exposure, such as in the parasitoid *Cotesia glomerata,* which developed twice as quickly in higher temperatures (Chen *et al.*, 2019). Other studies have shown negative effects, for instance in the solitary bee *Osmia lignaria*, which developed more slowly under 37°C heatwave conditions than the no-heatwave control (Melone *et al.*, 2024). However, in both of these cases, individuals were exposed to just one generation of heatwave rather than testing them after multiple (in our case 43) generations. Therefore, we may see a reduction in effect over time when individuals are exposed to more repeated heatwaves and become more resilient to environmental change (Ahrens *et al.*, 2021; Xu *et al.*, 2021).

In conclusion, we found that populations evolving under repeated heatwaves within naturally fluctuating environments can not only become resilient to exposure in the long term, but are also able to cope with rapid environmental change, reaching maximal performance when conditions suddenly become benign. This study highlights the importance of considering long-term diel fluctuations together with multi-generational exposure to heatwaves in order to better understand natural population responses to climatic warming.

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Conflict of Interest

The authors declare no conflict of interest.

References

Ahrens, C.W., Challis, A., Byrne, M., Leigh, A., Nicotra, A.B., Tissue, D., *et al.* 2021. Repeated extreme heatwaves result in higher leaf thermal tolerances and greater safety margins. *New Phytol.* **232**: 1212–1225.

Bagni, T., Siaussat, D., Maria, A., Fuentes, A., Couzi, P. & Massot, M. 2024. Fitness under high temperatures is overestimated when daily thermal fluctuation is ignored. *J. Therm. Biol.* **119**: 103806.

Barrett, R. & Stein, L.R. 2024. Short-term heat waves have long-term consequences for parents and offspring in stickleback. *Behav. Ecol.* **35**: arae036.

Bauweraerts, I., Wertin, T.M., Ameye, M., McGuire, M.A., Teskey, R.O. & Steppe, K. 2012. The effect of heat waves, elevated [<scp><scp>CO</scp></scp>$_2$] and low soil water availability on northern red oak (*Quercus rubra* L.) seedlings. *Glob. Change Biol.* **19**: 517–528.

Beck, C.W. & Blumer, L.S. 2014. A handbook on bean beetles, Callosobruchus maculatus. *Natl. Sci. Found. URL Httpwww Beanbeetles Orghandbook Pdf Last Accessed 16 June 2015.*

Berg, E.C., Lind, M.I., Monahan, S., Bricout, S. & Maklakov, A.A. 2019. Kin but less than kind: within-group male relatedness does not increase female fitness in seed beetles. *Proc. R. Soc. B Biol. Sci.* **286**: 20191664. Royal Society.

Berg, E.C. & Maklakov, A.A. 2012. Sexes suffer from suboptimal lifespan because of genetic conflict in a seed beetle. *Proc. R. Soc. B Biol. Sci.* **279**: 4296–4302.

Bozinovic, F., Medina, N.R., Alruiz, J.M., Cavieres, G. & Sabat, P. 2016. Thermal tolerance and survival responses to scenarios of experimental climatic change: changing thermal variability reduces the heat and cold tolerance in a fly. *J. Comp. Physiol. B* **186**: 581–587.

Breedveld, M.C., Devigili, A., Borgheresi, O. & Gasparini, C. 2023. Reproducing in hot water: Experimental heatwaves deteriorate multiple reproductive traits in a freshwater ectotherm. *Funct. Ecol.* **37**: 989–1004.

Brooks, M., E., Kristensen, K., Benthem, K., J. ,van, Magnusson, A., Berg, C., W., Nielsen, A., *et al.* 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *R J.* **9**: 378. Technische Universitaet Wien.

Buckley, L.B. & Kingsolver, J.G. 2021. Evolution of Thermal Sensitivity in Changing and Variable Climates. *Annu. Rev. Ecol. Evol. Syst.* **52**: 563–586.

Burggren, W.W. 2020. Phenotypic Switching Resulting From Developmental Plasticity: Fixed or Reversible? *Front. Physiol.* **10**. Frontiers.

Chen, C., Donner, S.H., Biere, A., Gols, R. & Harvey, J.A. 2019. Simulated heatwave conditions associated with global warming affect development and competition between hyperparasitoids. *Oikos* **128**: 1783–1792.

Chen, I.-C., Hill, J.K., Ohlemüller, R., Roy, D.B. & Thomas, C.D. 2011. Rapid Range Shifts of Species Associated with High Levels of Climate Warming. *Science* **333**: 1024–1026. American Association for the Advancement of Science.

COLEGRAVE, N. 1993. Does larval competition affect fecundity independently of its effect on adult weight? *Ecol. Entomol.* **18**: 275–277.

Colinet, H., Sinclair, B.J., Vernon, P. & Renault, D. 2015. Insects in Fluctuating Thermal Environments. *Annu. Rev. Entomol.* **60**: 123–140.

DAVID, J.R., ARARIPE, L.O., CHAKIR, M., LEGOUT, H., LEMOS, B., PÉTAVY, G., *et al.* 2005. Male sterility at extreme temperatures: a significant but neglected phenomenon for understanding *Drosophila* climatic adaptations. *J. Evol. Biol.* **18**: 838–846.

Dowle, M., Srinivasan, A., Gorecki, J., Chirico, M., Stetsenko, P., Short, T., *et al.* 2019. Package 'data. table.' *Ext. 'data Frame* **596**: 952. Citeseer.

Feder, M.E. & Hofmann, G.E. 1999. HEAT-SHOCK PROTEINS, MOLECULAR CHAPERONES, AND THE STRESS RESPONSE: Evolutionary and Ecological Physiology. *Annu. Rev. Physiol.* **61**: 243–282. Annual Reviews.

Firke, S. 2020. janitor: Simple tools for examining and cleaning dirty data. R package version 2.0. 1. Available form https://github. com/sfirk e/janitor.

Fox, C.W. 1993. Multiple Mating, Lifetime Fecundity and Female Mortality of the Bruchid Beetle, Callosobruchus maculatus (Coleoptera: Bruchidae). *Funct. Ecol.* **7**: 203. JSTOR.

Fox, C.W. & Messina, F.J. 2018. Evolution of larval competitiveness and associated life-history traits in response to host shifts in a seed beetle. *J. Evol. Biol.* **31**: 302–313.

French, K., Jansens, I.B., Ashcroft, M.B., Ecroyd, H. & Robinson, S.A. 2019. High tolerance of repeated heatwaves in Australian native plants. *Austral Ecol.* **44**: 597–608.

Gilchrist, G.W. 1995. Specialists and Generalists in Changing Environments. I. Fitness Landscapes of Thermal Sensitivity. *Am. Nat.* **146**: 252–270. The University of Chicago Press.

Gutschick, V.P. & BassiriRad, H. 2003. Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. *New Phytol.* **160**: 21–42.

Hartig, F. 2017. Package 'DHARMa.' *R Package*.

Hoffmann, A.A. & Bridle, J. 2021. The dangers of irreversibility in an age of increased uncertainty: revisiting plasticity in invertebrates. *Oikos* **2022**: e08715.

Hokanson, K.E.F., Kleiner, C.F. & Thorslund, T.W. 1977. Effects of Constant Temperatures and Diel Temperature Fluctuations on Specific Growth and Mortality Rates and Yield of Juvenile Rainbow Trout, *Salmo gairdneri*. *J. Fish. Res. Board Can.* **34**: 639–648.

Hurley, L.L., McDiarmid, C.S., Friesen, C.R., Griffith, S.C. & Rowe, M. 2018. Experimental heatwaves negatively impact sperm quality in the zebra finch. *Proc. R. Soc. B Biol. Sci.* **285**: 20172547. Royal Society.

Intergovernmental Panel on Climate Change (IPCC) (ed). 2023. Weather and Climate Extreme Events in a Changing Climate. In: *Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, pp. 1513–1766. Cambridge University Press, Cambridge.

Ivimey-Cook, E.R., Piani, C., Hung, W.-T. & Berg, E.C. 2024. Genetic background and thermal regime influence adaptation to novel environment in the seed beetle, Callosobruchus maculatus. *J. Evol. Biol.* **37**: 1–13.

Kefford, B.J., Ghalambor, C.K., Dewenter, B., Poff, N.L., Hughes, J., Reich, J., *et al.* 2022. Acute, diel, and annual temperature variability and the thermal biology of ectotherms. *Glob. Change Biol.* **28**: 6872–6888.

Ketola, T., Mikonranta, L., Zhang, J., Saarinen, K., Örmälä, A.-M., Friman, V.-P., *et al.* 2013. FLUCTUATING TEMPERATURE LEADS TO EVOLUTION OF THERMAL GENERALISM AND PREADAPTATION TO NOVEL ENVIRONMENTS. *Evolution* n/a-n/a.

Koller, M. 2016. **robustlmm**: An *R* Package for Robust Estimation of Linear Mixed-Effects Models. *J. Stat. Softw.* **75**.

Lenth, R., Singmann, H., Love, J., Buerkner, P. & Herve, M. 2019. Package ‘emmeans.’

Lind, M.I., Berg, E.C., Alavioon, G. & Maklakov, A.A. 2014. Evolution of differential maternal age effects on male and female offspring development and longevity. *Funct. Ecol.* **29**: 104–110.

Lüdecke, D. 2013. sjPlot: Data Visualization for Statistics in Social Science.

Lymbery, S.J., Wyber, B., Tomkins, J.L. & Simmons, L.W. 2020. No evidence for divergence in male harmfulness or female resistance in response to changes in the opportunity for dispersal. *J. Evol. Biol.* **33**: 966–978.

Ma, C., Wang, L., Zhang, W. & Rudolf, V.H.W. 2018. Resolving biological impacts of multiple heat waves: interaction of hot and recovery days. *Oikos* **127**: 622–633.

Ma, C.-S., Ma, G. & Pincebourde, S. 2021. Survive a Warming Climate: Insect Responses to Extreme High Temperatures. *Annu. Rev. Entomol.* **66**: 163–184.

Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., *et al.* 2017. Package ‘glmmTMB.’ *R Package Version 02 0.*

Martinet, B., Zambra, E., Przybyla, K., Lecocq, T., Anselmo, A., Nonclercq, D., *et al.* 2021. Mating under climate change: Impact of simulated heatwaves on the reproduction of model pollinators. *Funct. Ecol.* **35**: 739–752.

Matsubara, S. 2018. Growing plants in fluctuating environments: why bother? *J. Exp. Bot.* **69**: 4651–4654.

Mellanby, H. 1937. Experimental work on reproduction in the tsetse fly, Glossina palpalis. *Parasitology* **29**: 131–141.

Melone, G.G., Stuligross, C. & Williams, N.M. 2024. Heatwaves increase larval mortality and delay development of a solitary bee. *Ecol. Entomol.* **49**: 433–444.

Mitchell, R. 1991. The traits of a biotype of Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) from South India. *J. Stored Prod. Res.* **27**: 221–224. Elsevier.

Murali, G., Iwamura, T., Meiri, S. & Roll, U. 2023. Future temperature extremes threaten land vertebrates. *Nature* **615**: 461–467. Nature Publishing Group.

Niehaus, A.C., Angilletta, M.J., Sears, M.W., Franklin, C.E. & Wilson, R.S. 2012. Predicting the physiological performance of ectotherms in fluctuating thermal environments. *J. Exp. Biol.* **215**: 694–701.

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

Parmesan, C. & Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**: 37–42. Nature Publishing Group.

Perkins, S.E. & Alexander, L.V. 2013. On the Measurement of Heat Waves. *J. Clim.* **26**: 4500–4517.

Perkins-Kirkpatrick, S.E. & Lewis, S.C. 2020. Increasing trends in regional heatwaves. *Nat. Commun.* **11**: 3357. Nature Publishing Group.

R Core Team. 2021. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rankin, T.L. & Sponaugle, S. 2011. Temperature Influences Selective Mortality during the Early Life Stages of a Coral Reef Fish. *PLOS ONE* **6**: e16814. Public Library of Science.

Ratz, T., Chechi, T.S., Dimopoulou, A.-I., Sedlmair, S.D. & Tuni, C. 2024. Heatwaves inflict reproductive but not survival costs to male insects. *J. Exp. Biol.* **227**: jeb246698.

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. *J. Exp. Biol.* **207**: 2735–2743.

Ruthrof, K.X., Breshears, D.D., Fontaine, J.B., Froend, R.H., Matusick, G., Kala, J., *et al.* 2018. Subcontinental heat wave triggers terrestrial and marine, multi-taxa responses. *Sci. Rep.* **8**: 13094. Nature Publishing Group.

Sales, K., Gage, M.J.G. & Vasudeva, R. 2024. Experimental evolution reveals that males evolving within warmer thermal regimes improve reproductive performance under heatwave conditions in a model insect. *J. Evol. Biol.* voae116.

Sales, K., Vasudeva, R., Dickinson, M.E., Godwin, J.L., Lumley, A.J., Michalczyk, Ł., *et al.* 2018. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nat. Commun.* **9**: 4771. Nature Publishing Group.

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. *R. Soc. Open Sci.* **8**: 201717. Royal Society.

Schaum, C.-E., Buckling, A., Smirnoff, N., Studholme, D.J. & Yvon-Durocher, G. 2018. Environmental fluctuations accelerate molecular evolution of thermal tolerance in a marine diatom. *Nat. Commun.* **9**: 1719. Nature Publishing Group.

Schaum, C.-E., Buckling, A., Smirnoff, N. & Yvon-Durocher, G. 2022. Evolution of thermal tolerance and phenotypic plasticity under rapid and slow temperature fluctuations. *Proc. R. Soc. B* **289**: 20220834. The Royal Society.

Siegle, M.R., Taylor, E.B. & O'Connor, M.I. 2022. Heat Wave Intensity Drives Sublethal Reproductive Costs in a Tidepool Copepod. *Integr. Org. Biol.* **4**: obac005.

Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willett, C.S., Slotsbo, S., *et al.* 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol. Lett.* **19**: 1372–1385.

Sjoberg, D. 2020. hablar: Non-Astonishing Results in R.

Smale, D.A. & Wernberg, T. 2013. Extreme climatic event drives range contraction of a habitat-forming species. *Proc. R. Soc. B Biol. Sci.* **280**: 20122829. Royal Society.

Smith, M.D. 2011. The ecological role of climate extremes: current understanding and future prospects. *J. Ecol.* **99**: 651–655.

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

Stillman, J.H. 2019. Heat Waves, the New Normal: Summertime Temperature Extremes Will Impact Animals, Ecosystems, and Human Communities. *Physiology* **34**: 86–100. American Physiological Society.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., *et al.* 2004. Extinction risk from climate change. *Nature* **427**: 145–148. Nature Publishing Group.

Ursprung, C., den Hollander, M. & Gwynne, D.T. 2009. Female seed beetles, Callosobruchus maculatus, remate for male-supplied water rather than ejaculate nutrition. *Behav. Ecol. Sociobiol.* **63**: 781–788. Springer.

Ushey, K. 2023. renv: Project Environments.

Vamosi, S. & Lesack, T. 2007. Direct effects of larval competition on development time and fecundity in seed beetles. *Evol. Ecol. Res.* **9**: 1131–1143.

VAMOSI, S.M. 2005. Interactive effects of larval host and competition on adult fitness: an experimental test with seed beetles (Coleoptera: Bruchidae). *Funct. Ecol.* **19**: 859–864.

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. *Philos. Trans. R. Soc. B Biol. Sci.* **372**: 20160134. Royal Society.

Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harley, C.D.G., McCann, K.S., *et al.* 2014. Increased temperature variation poses a greater risk to species than climate warming. *Proc. R. Soc. B Biol. Sci.* **281**: 20132612.

Walsh, B.S., Parratt, S.R., Hoffmann, A.A., Atkinson, D., Snook, R.R., Bretman, A., *et al.* 2019. The Impact of Climate Change on Fertility. *Trends Ecol. Amp Evol.* **34**: 249–259.

Wang, C., Li, Z., Chen, Y., Li, Y., Ouyang, L., Zhu, J., *et al.* 2024. Changes in Global Heatwave Risk and Its Drivers Over One Century. *Earths Future* **12**: e2024EF004430.

Weaving, H., Terblanche, J.S. & English, S. 2024. Heatwaves are detrimental to fertility in the viviparous tsetse fly. *Proc. R. Soc. B Biol. Sci.* **291**: 20232710. Royal Society.

Wickham, H. 2011. ggplot2. *WIREs Comput. Stat.* **3**: 180–185. Wiley Online Library.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., *et al.* 2019. Welcome to the Tidyverse. *J. Open Source Softw.* **4**: 1686.

Xu, Y., Zhang, Y., Liang, J., He, G., Liu, X., Zheng, Z., *et al.* 2021. Impacts of marine heatwaves on pearl oysters are alleviated following repeated exposure. *Mar. Pollut. Bull.* **173**: 112932.

Zhang, W., Chang, X.-Q., Hoffmann, AryA., Zhang, S. & Ma, C.-S. 2015. Impact of hot events at different developmental stages of a moth: the closer to adult stage, the less reproductive output. *Sci. Rep.* **5**: 10436. Nature Publishing Group.