Journal of Evolutionary Biology, 2024, **37**, 1–13
https://doi.org/10.1093/jeb/voad009
Advance access publication 7 December 2023
Research Article

JOURNAL OF
Evolutionary Biology

Genetic background and thermal regime influence adaptation to novel environment in the seed beetle, *Callosobruchus maculatus*

Edward R. Ivimey-Cook[1], Claudio Piani[2], Wei-Tse Hung[2], Elena C. Berg[2]

[1]School of Biological Sciences, One Health & Veterinary Medicine, University of Glasgow, Scotland, United Kingdom
[2]Department of Computer Science, Mathematics, and Environmental Science, The American University of Paris, Paris, France

Corresponding author: Edward R. Ivimey-Cook, School of Biological Sciences, One Health & Veterinary Medicine, Graham Kerr Building, University of Glasgow, Glasgow, Scotland G12 8QQ, United Kingdom. Email: e.ivimeycook@gmail.com

Abstract

Climate change is associated with the increase in both the mean and variability of thermal conditions. Therefore, the use of more realistic fluctuating thermal regimes is the most appropriate laboratory method for predicting population responses to thermal heterogeneity. However, the long- and short-term implications of evolving under such conditions are not well understood. Here, we examined differences in key life-history traits among populations of seed beetles (*Callosobruchus maculatus*) that evolved under either constant control conditions or in an environment with fluctuating daily temperatures. Specifically, individuals from two distinct genetic backgrounds were kept for 19 generations at one of two temperatures, a constant temperature (T = 29 °C) or a fluctuating daily cycle (T_{mean} = 33 °C, T_{max} = 40 °C, and T_{min} = 26 °C), and were assayed either in their evolved environment or in the other environment. We found that beetles that evolved in fluctuating environments but were then switched to constant 29 °C conditions had far greater lifetime reproductive success compared with beetles that were kept in their evolved environments. This increase in reproductive success suggests that beetles raised in fluctuating environments may have evolved greater thermal breadth than control condition beetles. In addition, the degree of sexual dimorphism in body size and development varied as a function of genetic background, evolved thermal environment, and current temperature conditions. These results not only highlight the value of incorporating diel fluctuations into climate research but also suggest that populations that experience variability in temperature may be better able to respond to both short- and long-term changes in environmental conditions.

Keywords: thermal adaptation, fluctuating temperatures, thermal breadth, climate change, *Callosobruchus maculatus*

Introduction

Climate change is leading to a rise in both the mean and variability of global temperatures (Folguera et al., 2011; Kotz et al., 2021; IPCC, 2021; Morón Lugo et al., 2020; Vasseur et al., 2014; Wang & Dillon, 2014).

Until recently, the majority of laboratory studies have focused solely on variation in mean temperature, maintaining populations at constant conditions for the duration of the experimental assay (Bauerfeind & Fischer, 2014; Colinet et al., 2015; Enders & Boisclair, 2016; Fischer et al., 2011; Folguera et al., 2011; Matsubara, 2018; Thompson et al., 2013). However, the impact of increased temperatures, which have been shown to negatively impact various life-history traits across a range of species (Alfonso et al., 2021; Bauerfeind & Fischer, 2014; Berger et al., 2017; Chen et al., 2018; Godwin et al., 2020; Ivimey-Cook et al., 2021; Klepsatel et al., 2019; Ontoria et al., 2019; Rogell et al., 2014; Vasudeva et al., 2014), are predicted to intensify as the environment becomes more variable (Bozinovic et al., 2016; Deutsch et al., 2008; Morón Lugo et al., 2020; Renault et al., 2022; Slein et al., 2023; Vasseur et al., 2014). Indeed, in an analysis of 38 globally distributed invertebrates, Vasseur et al. (2014) found that a change in mean temperature explained only 32% of future species performance, whereas combining both the mean and variance simultaneously explained 93%.

Fortunately, there is now a well-accepted requirement that empirical studies incorporate more realistic thermal regimes into their experimental design, including changes to both the mean and variability of temperatures (Hokanson et al., 1977; Niehaus et al., 2012; Paaijmans et al., 2013; Bozinovic et al., 2016; Matsubara, 2018; Schaum et al., 2018, 2022; Buckley & Kingsolver, 2021). Populations exposed to fluctuating rather than constant conditions have been shown to exhibit marked life-history differences (Bauerfeind & Fischer, 2014; Buckley & Kingsolver, 2021; Folguera et al., 2011; Hokanson et al., 1977; Kellermann & van Heerwaarden, 2019; Matsubara, 2018; Schaum et al., 2018, 2022). For example, thermal reaction norms of the mosquito *Anopheles stephensi* under fluctuating thermal regimes were fundamentally different in shape from those under constant temperatures (Paajimans et al., 2013). Similarly, in the tropical butterfly *Bicyclus anyana*, life-history traits, such as development time, pupal weight, and wing length, were strongly influenced by both the mean and the amplitude of the experimental thermoperiod. More specifically, thermoperiods with increasing amplitudes led to faster development time, which in turn reduced pupal weight and wing length (Brakefield & Mazzotta, 1995).

Most studies of thermal variation have focused on very short timescales, which a recent meta-analysis suggests might underestimate the impacts of climate change on life-history

Received July 27, 2022; revised October 16, 2023; accepted November 24, 2023
© The Author(s) 2023. Published by Oxford University Press on behalf of the European Society of Evolutionary Biology. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

traits (Slein et al., 2023). Thus, there is still a need to understand how long-term evolution under naturally variable conditions impacts the ability to persist and adapt to novel temperatures, compared to long-term evolution under more constant and benign conditions. To this end, we investigated the long-term effects of fluctuating diel temperatures on key life-history traits of the seed beetle (*Callosobruchus maculatus*) from two distinct genetic backgrounds. In addition, we examined the short-term effects of exposure to a novel environment for beetles that had evolved under two different thermal regimes. Beetles were either kept at a constant control temperature of 29 °C or exposed to diel variation that fluctuated around a mean temperature of 33 °C. Beetles were either kept in these two environments for the entirety of the experiment or were *switched* to the other environment for two generations without selection prior to assaying development time, body mass, and reproductive fitness. This experimental design allowed us to ask several questions pertaining to thermal performance, genetic background, and sexual dimorphism both in the long term (after evolution under constant or fluctuating conditions) and in the short term (after exposure to a novel environment).

First, we can investigate whether long-term evolution under predictable fluctuating conditions selects individuals with either enhanced or reduced thermal breadth. A species' thermal breadth can influence performance and fitness (one measure of which is lifetime reproductive success [LRS]) both in the long term, with the ability to survive and persist under periods of diel variation, or in the short term, upon exposure to novel environmental conditions. We may expect several differing responses to evolution under thermal fluctuations. For instance, species could broaden their thermal breadth in order to match the fluctuating environment (Bonnefond et al., 2017; Gilchrist, 1995; Kassen, 2002; Ketola et al., 2014; Lynch & Gabriel, 1987; Shah et al., 2017), which may ultimately help with persistence and performance under stressful diel fluctuations and exposure to novel conditions. Indeed, fluctuating environments have been shown to increase the expression of heat-shock proteins in comparison to stable environments (Ketola et al., 2004). However, this broadening of thermal tolerance may result in lowered optimal performance (often referred to as the "jack-of-all-trades a master of none"; see Huey & Hertz, 1984; Huey & Kingsolver, 1989; Lynch & Gabriel, 1987) and could manifest in stable but not maximized fitness across all environments, including when exposed to short-term novel conditions (Bennett et al., 1992; Berger et al., 2014; Grether, 2005; Van Tienderen, 1991).

Interestingly, a trade-off between thermal breadth and maximal performance has not always been found (Angilletta et al., 2003; Hughes et al., 2007; Berger et al., 2014; but see Bennett et al., 1992), suggesting that we may see the evolution of superior performance across all temperatures in comparison to those evolved in more homogenous environments ("jack-of-all-temperatures master of all"; see Huey & Hertz, 1984). Conversely, we may see a narrowing of thermal breadth where critical functions are contained when diel fluctuations are more favourable, often termed the "diel narrowing hypothesis" (Gilchrist, 1995; Kefford et al., 2022; New et al., 2014). If selection acts on organisms to reproduce when conditions are optimal, we may see analogous or even increased performance among individuals that evolved under specific constant conditions (Gilchrist, 1995; Ketola et al., 2013, 2014), which has been suggested to lead to thermal specialization (Bennett et al., 1992; Gilchrist, 1995; Kassen, 2002). Ultimately, we will determine whether fluctuating thermal conditions select environmentally robust individuals (i.e., large thermal breadth with little change between environments) or individuals that have narrowed their niche (i.e., better performance only in optimal conditions).

Second, we could ask whether genetic background influences a population's response to long-term thermal regimes and exposure to novel environmental conditions. Although we had no a priori predictions about if and how any population-level differences would manifest, previous research has highlighted the substantial genotypic and phenotypic differences that exist between populations of *C. maculatus* (Fox, Bush, et al., 2004; Messina, 1990; Rankin & Arnqvist, 2008). Therefore, we may expect similar variation, with population-specific patterns emerging for all measured life-history traits across both the long- and short-term timescales.

Third, we can examine whether sexual dimorphism in body size and development time changes after long- and short-term exposure to constant or fluctuating environments. In this species, these two measures of sexual dimorphism are positively correlated (Hallsson & Björklund, 2012). Although females are consistently bigger than males across all conditions, body size dimorphism has been shown to vary with rearing temperature and environmental conditions (Hallsson & Björklund, 2012; Stillwell & Fox, 2007). Two hypotheses could explain potential differences in sexually dimorphic traits in this species and involve various assumptions surrounding the sex-specific importance of body mass (more important to female fitness as heavier females are more fecund) and developmental time (more important to male fitness as quicker development leads to faster access to potential mates) (Ceballos & Valenzuela, 2011; Hallsson & Björklund, 2012; Savalli & Fox, 1999). Under condition dependence (or directional selection), it is predicted that as female mass is under stronger selection, it will exhibit greater sensitivity to environmental conditions than in males, resulting in decreased sexual dimorphism for body size at higher temperatures. Conversely, for developmental time, as male development will be under stronger selection, sexual dimorphism is predicted to increase during periods of high temperatures and diel fluctuations (Hallsson & Björklund, 2012; Stillwell et al., 2010). Alternatively, if adaptive canalization (or stabilizing selection) is occurring, sexual dimorphism for body size is predicted to increase under periods of higher temperature; female mass will remain constant, while male mass varies across environmental treatments. For development time, under the same hypothesis, males are expected to be less responsive to environmental conditions compared with females, leading to a reduction in sexual dimorphism at higher temperatures (Hallsson & Björklund, 2012; Stillwell et al., 2010).

Lastly, we may predict specific changes to the absolute values of body size and developmental time. The temperature–size rule (Atkinson, 1994; Colinet et al., 2015; Verberk et al., 2021) dictates that higher temperatures should lead to faster development with a correlated decrease in body size. As a result, we could see a similar pattern emerge after both short- and long-term exposures to the higher mean temperatures associated with diel fluctuations. However, it is important to acknowledge that the amplitude of fluctuation in addition to potential thermal injury can cause a variety of reactions, including delayed development coupled with smaller body size (Chen et al., 2019; Colinet et al., 2015; Kjærsgaard et al., 2013).

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

Methods

Study system

Callosobruchus maculatus is an agricultural pest that originates from Africa and Asia but is currently found throughout the world. Adult seed beetles are facultatively aphagous, i.e., they do not require food or water to survive and acquire all the resources they need during the larval stage within the bean. Aphagous adults can live up to 2 weeks, but adults with access to nutrients can live 3 weeks or more (Fox, 1993; Ursprung et al., 2009). Females lay their eggs on the surface of dried legumes, such as mung beans (*Vigna radiata*) or black-eyed beans (*Vigna unguiculata*). Larvae from the fertilized eggs burrow into the bean and eclose as adults 23–27 days later. Females of this species start to mate and lay eggs as soon as they emerge (Beck & Blumer, 2014).

In this experiment, we used two different strains of *C. maculatus* from differing genetic backgrounds: South India (SI) USA and SI Leicester (hereafter known as USA and LEIC, respectively). Both of these initially derived from a population of beetles collected in Tirunelveli, India, in 1979 (Mitchell, 1991) but were subsequently raised at either the University of Kentucky, USA, or the University of Leicester, UK (from 1992; Fricke & Arnqvist, 2004), respectively. We obtained these strains from Uppsala University in Sweden in 2015 and maintained them at the American University of Paris for approximately 25 generations before establishing the lines for the current experiment. During this time, beetles were cultured exclusively on mung beans and kept in climate chambers at a constant 29 °C, 50% relative humidity, and a 12:12h light:dark cycle.

Beetles were housed at aphagy in 1-L jars with 250 g of beans, and approximately 250–350 newly hatched beetles were transferred to new jars with fresh beans every 24 days on a continuous basis. Sufficient beans were provided so that females could lay just one egg per bean, thereby eliminating competition among multiple larvae within a bean (Berg & Maklakov, 2012; Berg et al., 2019). This is important as larval competition has been found to significantly reduce the fitness (Vamosi & Lesack, 2007) and mass (Colegrave, 1993; Vamosi, 2005) of individuals at emergence. Indeed, in populations where larval competition is known to be high, females readily avoid laying additional eggs on previously laid seeds (Fox & Messina, 2018).

Thermal evolution lines and assay conditions

To create our treatment groups, we subdivided beetles from the USA and LEIC genetic backgrounds into eight replicates ($n = 4$ for each genetic background) (Figure 1). We then maintained the eight replicates for 19 generations (i.e., approximately 15 months) prior to the experiment, a standard timeframe for experimental evolution in this species (Berg & Maklakov, 2012; Lind et al., 2015). During those 19 generations, beetles were kept at the same humidity and light cycle conditions as above but differed in their long-term thermal regime (hereafter known as Thermal Regime; Figure 1): Two replicates from both USA and LEIC ($n = 4$) were maintained at a "Constant" thermal regime of 29 °C, and two replicates from both USA and LEIC ($n = 4$) were kept under a "Fluctuating" regime where individuals were subject to a daily temperature cycle consisting of 12 separate 2-hr periods of constant temperature T_i,

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

where $T_{\text{mean}} = 33$ °C, $\Delta T = 7$ °C, and $i = 0, 1 \ldots 11$. This was a stepwise sinusoidal temperature cycle with $T_{\text{max}} = 40$ and $T_{\text{min}} = 26$ that mimics typical late spring conditions in southern India, where this species evolved (see Supplementary Methods for further information on the rate of change experienced by different groups of beetles under these conditions). Importantly, our definition of environmental fluctuation differs from previous work on this species that used the same term (see Hallsson & Björklund, 2012). While we use the term to represent daily sinusoidal fluctuations around a constant mean, representative of the natural environment that a seed beetle could experience, Hallsson and Björklund (2012) used the term to describe the addition of thermal noise around a linear increase in temperature.

Prior to conducting assays of body size, development time, and LRS (see next section), we divided each individual replicate (listed above, $n = 8$) into two, thereby creating 16 replicate populations in total. For each replicate, one group was kept at the same conditions to which it had evolved during the previous 19 generations, and the other was allowed to acclimatize for two generations to the other set of conditions. We did this to separate genetic adaptation from phenotypic plasticity, i.e., to control for the influence of parental effects (Lind et al., 2015; Lymbery et al., 2020). Furthermore, to remove the possible effects of natural selection via assortative mating, and thus rule out the possibility that rapid genetic responses might influence our measurements for the "acclimatized" treatments, we randomly paired 50 pairs of virgin beetles during each of the two generations prior to assays (Figure 1). The assay conditions were as follows (followed in each case by the notation of Thermal Regime–Assay Environment):

1. beetles evolved and assayed in Constant conditions (Constant–Constant);
2. beetles evolved and assayed in Fluctuating conditions (Fluctuating–Fluctuating);
3. beetles evolved in Constant conditions that were then acclimatized to Fluctuating conditions for two generations without selection prior to being assayed (Constant–Fluctuating); and
4. beetles evolved in Fluctuating conditions that were then acclimatized to Constant conditions for two generations without selection prior to being assayed (Fluctuating–Constant).

For the Fluctuating–Constant and the Constant–Fluctuating assays, the following steps were performed. First, we moved beans with fertilized eggs from jars into a 48-well virgin chamber (aerated plastic culture plates with a separate well for each individual) with one bean per well. When enough males and females had hatched (from Days 21 to 24 after hatch), we paired 60 males and 60 females in 60-mm Petri dishes with 100 beans in each dish and placed these in the assay environment chamber. As in the jars, we made sure that there were sufficient number of beans in each dish for females to lay just one egg per bean, thereby reducing larval competition (see above). To further reduce competition by larvae, we removed adult beetle pairs from the petri dishes after 72 hr.

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

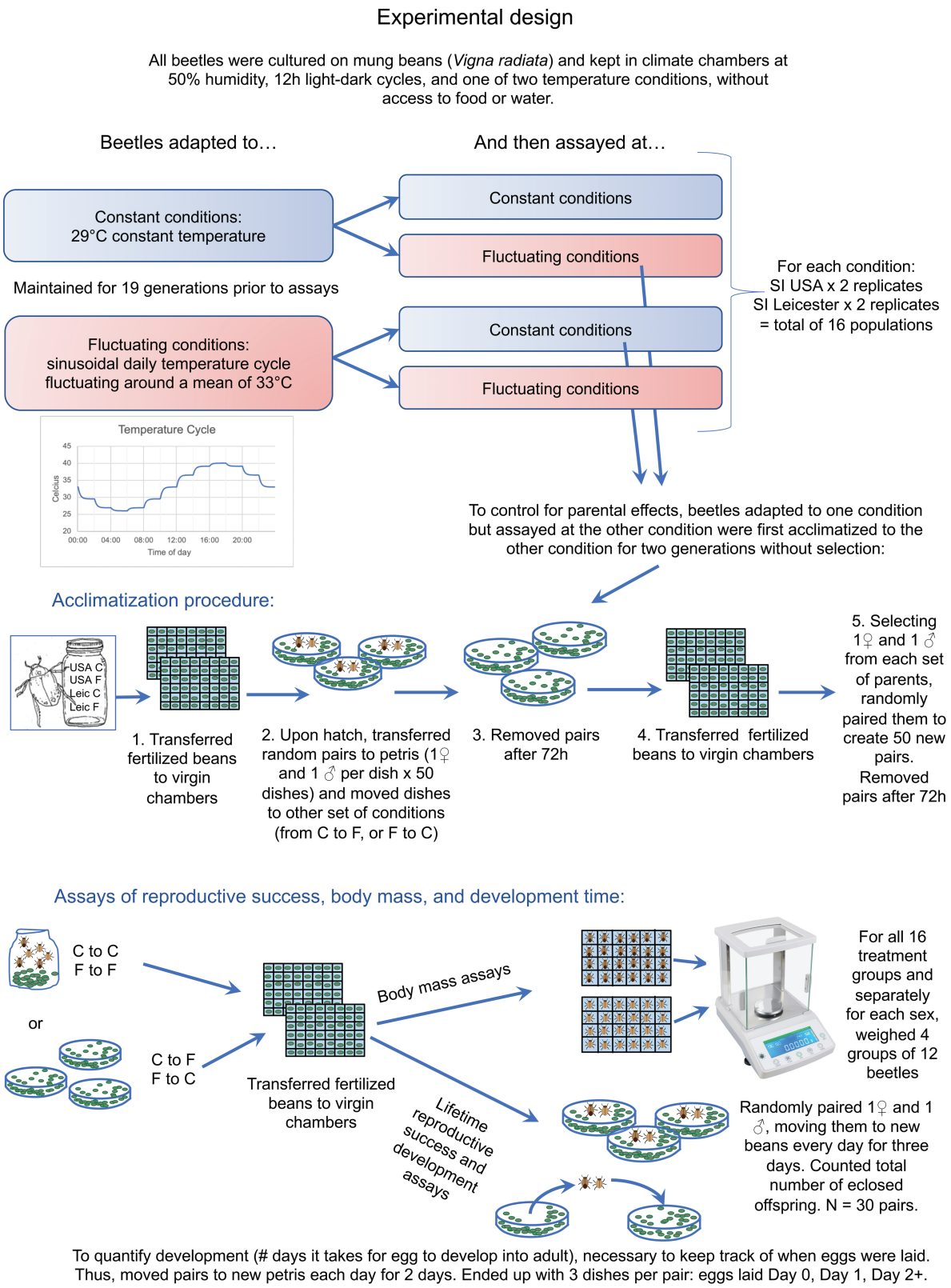

Figure 1. An overview of the experimental design used to generate beetles involved in the various life-history assays from the 16 sampling populations.

After 19 days, before any offspring eclosed, we chose 50 Petri dishes with sufficient numbers of eggs to allow us to transfer 48 beans with eggs on them from each dish into individual marked 48-well virgin chambers. One day after eclosion, we randomly selected and paired 50 females and 50 males and put them into 50-mm petri dishes with 80 beans. After 72 hr, the adult males and females were removed from the petri dishes. After 19 days, prior to eclosion, beans with fertilized eggs from each petri dish were transferred into 48-well virgin chambers. We then marked the date of hatch and sex of all

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

offspring and conducted assays of development time, body mass, and reproductive fitness (Fig. 1).

For the Constant–Constant and Fluctuating–Fluctuating treatments, we skipped the acclimatization steps and simply moved beans with fertilized eggs into virgin chambers prior to hatch, selecting 1-day-old males and females for all subsequent assays.

Body mass assay

One day after hatching, for each of the 16 sampling populations, males and females were separately weighed in two batches of 12 beetles each using an Ohaus Pioneer Plus Analytical Balance (Model PA214C), to the nearest 0.0001 g. This was then repeated four times for each of the replicate populations, aside from the USA Control, which was repeated eight times (Fig. 1). Beetles from the body mass assay were not used for the other assays.

Development time and fitness assays

Development time and fitness assays were done concurrently (Fig. 1). We paired 30 one-day-old male and female beetles and placed them into 60-mm Petri dishes filled with 85 beans. After 24 hr, each pair was moved together into a new 60-mm petri dish filled with 75 beans, and the first dish was set aside in the same climate chamber. The next day, this procedure was repeated. The beetles were left in the third and final set of dishes until they died. The first dishes for each pair were marked "Day 0," the second dishes were marked "Day 1," and the third and final dishes were marked "Day 2+." After 19 days for each dish, before any new beetles could hatch, all the beans with fertilized eggs were placed into virgin chambers, which were monitored daily for 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. To calculate LRS, we calculated the number of male and female offspring that emerged for each pair, from all three sets of dishes (Days 0, 1, and 2+).

Statistical analyses

All statistical analyses were performed in R v. 4.2.3 (R Core Team, 2016) using glmmTMB v. 1.1.8 (Brooks et al., 2017; Magnusson et al., 2019), with pairwise interactions and slopes compared using emmeans v. 1.8.9 (Russell et al., 2018), which were corrected for multiple comparisons using the "mvt" function from emmeans, which refers to the multivariate *t* distribution. ggplot2 v. 3.4.4 (Wickham, 2011) was used for all graphical visualization. The overall effects of each variable were identified using the Type 3 ANOVA function from car v. 3.1-2 package with sum-to-zero contrasts (where possible; Fox et al., 2012). No model selection on any main model parameters or higher-order interactions took place. However, as we had no a priori reason to expect zero inflation (ZI) to be due to a particular predictor, we performed model selection on the zero-inflated parameters if ZI was indeed identified (see below). Lastly, if significant dispersion was detected even after accounting for ZI, we also performed model selection to identify the best-fitting dispersion parameters (all model selection tables are located in Supplementary Material).

To answer questions relating to thermal performance, genetic background, and sexual dimorphism, we fit the following models to the following traits:

1. To investigate short- and long-term thermal performance under constant or diel fluctuations, we fit two models involving age-specific reproduction and LRS. More specifically, for both traits, we fit generalized linear mixed-effects model with a Poisson distribution and all higher-order interactions between the fixed factors of genetic background, thermal regime, and assay environment. In addition to a linear covariate of day (with all higher-order interactions included), a random intercept of individual ID was also added to the age-specific reproduction model to account for repeated measures. This random effect of individual ID was nested within a 16-level factor of the sampling population to account for the non-independence of individuals from the same rearing environment prior to assaying (for LRS, only this 16-level factor was added). Both models were checked for overdispersion and ZI using DHARMa v. 0.4.1 (Hartig, 2020) by simulating the residuals of a Poisson model with and without an object-level random effect. If significant ZI was identified, a variety of error distributions (including Poisson, negative binomial, generalized Poisson, and Conway–Maxwell–Poisson) with/without zero-inflated parameters were then fitted and the best model was identified by Akaike's information criterion (see Supplementary Material for model selection). Tested zero-inflated parameters included the main effects of genetic background, thermal regime, assay environment, and, if age-specific reproduction, the linear covariate of day.
2. When considering if genetic background influenced a population's response to long-term thermal regimes and exposure to novel environmental conditions, a two-factor variable of genetic background (USA/Leicester) was added to all models (along with all higher-order interactions).
3. Lastly, to examine whether sexual dimorphism was altered after short- and long-term exposure to constant or fluctuating environments, we fit two models involving the body mass of parents and the developmental time of offspring. For development time and body mass, a Gaussian linear mixed-effects model was fit (after log-transforming development time to reduce right skew) with the fixed effects of genetic background (USA/Leicester), thermal regime (Constant/Fluctuating), assay environment (Constant/Fluctuating), sex (M/F), and all higher-order interactions. The same 16-level random effect was added as above. Lastly, for the models involving development time, the random effect of parental ID was added to account for potential pseudoreplication and was nested within the 16-level factor of the sampling population.

Results

The highest-order interaction involving genetic background, thermal regime, and assay environment (see Supplementary Tables S1C and D/G and H, S2A and B, and S3A and B; for body mass and development time, sex was also included; for age-specific reproduction, day was also included), was only statistically significant when analysing age-specific reproduction (age-specific reproduction: $p < .001$; body mass: $p = .124$; LRS: $p = .433$; development time: $p = .765$). However, as we were interested in specific comparisons (e.g., between males

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

and females or between genetic backgrounds) and to avoid model reduction, we instead interpreted estimated marginal means (EMMs, adjusted for multiple comparisons) from these models regardless of overall significance. In all cases, results are presented as Thermal Regime–Assay Environment.

Thermal performance

For beetles assayed under constant conditions, LRS was highest for those that evolved in fluctuating environments, and this pattern held across both genetic backgrounds (LEIC Fluctuating–Constant—LEIC Constant–Constant = 17.16, $p < .001$; USA Fluctuating–Constant—USA Constant–Constant = 23.52, $p < .001$; Figure 2A; Supplementary Table S1E). For those assayed under fluctuating environments, no differences were found between thermal regimes (both comparisons $p > .05$; Figure 2A). Indeed, differences between assay environments only became detectable for individuals that had evolved under fluctuating conditions (LEIC Fluctuating–Constant—LEIC Fluctuating–Fluctuating = −24.67, $p < .001$; USA Fluctuating–Constant—USA Fluctuating–Fluctuating = −28.17, $p < .001$; Figure 2A; Supplementary Table S1E). These differences suggest a contrasting ability of individuals to deal with short-term changes in environmental conditions. While constant-evolved beetles performed similarly across assay environments, the fluctuating-evolved beetles performed better under constant conditions. Lastly, long-term exposure to diel fluctuations (Fluctuating–Fluctuating) did not appear to adversely reduce thermal performance; those beetles performed similarly to beetles that had evolved under and been assayed in constant conditions (Constant–Constant) (all comparisons $p > .05$; Figure 2A; Supplementary Table S1E).

Genetic background (all traits)

No statistical differences were found in body mass between the two genetic backgrounds across treatments (p for all EMM comparisons > .05; Figure 3A and B; Supplementary Table S2C). However, the relative difference between male and female body size (discussed further below) was background specific. Beetles from the LEIC population exposed to Fluctuating–Fluctuating conditions showed a reduction in male–female body size differences (LEIC F-M difference: −2.08, $p = 1.000$; USA F-M Estimate: 18.55, $p < .001$; Figure 3B; Supplementary Table S2C). For development time, there were several differences between the two genetic backgrounds. In the Constant–Constant treatment, males and females from the LEIC population developed faster than males and females from the USA population (F-Constant–Constant LEIC–USA difference: 0.992, $p < .001$; M-Constant–Constant LEIC–USA difference: 0.985,

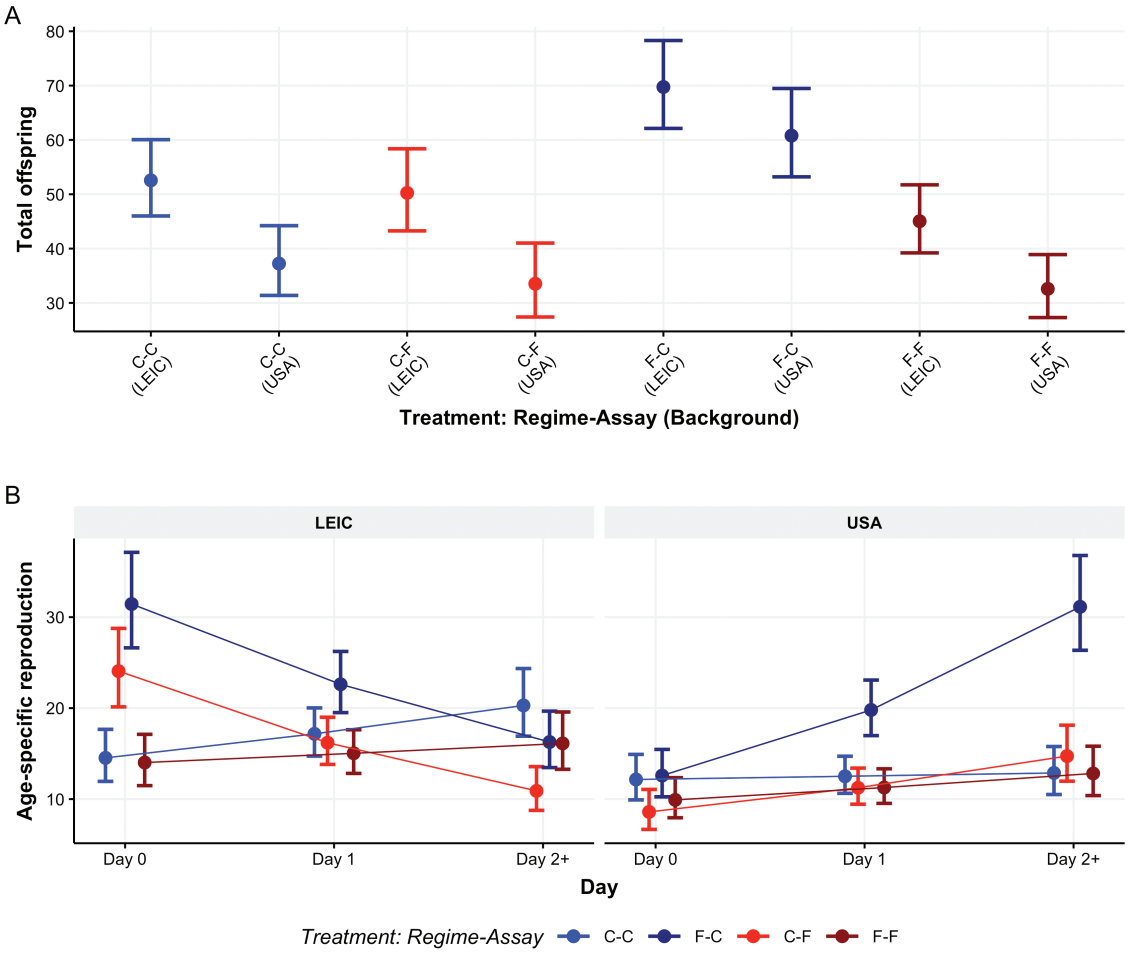

Figure 2. (A) Lifetime reproductive success (LRS). (B) Age-specific reproduction of individuals from Leicester (left) or USA (right). Colours correspond to each combination of Regime–Assay Environment. Points with error bars represent estimated marginal mean values with accompanying standard errors that have accounted for multiple comparisons using a multivariate t distribution (see Methods).

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

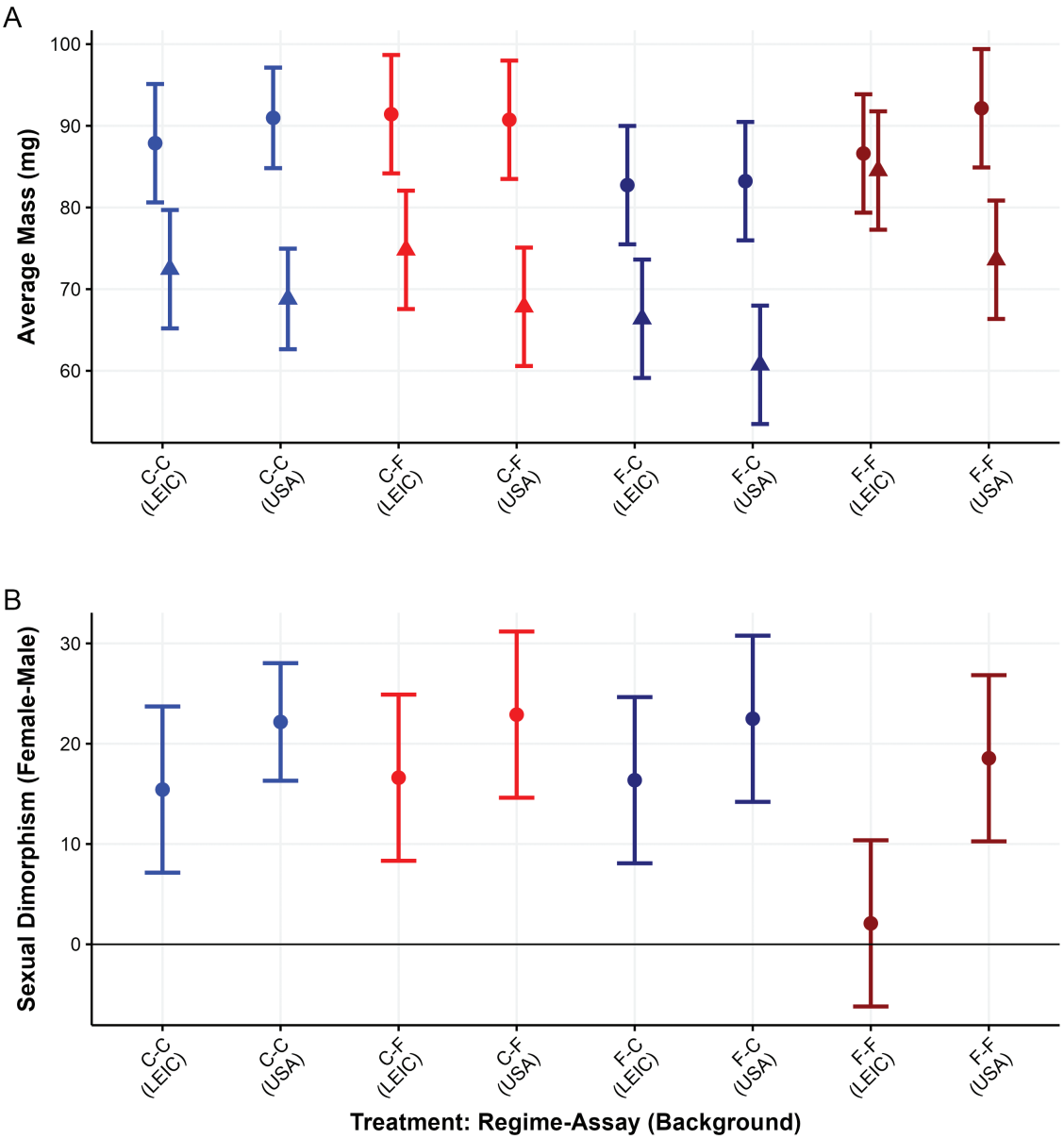

Figure 3. (A) Average body mass of 12 male (triangle) or female (circle) beetles; (B) average difference between female and male body mass. The line across zero denotes no difference, while points above the line indicate heavier females. Colours correspond to each combination of Regime–Environment. Points with error bars represent estimated marginal mean values with accompanying *SE* that have accounted for multiple comparisons using a multivariate *t* distribution (see Methods).

$p < .001$; Figure 4A; Supplementary Table S3C). A similar difference was found between the two backgrounds in the Fluctuating–Constant treatment, with LEIC developing faster than USA (F-Fluctuating–Constant LEIC–USA difference: −0.924, $p < .001$; M-Fluctuating–Constant LEIC–USA difference: 0.929, $p < .001$; Figure 4A; Supplementary Table S3C). Both backgrounds showed the same differences between male and female development time (discussed further below; Figure 4B; Supplementary Table S3C).

Lastly, there were significant differences in the thermal performance of the two populations (Figure 2A). With the exception of the Fluctuating–Constant treatment, LEIC individuals had greater LRS than USA individuals (all comparisons significant to $p < .001$; Figure 2A and B; Supplementary Table S1E). This was in part due to large differences in the reproductive schedule between the two genetic backgrounds, particularly those from the Constant–Fluctuating and Fluctuating–Constant treatments (Figure 2B). Interestingly, Fluctuating–Constant beetles showed opposing reproductive strategies, with those from LEIC exhibiting a decline and those from USA exhibiting an increase in reproduction over time. This ultimately contributed to the lack of difference in LRS (Figure 2A and B).

Sexual dimorphism (body mass and development time)

Within treatments, females had higher body mass in all but one (see below) of the regime–assay–background combinations (all comparisons $p < .001$; Figure 3A; Supplementary Table S2). The relative differences between sexes, or degree of sexual dimorphism, were similar across the various treatments (all pairwise contrasts $p > .05$: Figure 3B; Supplementary

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

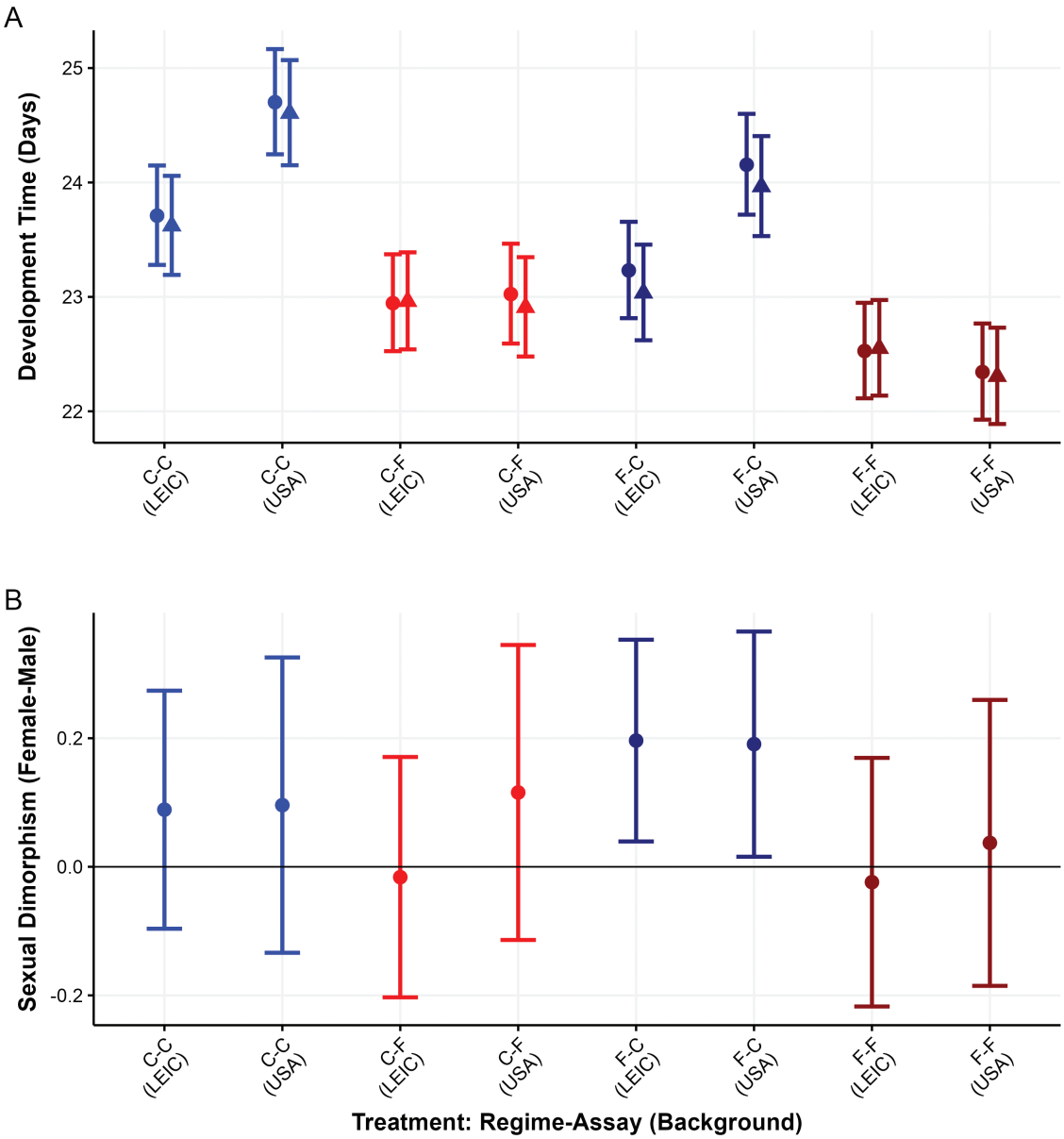

Figure 4. (A) Average development time in days of male (triangle) or female (circle) beetles; (B) average difference between female and male development time. The line across zero denotes no difference, while points above the line indicate that females had longer development. Colours correspond to each combination of Regime–Environment. Points with error bars represent estimated marginal mean values with accompanying *SE* that have accounted for multiple comparisons using a multivariate *t* distribution (see Methods).

Table S2D) aside from Fluctuating–Fluctuating beetles from the LEIC population, which exhibited significantly reduced sexual dimorphism in body size (all contrasts $p < .05$; Supplementary Table S2D).

Body size also differed between treatments. LEIC males from the Fluctuating–Fluctuating treatment were heavier than males from the two treatments assayed in constant environments (p for all EMM comparisons > .05; Constant–Constant and Fluctuating–Constant; Supplementary Table S2C). A similar trend was found for USA males, albeit without the difference between Fluctuating–Fluctuating and Constant–Constant treatments ($p = .948$; Supplementary Table S2C). In contrast, there were no significant differences in female body size between treatments (p for all EMM comparisons > .05; Figure 4A and B; Supplementary Table S2C).

For all but one treatment, males and females had similar development times (p for all EMM comparisons > .05; Figure 4A and B; Supplementary Table S3C). However, for both genetic backgrounds, there was increased sexual dimorphism in the Fluctuating–Constant treatment, with males developing faster than females (Fluctuating–Constant M-F p values: LEIC = .003, USA = .020; Figure 4B; Supplementary Table S3C). However, this relative difference between male and female development time did not differ statistically from the other treatment differences (p for all EMM comparisons $p > .05$; Figure 4B; Supplementary Table S3D). For development time, there were large differences between treatment groups across both sexes and genetic backgrounds. In general, both sexes took longer to develop when individuals were assayed at Constant temperatures, particularly those from the USA background (all comparisons $p < .001$; Figure 4A; Supplementary Table S3C) and were faster to develop when evolved and assayed under Fluctuating conditions (Figure 4A; Supplementary Table S3C).

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

Discussion

The aim of this experiment was to evaluate both the short- and long-term impacts of evolving under realistic thermal fluctuations on the life-history traits of *C. maculatus* from two genetic backgrounds. First, we asked whether evolving under diel fluctuations would select for individuals with either enhanced or reduced thermal breadth. Early hypotheses (Lewontin, 1974; Nevo, 1978) suggested that evolution in fluctuating environments should select for genetically variable individuals that are robust to a wide range of thermal conditions with wide thermal performance curves (TPCs; Jin & Agustí, 2018; Kassen, 2002). In this experiment, individuals that evolved under diel fluctuations and were subsequently exposed to novel benign conditions (Fluctuating–Constant) had higher reproductive fitness (manifesting in higher LRS) than those that evolved in a constant environment and were then exposed either to novel fluctuating conditions (Constant–Fluctuating) or kept in a constant environment (Constant–Constant). These results are broadly in line with previous research conducted on the opportunistic bacterial pathogen, *Serratia marcescens* (Ketola et al., 2013), where bacterial clones that evolved under fluctuating conditions had faster growth and higher yield than those grown under constant environments, even when introduced into the same control temperature of 31 °C (Ketola et al., 2013). This increased growth may be due to far greater selective pressure to remove mutations that could otherwise accumulate in more constant and benign environments (Ketola et al., 2013).

Moreover, our results suggest that individuals that evolved in Fluctuating thermal environments were able to tolerate and survive at wide thermal breadth and perform in an analogous manner to those evolved and assayed under Constant conditions, but they reached maximal performance and experienced increased LRS only when conditions were benign and constant. This suggests that diel fluctuations select not only for a broadening of thermal breadth, presumably by upregulation of heat-shock proteins (Ketola et al., 2004), but also for the maximization of fitness under optimal conditions (Gilchrist, 1995; Kefford et al., 2022; New et al., 2014). This increase in reproductive performance could in part be due to differential gene expression as a result of exposure to a novel thermal environment (also known as cryptic genetic variability, McGuigan & Sgro, 2009; Paaby & Rockman, 2014; Zheng et al., 2019). Individuals from the Constant–Fluctuating environment may have expressed a similar form of cryptic genetic variation, since surprisingly, individuals suffered no cost to fitness upon switching from a benign constant to a stressful fluctuating environment and performed in a similar manner to those that evolved and were tested under fluctuating thermal conditions. However, we note that the relative merits of cryptic genetic variability have been queried and require additional empirical testing (Edwards & Yang, 2021; Johansson et al., 2021; McGuigan & Sgro, 2009). It may also be that selective mortality of eggs or larvae during the acclimatization process in Constant–Fluctuating treatments led to similar LRS as in the long-term evolution in Fluctuating–Fluctuating; however, we lack the data to address this. Importantly, not detecting any costs does not mean that they do not exist or are not manifesting in another trait that was not measured such as immune status (Ketola et al., 2013). Alternatively, there might not be a substantial cost to fitness if individuals are able to accurately predict future environmental conditions and avoid the expression of suboptimal genes (Auld et al., 2010; Hoffmann & Bridle, 2022). Quantifying these costs would require an experimental design with additional thermal regimes beyond the scope of this current experiment.

Second, we examined whether genetic background might influence a population's response to different thermal regimes. As mentioned previously, such population-level variation among *C. maculatus* has been found to impact larval competition (Messina, 1991), various morphometric measures (Rankin & Arnqvist, 2008), egg-to-adult survival and offspring production (Rankin & Arnqvist, 2008), and adult longevity and mortality (Fox, Bush, et al., 2004, Fox, Czesak, et al., 2004). Here, we found population-level differences in (a) body mass, particularly an absence of difference between males and females in LEIC, when individuals evolved and were subsequently assayed under Fluctuating conditions; (b) development time, with LEIC typically developing faster than USA; and (c) thermal performance, with LEIC beetles exhibiting higher performance across all but one treatment group. Intriguingly, while a switch from a fluctuating regime to a constant assay environment (Fluctuating–Constant) was associated with an increase in LRS across both genetic backgrounds, there was a noticeable difference in the age-specific reproductive schedules between USA and LEIC beetles. LEIC beetles produced more offspring on the first day (Day 0) and fewer on Days 1 and Days 2+, while the opposite was observed for USA beetles. Why such a difference in reproductive schedule exists warrants deeper investigation and suggests the need for a more direct measure of the genetic variance between beetles from different populations. However, together, this clearly highlights the benefits of comparing and contrasting results from multiple strains, as the response of species to environmental change can often be highly background specific.

Third, we examined changes in the absolute values of two correlated traits, body mass and development time, in addition to relative levels of sexual dimorphism in response to diel fluctuations. Long-term exposure to fluctuating conditions (Fluctuating–Fluctuating) led to an increase in male body size, but this occurred without a corresponding increase in development time. This could be explained if diel fluctuations select males with rapid growth rates (Schaum et al., 2022) that develop quickly and attain a large body size. This change in mass for males makes logical sense, as large size provides both a fitness advantage due to sexual selection (Andersson & Iwasa, 1996) but, more importantly, offers protection from desiccation (Chown & Gaston, 2010; Le Lagadec et al., 1998), which would be crucial for surviving the upper limits of diel fluctuation. Females showed a similar lack of trade-off between development time and body mass. They developed faster in long-term fluctuating environments (and slower in Constant–Constant), without a corresponding change in body mass between treatments. However, one constraint of our experimental design is that mass and development time were measured in different individuals, so they cannot be correlated within individuals. A future extension to this research should endeavour to investigate the trade-offs between these two correlated traits on an individual basis.

Sexual dimorphism also differed depending on the treatment group. Similar to Hallsson and Björklund (2012), we found that sexual dimorphism in adult body size decreased when individuals evolved and were assayed under diel fluctuations (Fluctuating–Fluctuating). Males exhibited increased body size under these conditions, whereas females showed a constant body size across all treatment groups. However, as

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

noted above, this increase in male body size only occurred in one of the sampled genetic backgrounds (LEIC). In addition, we found that sexual dimorphism in development time increased when individuals evolved under diel fluctuations and were assayed in constant environments (Fluctuating–Constant), with males exhibiting faster development than females. Although not conclusive, this could suggest some combination of adaptive canalization and condition-dependence acting on males and females that have evolved under long-term diel fluctuations in temperature, a finding that is generally supported by previous work in *C. maculatus* by Hallsson and Björklund (2012). However, we note that in this study we were unable to calculate coefficients of environmental and genetic variation owing to a lack of full-sibling design. Nonetheless, our results suggest that long-term evolution in fluctuating environments can significantly influence dynamics between males and females and that sex-specific responses to thermal variation are likely to occur.

Conclusions

Our work adds to the growing pool of studies that suggest that successfully mimicking natural conditions within the laboratory is paramount to understanding how species life histories will change in response to climate change. Although it is beyond the scope of the current experiment, assaying individuals at an array of temperature conditions would provide an even more comprehensive understanding of the evolution of thermal breadth (e.g., through the formation of a species-specific TPC; Huey & Kingsolver, 1989; MacLean et al., 2019; Sinclair et al., 2016). Nevertheless, as temperatures increase and become more variable, the incorporation of more realistic conditions into laboratory studies will allow for more accurate predictions of how wild species will respond to climate change. In addition, it will allow us to produce far more precise species- and population-specific thermal reaction norms (Buckley & Kingsolver, 2021; Paaijmans et al., 2013) that could eventually include a host of additional environmental variables that may also vary (Buckley & Kingsolver, 2021). Furthermore, carefully considering the genetic background, coupled with information on past and present environmental conditions, will give us additional valuable information about individual responses to climate change under conditions that mimic the natural environment.

Supplementary material

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

Data availability

Data have been uploaded to Dryad (https://doi.org/10.5061/dryad.f1vhhmgz7). Code used to run the analysis and produce the graphs is located on Zenodo at https://zenodo.org/doi/10.5281/zenodo.10118422.

Author contributions

Edward Ivimey-Cook (Data curation [equal], Formal analysis [lead], Writing—original draft [equal], Writing—review & editing [equal]), Claudio Piani (Conceptualization [equal], Methodology [supporting], Project administration [equal], Writing—original draft [supporting], Writing—review & editing [support], supervision [equal], Tony Wei-Tse Hung (Investigation [supporting], Methodology [supporting]), and Elena Berg (Conceptualization [equal], Data curation [equal], Formal analysis [supporting], Funding acquisition [lead], Methodology [lead], Project administration [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal])

Funding

This study was supported by The American University of Paris.

Acknowledgments

We thank Sophie Bricout for helping to maintain the beetle populations and Sophie Bricout, Linda Martz, Shannon Monahan, Sarah Sidi, and Xaviera Steele for their assistance with the experimental assays. We also thank Ram Vasudeva, Kynan Delaney, Joel Pick, and David Berger for helpful discussions and statistical advice. Lastly, we also want to thank the journal editors and two anonymous reviewers for their helpful comments and suggestions.

Conflicts of interest

None declared.

References

Alfonso, S., Gesto, M., & Sadoul, B. (2021). Temperature increase and its effects on fish stress physiology in the context of global warming. *Journal of Fish Biology*, *98*(6), 1496–1508. https://doi.org/10.1111/jfb.14599

Andersson, M., & Iwasa, Y. (1996). Sexual selection. *Trends in Ecology and Evolution*, *11*(2), 53–58. https://doi.org/10.1016/0169-5347(96)81042-1

Angilletta, M. J., Wilson, R. S., Navas, C. A., & James, R. S. (2003). Tradeoffs and the evolution of thermal reaction norms. *Trends in Ecology and Evolution*, *18*(5), 234–240. https://doi.org/10.1016/s0169-5347(03)00087-9

Atkinson, D. (1994). Temperature and organism size—A biological law for ectotherms? *Advances in Ecological Research*, *25*, 1–58.

Auld, J. R., Agrawal, A. A., & Relyea, R. A. (2010). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1681), 503–511. https://doi.org/10.1098/rspb.2009.1355

Bauerfeind, S. S., & Fischer, K. (2014). Simulating climate change: Temperature extremes but not means diminish performance in a widespread butterfly. *Population Ecology*, *56*, 239–250.

Beck, C. W., & Blumer, L. S. (2014). *A handbook on bean beetles, Callosobruchus maculatus*. National Science Foundation. https://www.beanbeetle.org/new_website/wp-content/images/handbook.pdf

Bennett, A. F., Lenski, R. E., & Mittler, J. E. (1992). Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. *Evolution*, *46*(1), 16–30. https://doi.org/10.1111/j.1558-5646.1992.tb01981.x

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. *Proceedings of the Royal Science B: Biological Sciences*, *286*(1910), 20191664. https://doi.org/10.1098/rspb.2019.1664

Berg, E. C., & Maklakov, A. A. (2012). Sexes suffer from suboptimal lifespan because of genetic conflict in a seed beetle. *Proceedings of*

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

the Royal Science B: Biological Sciences, *279*(1745), 4296–4302. https://doi.org/10.1098/rspb.2012.1345

Berger, D., Stångberg, J., Grieshop, K., Martinossi-Allibert, I., & Arnqvist, G. (2017). Temperature effects on life-history trade-offs, germline maintenance and mutation rate under simulated climate warming. *Proceedings of the Royal Science B: Biological Sciences*, *284*(1866), 20171721. https://doi.org/10.1098/rspb.2017.1721

Berger, D., Walters, R. J., & Blanckenhorn, W. U. (2014). Experimental evolution for generalists and specialists reveals multivariate genetic constraints on thermal reaction norms. *Journal of Evolutionary Biology*, *27*(9), 1975–1989. https://doi.org/10.1111/jeb.12452

Bonnefond, H., Grimaud, G., Rumin, J., Bougaran, G., Talec, A., Gachelin, M., Boutoute, M., Pruvost, E., Bernard, O., & Sciandra, A. (2017). Continuous selection pressure to improve temperature acclimation of *Tisochrysis lutea*. *PLoS One*, *12*(9), e0183547. https://doi.org/10.1371/journal.pone.0183547

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. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *186*(5), 581–587. https://doi.org/10.1007/s00360-016-0980-6

Brakefield, P. M., & Mazzotta, V. (1995). Matching field and laboratory environments: Effects of neglecting daily temperature variation on insect reaction norms. *Journal of Evolutionary Biology*, *8*, 559–573.

Brooks, M. E., Kristensen, K., van Benthem, K. J., … & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378–400. https://doi.org/10.32614/rj-2017-066

Buckley, L. B., & Kingsolver, J. G. (2021). Evolution of thermal sensitivity in changing and variable climates. *Annual Review of Ecology, Evolution, and Systematics*, *52*, 563–586.

Ceballos, C. P., & Valenzuela, N. (2011). The role of sex-specific plasticity in shaping sexual dimorphism in a long-lived vertebrate, the snapping turtle *Chelydra serpentina*. *Evolutionary Biology*, *38*, 163–181.

Chen, H., Zheng, X., Luo, M., … & Zhou, Z. (2018). Effect of short-term high-temperature exposure on the life history parameters of *Ophraella communa*. *8*(1), 13969.

Chen, W., Yang, L., Ren, L., Shang, Y., Wang, S., & Guo, Y. (2019). Impact of constant versus fluctuating temperatures on the development and life history parameters of *Aldrichina grahami* (Diptera: Calliphoridae). *Insects*, *10*(7), 184. https://doi.org/10.3390/insects10070184

Chown, S. L., & Gaston, K. J. (2010). Body size variation in insects: A macroecological perspective. *Biological Reviews of the Cambridge Philosophical Society*, *85*(1), 139–169. https://doi.org/10.1111/j.1469-185X.2009.00097.x

Colegrave, N. (1993). Does larval competition affect fecundity independently of its effect on adult weight? *Ecological Entomology*, *18*(3), 275–277. https://doi.org/10.1111/j.1365-2311.1993.tb01101.x

Colinet, H., Sinclair, B. J., Vernon, P., & Renault, D. (2015). Insects in fluctuating thermal environments. *Annual Review of Entomology*, *60*, 123–140. https://doi.org/10.1146/annurev-ento-010814-021017

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & 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

Edwards, C. B., & Yang, L. H. (2021). Evolved phenological cueing strategies show variable responses to climate change. *American Naturalist*, *197*, E1–E16.

Enders, E. C., & Boisclair, D. (2016). Effects of environmental fluctuations on fish metabolism: Atlantic salmon *Salmo salar* as a case study. *Journal of Fish Biology*, *88*(1), 344–358. https://doi.org/10.1111/jfb.12786

Fischer, K., Kölzow, N., Höltje, H., & Karl, I. (2011). Assay conditions in laboratory experiments: Is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? *Oecologia*, *166*(1), 23–33. https://doi.org/10.1007/s00442-011-1917-0

Folguera, G., Bastías, D. A., Caers, J., Rojas, J. M., Piulachs, M. -D., Bellés, X., & Bozinovic, F. (2011). An experimental test of the role of environmental temperature variability on ectotherm molecular, physiological and life-history traits: Implications for global warming. *Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology*, *159*(3), 242–246. https://doi.org/10.1016/j.cbpa.2011.03.002

Fox, C. W. (1993). Multiple mating, lifetime fecundity and female mortality of the bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Functional Ecology*, *7*(2), 203–208. https://doi.org/10.2307/2389888

Fox, C. W., Bush, M. L., Roff, D. A., & Wallin, W. G. (2004). Evolutionary genetics of lifespan and mortality rates in two populations of the seed beetle, *Callosobruchus maculatus*. *Heredity*, *92*(3), 170–181. https://doi.org/10.1038/sj.hdy.6800383

Fox, C. W., Czesak, M. E., & Wallin, W. G. (2004). Complex genetic architecture of population differences in adult lifespan of a beetle: Nonadditive inheritance, gender differences, body size and a large maternal effect. *Journal of Evolutionary Biology*, *17*(5), 1007–1017. https://doi.org/10.1111/j.1420-9101.2004.00752.x

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. *Journal of Evolutionary Biology*, *31*(2), 302–313. https://doi.org/10.1111/jeb.13222

Fox, J., Weisberg, S., Adler, D., ... & Heiberger, R. (2012). *Package 'car'*. Vienna: R Foundation for Statistical Computing, 16.

Fricke, C., & Arnqvist, G. (2004). Divergence in replicated phylogenies: The evolution of partial post-mating prezygotic isolation in bean weevils. *Journal of Evolutionary Biology*, *17*(6), 1345–1354. https://doi.org/10.1111/j.1420-9101.2004.00757.x

Gilchrist, G. W. (1995). Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. *American Naturalist*, *146*(2), 252–270. https://doi.org/10.1086/285797

Godwin, S. C., Fast, M. D., Kuparinen, A., Medcalf, K. E., & Hutchings, J. A. (2020). Increasing temperatures accentuate negative fitness consequences of a marine parasite. *Scientific Reports*, *10*(1), 18467. https://doi.org/10.1038/s41598-020-74948-3

Grether, G. F. (2005). Environmental change, phenotypic plasticity, and genetic compensation. *American Naturalist*, *166*(4), E115–E123. https://doi.org/10.1086/432023

Hallsson, L. R., & Björklund, M. (2012). Selection in a fluctuating environment and the evolution of sexual dimorphism in the seed beetle *Callosobruchus maculatus*. *Journal of Evolutionary Biology*, *25*(8), 1564–1575. https://doi.org/10.1111/j.1420-9101.2012.02541.x

Hartig, F. (2020). *DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models. R package*.

Hoffmann, A. A., & Bridle, J. (2022). The dangers of irreversibility in an age of increased uncertainty: revisiting plasticity in invertebrates. *Oikos*, *2022*(4), 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*. *Journal of the Fisheries Research Board of Canada*, *34*(5), 639–648. https://doi.org/10.1139/f77-100

Huey, R. B., & Hertz, P. E. (1984). Is a jack-of-all-temperatures a master of none? *Evolution*, *38*(2), 441–444. https://doi.org/10.1111/j.1558-5646.1984.tb00302.x

Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology and Evolution*, *4*(5), 131–135. https://doi.org/10.1016/0169-5347(89)90211-5

Hughes, B. S., Cullum, A. J., & Bennett, A. F. (2007). An experimental evolutionary study on adaptation to temporally fluctuating pH in Escherichia coli. *Physiological and Biochemical Zoology*, *80*(4), 406–421.

IPCC, 2021: *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report*

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2391 pp. doi:10.1017/9781009157896.

Ivimey-Cook, E., Bricout, S., Candela, V., Maklakov, A. A., & Berg, E. C. (2021). Inbreeding reduces fitness of seed beetles under thermal stress. *Journal of Evolutionary Biology*, *34*(9), 1386–1396.

Jin, P., & Agustí, S. (2018). Fast adaptation of tropical diatoms to increased warming with trade-offs. *Scientific Reports*, *8*(1), 17771. https://doi.org/10.1038/s41598-018-36091-y

Johansson, F., Watts, P. C., Sniegula, S., & Berger, D. (2021). Natural selection mediated by seasonal time constraints increases the alignment between evolvability and developmental plasticity. *Evolution*, *75*(2), 464–475. https://doi.org/10.1111/evo.14147

Kassen, R. (2002). The experimental evolution of specialists, generalists, and the maintenance of diversity. *Journal of Evolutionary Biology*, *15*(2), 173–190. https://doi.org/10.1046/j.1420-9101.2002.00377.x

Kefford, B. J., Ghalambor, C. K., Dewenter, B., Poff, N. L., Hughes, J., Reich, J., & Thompson, R. (2022). Acute, diel, and annual temperature variability and the thermal biology of ectotherms. *Global Change Biology*, *28*(23), 6872–6888. https://doi.org/10.1111/gcb.16453

Kellermann, V., & van Heerwaarden, B. (2019). Terrestrial insects and climate change: Adaptive responses in key traits. *Physiological Entomology*, *44*, 99–115.

Ketola, T., Kellermann, V. M., Loeschcke, V., López-Sepulcre, A., & Kristensen, T. N. (2014). Does environmental robustness play a role in fluctuating environments? *Evolution*, *68*(2), 587–594. https://doi.org/10.1111/evo.12285

Ketola, T., Mikonranta, L., Zhang, J., ... & Laakso, J. (2013). Fluctuating temperature leads to evolution of thermal generalism and preadaptation to novel environments. *Evolution*, *67*(10), 2936–2944.

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*, 2936–2944.

Kjærsgaard, A., Pertoldi, C., Loeschcke, V., & Blanckenhorn, W. U. (2013). The effect of fluctuating temperatures during development on fitness-related traits of *Scatophaga stercoraria* (Diptera: Scathophagidae). *Environmental Entomology*, *42*(5), 1069–1078. https://doi.org/10.1603/EN13074

Klepsatel, P., Girish, T. N., Dircksen, H., & Gáliková, M. (2019). Reproductive fitness of Drosophila is maximised by optimal developmental temperature. *Journal of Experimental Biology*, *222*(10), jeb202184.

Kotz, M., Wenz, L., & Levermann, A. (2021). Footprint of greenhouse forcing in daily temperature variability. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(32), e2103294118. https://doi.org/10.1073/pnas.2103294118

Le Lagadec, M. D., Chown, S. L., & Scholtz, C. H. (1998). Desiccation resistance and water balance in southern African keratin beetles (Coleoptera, Trogidae): The influence of body size and habitat. *Journal of Comparative Physiology B, Biochemical, Systemic, and Environmental Physiology*, *168*, 112–122.

Lewontin, R. C. (1974). *The genetic basis of evolutionary change*. Columbia University Press.

Lind, M. I., Berg, E. C., Alavioon, G., & Maklakov, A. A. (2015). Evolution of differential maternal age effects on male and female offspring development and longevity. *Functional Ecology*, *29*, 104–110.

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. *Journal of Evolutionary Biology*, *33*(7), 966–978. https://doi.org/10.1111/jeb.13628

Lynch, M., & Gabriel, W. (1987). Environmental tolerance. *American Naturalist*, *129*, 283–303.

MacLean, H. J., Sørensen, J. G., Kristensen, T. N., Loeschcke, V., Beedholm, K., Kellermann, V., & Overgaard, J. (2019). Evolution and plasticity of thermal performance: An analysis of variation in thermal tolerance and fitness in 22 *Drosophila* species. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, *374*(1778), 20180548. https://doi.org/10.1098/rstb.2018.0548

Magnusson, A., Skaug, H.J., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., et al. (2019). *Package 'glmmTMB. R Package Version 01*.

Matsubara, S. (2018). Growing plants in fluctuating environments: Why bother? *Journal of Experimental Botany*, *69*(20), 4651–4654. https://doi.org/10.1093/jxb/ery312

McGuigan, K., & Sgro, C. M. (2009). Evolutionary consequences of cryptic genetic variation. *Trends in Ecology and Evolution*, *24*(6), 305–311. https://doi.org/10.1016/j.tree.2009.02.001

Messina, F.J. (1990). Alternative life-histories in *Callosobruchus maculatus*: Environmental and genetic bases. In: K. Fujii, A. M. R. Gatehouse, C. D. Johnson, R. Mitchel, & T. Yoshida (Eds.), *Bruchids and legumes: Economics, ecology and coevolution* (pp. 303–315). Springer.

Messina, F. J. (1991). Competitive interactions between larvae from divergent strains of the cowpea weevil (Coleoptera: Bruchidae). *Environmental Entomology*, *20*(5), 1438–1443. https://doi.org/10.1093/ee/20.5.1438.

Mitchell, R. (1991). The traits of a biotype of *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) from South India. *Journal of Stored Products Research*, *27*(4), 221–224. https://doi.org/10.1016/0022-474x(91)90004-v

Morón Lugo, S. C., Baumeister, M., Nour, O. M., Wolf, F., Stumpp, M., & Pansch, C. (2020). Warming and temperature variability determine the performance of two invertebrate predators. *Scientific Reports*, *10*(1), 6780. https://doi.org/10.1038/s41598-020-63679-0

Nevo, E. (1978). Genetic variation in natural populations: Patterns and theory. *Theoretical Population Biology*, *13*(1), 121–177. https://doi.org/10.1016/0040-5809(78)90039-4

New, A. M., Cerulus, B., Govers, S. K., Perez-Samper, G., Zhu, B., Boogmans, S., Xavier, J. B., & Verstrepen, K. J. (2014). Different levels of catabolite repression optimize growth in stable and variable environments. *PLoS Biology*, *12*(1), e1001764. https://doi.org/10.1371/journal.pbio.1001764

Niehaus, A. C., Angilletta Jr, M. J., Sears, M. W., Franklin, C. E., & Wilson, R. S. (2012). Predicting the physiological performance of ectotherms in fluctuating thermal environments. *Journal of Experimental Biology*, *215*(4), 694–701.

Ontoria, Y., Cuesta-Gracia, A., Ruiz, J. M., Romero, J., & Pérez, M. (2019). The negative effects of short-term extreme thermal events on the seagrass *Posidonia oceanica* are exacerbated by ammonium additions. *PLoS One*, *14*(9), e0222798. https://doi.org/10.1371/journal.pone.0222798

Paaby, A. B., & Rockman, M. V. (2014). Cryptic genetic variation: Evolution's hidden substrate. *Nature Reviews Genetics*, *15*(4), 247–258. https://doi.org/10.1038/nrg3688

Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., & 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

R Core Team. (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rankin, D. J., & Arnqvist, G. (2008). Sexual dimorphism is associated with population fitness in the seed beetle *Callosobruchus maculatus*. *Evolution*, *62*(3), 622–630. https://doi.org/10.1111/j.1558-5646.2007.00315.x

Renault, D., Leclerc, C., Colleu, M. -A., Boutet, A., Hotte, H., Colinet, H., Chown, S. L., & Convey, P. (2022). The rising threat of climate change for arthropods from Earth's cold regions: Taxonomic rather than native status drives species sensitivity. *Global Change Biology*, *28*(20), 5914–5927. https://doi.org/10.1111/gcb.16338

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025

Rogell, B., Widegren, W., Hallsson, L. R., Berger, D., Björklund, M., & Maklakov, A. A. (2014). Sex-dependent evolution of life-history traits following adaptation to climate warming. *Functional Ecology*, *28*, 469–478.

Russell, L., Henrik, S., Jonathon, L., ... & Maxime, H. (2018). Estimated marginal means, aka least-squares means. *The American Statistician*, *34*, 216–221.

Savalli, U. M., & Fox, C. W. (1999). The effect of male size, age, and mating behavior on sexual selection in the seed beetle *Callosobruchus maculatus*. *Ethology Ecology and Evolution*, *11*, 49–60.

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. *Nature Communications*, *9*(1), 1719. https://doi.org/10.1038/s41467-018-03906-5

Schaum, C. -E., Buckling, A., Smirnoff, N., & Yvon-Durocher, G. (2022). Evolution of thermal tolerance and phenotypic plasticity under rapid and slow temperature fluctuations. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1980), 20220834. https://doi.org/10.1098/rspb.2022.0834

Shah, A. A., Gill, B. A., Encalada, A. C., Flecker, A. S., Funk, W. C., Guayasamin, J. M., Kondratieff, B. C., Poff, N. L., Thomas, S. A., Zamudio, K. R., & Ghalambor, C. K. (2017). Climate variability predicts thermal limits of aquatic insects across elevation and latitude. *Functional Ecology*, *31*(11), 2118–2127. https://doi.org/10.1111/1365-2435.12906

Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., Dong, Y., Harley, C. D. G., Marshall, D. J., Helmuth, B. S., & Huey, R. B. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters*, *19*(11), 1372–1385. https://doi.org/10.1111/ele.12686

Slein, M. A., Bernhardt, J. R., O'Connor, M. I., & Fey, S. B. (2023). Effects of thermal fluctuations on biological processes: A meta-analysis of experiments manipulating thermal variability. *Proceedings of the Royal Society B: Biological Sciences*, *290*(1992), 20222225. https://doi.org/10.1098/rspb.2022.2225

Stillwell, R. C., Blanckenhorn, W. U., Teder, T., Davidowitz, G., & Fox, C. W. (2010). Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: From physiology to evolution. *Annual Review of Entomology*, *55*, 227–245. https://doi.org/10.1146/annurev-ento-112408-085500

Stillwell, R. C., & Fox, C. W. (2007). Environmental effects on sexual size dimorphism of a seed-feeding beetle. *Oecologia*, *153*(2), 273–280. https://doi.org/10.1007/s00442-007-0724-0

Thompson, R. M., Beardall, J., Beringer, J., Grace, M., & Sardina, P. (2013). Means and extremes: Building variability into community-level climate change experiments. *Ecology Letters*, *16*(6), 799–806. https://doi.org/10.1111/ele.12095

Ursprung, C., Den Hollander, M., & Gwynne, D. T. (2009). Female seed beetles, *Callosobruchus maculatus*, remate for male-supplied water rather than ejaculate nutrition. *Behavior, Ecology and Sociobiology*, *63*, 781–788.

Vamosi, S., & Lesack, T. (2007). Direct effects of larval competition on development time and fecundity in seed beetles. *Evolutionary Ecology Research*, *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). *Functional Ecology*, *19*(5), 859–864. https://doi.org/10.1111/j.1365-2435.2005.01029.x

Van Tienderen, P. H. (1991). Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution*, *45*(6), 1317–1331. https://doi.org/10.1111/j.1558-5646.1991.tb02638.x

Vasseur, D. A., DeLong, J. P., Gilbert, B., Greig, H. S., Harley, C. D. G., McCann, K. S., Savage, V., Tunney, T. D., & O'Connor, M. I. (2014). Increased temperature variation poses a greater risk to species than climate warming. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1779), 20132612. https://doi.org/10.1098/rspb.2013.2612

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

Verberk, W. C. E. P., Atkinson, D., Hoefnagel, K. N., Hirst, A. G., Horne, C. R., & Siepel, H. (2021). Shrinking body sizes in response to warming: Explanations for the temperature–size rule with special emphasis on the role of oxygen. *Biological Reviews of the Cambridge Philosophical Society*, *96*(1), 247–268. https://doi.org/10.1111/brv.12653

Wang, G., & Dillon, M. E. (2014). Recent geographic convergence in diurnal and annual temperature cycling flattens global thermal profiles. *Nature Climate Change*, *4*(11), 988–992. https://doi.org/10.1038/nclimate2378

Wickham, H. (2011). "*ggplot2*." Wiley interdisciplinary reviews: computational statistics 3.2, pp. 180–185.

Zheng, J., Payne, J. L., & Wagner, A. (2019). Cryptic genetic variation accelerates evolution by opening access to diverse adaptive peaks. *Science*, *365*(6451), 347–353. https://doi.org/10.1126/science.aax1837

Downloaded from https://academic.oup.com/jeb/article/37/1/1/7459832 by University of East Anglia user on 08 September 2025