1 **Genetic and thermal variation influences adaptation to fluctuating**
2 **temperature in the seed beetle, *Callosobruchus maculatus***

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Summary

The impacts of climate change on biological systems are notoriously difficult to measure, and laboratory studies often do not realistically represent natural fluctuations in environmental conditions. To date, most experimental studies of thermal adaptation test populations at constant temperatures, or they make incremental changes to an otherwise constant mean background state. To address this, we examined the long-term effects of stressful fluctuating daily temperature on several key life history traits in two laboratory populations of the seed beetle, *Callosobruchus maculatus*. These populations were kept for 19 generations at either a constant control temperature, $T=29^{\circ}\text{C}$, or at a fluctuating daily cycle with $T_{\text{mean}} = 33^{\circ}\text{C}$, $T_{\text{max}}=40$, and $T_{\text{min}}=26$. The latter being a simple representation of daily temperatures in southern central India in May, where this species originally evolved. We found that beetles that had evolved in stressful environments were smaller in body size when switched to a constant 29°C and had far greater reproductive fitness in comparison to beetles from both the constant control and continuously stressful 33°C environments. This suggests that beetles raised in environments with stressful fluctuating temperatures were more phenotypically plastic and had greater genetic variability than control treatment beetles and indicates that populations that experience fluctuations in temperature may be better able to respond to short-term changes in environmental conditions.

45 **Introduction**

46 An increase in mean temperature negatively impacts a multitude of life history
47 traits in both plants (Hedhly *et al.*, 2009; Hatfield & Prueger, 2015) and animals
48 (Bauerfeind & Fischer, 2014; Berger *et al.*, 2017; Chen *et al.*, 2018; Lopez &
49 Adair, 2019; Ivimey-Cook *et al.*, 2021). In particular, heat stress caused by high
50 temperatures outside of a species' thermal optimum can disrupt reproduction in
51 mammals (Hansen, 2009) and fish (Lopez & Adair, 2019); inhibit growth and
52 development in plants (Hatfield & Prueger, 2015); and reduce longevity and
53 survival in insects (Berger *et al.*, 2017; Ivimey-Cook *et al.*, 2021).

54 Elevated temperatures, one of the main outcomes of rising anthropogenic
55 gas emissions (Lashof & Ahuja, 1990; Pereira *et al.*, 2012; Pachauri *et al.*, 2014),
56 can influence populations both directly, by increasing the rate of genome wide *de*
57 *novo* mutations (Berger *et al.*, 2017, 2021), or indirectly, by triggering
58 phenological mismatches between species and prey (Visser *et al.*, 1998, 2012;
59 see also Parmesan, 2006 for an extensive review). This increase in
60 environmental stress leads populations to either adapt to novel conditions through
61 phenotypic plasticity, to shift their geographic boundaries to match thermal
62 tolerance, or to go extinct (Holt, 1990; Pereira *et al.*, 2012; Hetem *et al.*, 2014;
63 Trisos *et al.*, 2020). Species in tropical habitats are predicted to be especially
64 vulnerable, as they have evolved far more narrow and specialized thermal ranges
65 (Janzen, 1967; Deutsch *et al.*, 2008; Walters *et al.*, 2012).

66 However, not only are the Earth's mean temperatures rising, but they are
67 also becoming more variable (Folguera *et al.*, 2011; Vasseur *et al.*, 2014;
68 Masson-Delmotte *et al.*, 2021). This increase in environmental variability is likely
69 to intensify the already detrimental impacts of elevated temperatures (Vasseur *et*
70 *al.*, 2014). Building on work by Deutsch *et al.* (2008), Vasseur *et al.* (2014) found
71 that changes to both the mean and variation in environmental temperature
72 produced long-term changes to population fitness. More specifically, performance

data collected from 38 globally distributed invertebrate species indicated that whilst increasing mean temperature had a mixture of both positive and negative effects on performance, which were largely latitude-dependent, increased variation was associated with more negative effects (Vasseur *et al.*, 2014). In addition, the authors highlighted the relative contribution of mean and variance in determining projected performance in the years 2050-2059. They found that a change in mean temperature explained only 32% of future species performance, whereas combining both the mean and variance simultaneously explained 93%. This highlights the importance of understanding how species react not only to changes in mean temperature, but also to changes in temperature *variation* in order to form accurate predictions of a population's response to climate change.

Studying the effects of climate warming and the resulting adaptive responses of organisms is often confined either to field studies, which normally consist of a multitude of uncontrollable ecological variables, or to laboratory studies, which have the benefit of increased experimental control but typically rely on the use of constant mean temperatures with very little consideration of natural fluctuations (Fischer *et al.*, 2011; Folguera *et al.*, 2011; Thompson *et al.*, 2013). As a result, populations could respond in an entirely different manner when exposed to fluctuating rather than constant thermal stress in the laboratory (Folguera *et al.*, 2011; Bauerfeind & Fischer, 2014). For example, reaction norms of the mosquito *Anopheles stephensi* under fluctuating thermal regimes were fundamentally different to those under constant temperatures (Paajimans *et al.*, 2013). Similarly, in the tropical butterfly *Bicyclus anyana*, life history traits were strongly influenced by both the mean and the amplitude of the experimental thermoperiod (Brakefield & Mazzotta, 1995). Indeed, the use of constant temperatures might significantly underestimate the impact of global warming on life history evolution (Fischer *et al.* 2011). These concerns clearly highlight the need to integrate more realistic thermal regimes into empirical studies (Niehaus *et*

al., 2012; Paaijmans *et al.*, 2013) in order to fully understand how species are responding to climate change (specifically increases in mean temperature and increased variability) under more natural conditions (Folguera *et al.*, 2011; Colinet *et al.*, 2015).

To this end, we investigated the long-term effects of thermal stress caused by realistically fluctuating temperatures on the phenotypic plasticity and adaptation of two laboratory populations of seed beetle, *Callosobruchus maculatus*. Two different thermal regimes were used: in one regime, beetles were kept at a constant control temperature of 29°C, and in the second regime we exposed beetles to a more stressful and realistic fluctuating daily cycle with a mean temperature of 33°C. This temperature regime was chosen because it is representative of a May day in southern India, where the laboratory lines were originally collected. The two beetle populations were then 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 several key life history traits (namely, development time, body mass and various measures of fitness).

Previous work in the same species of beetle has highlighted the negative effect of elevated temperatures on various life history traits (Rogell *et al.*, 2014; Vasudeva *et al.*, 2014; Ivimey-Cook *et al.*, 2021). In addition, a series of papers in by Hallsson and Björklund (2012) investigated how increasing temperature with and without environmental fluctuation influenced reproductive success, development time, and body size. Whilst predominantly focusing on the amount of treatment-specific genetic and environmental variance for each trait, they found that fluctuating environments had a significantly negative effect on fecundity but little to no effect on hatching success, development time and body mass. Importantly, our definition of fluctuating environments differs from theirs. Whilst

the term employed here represents 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 from 30°C to 36°C. As such, we may expect entirely different life-history responses to higher mean temperatures that are also fluctuating around that mean.

In particular, the heterogeneity of the environment an organism experiences, along with the associated costs and benefits of maintaining differing widths of thermal tolerance, can dramatically alter which genotypic strategies are favoured by selection. For instance, stabile and homogenous environments have been shown to lead to the evolution of specialists (Reboud & Bell, 1997) who reduce the width of their thermal niche in order to maximise performance. The particular direction of this specialization is largely dependent on the optimum temperature of the organism, be that hot: “Hotter is Better” hypothesis (Kingsolver & Huey, 2008, Fig. 1), or cold: “Colder is Better” hypothesis (Huey *et al.*, 1999, Fig. 1). Fluctuating environments may instead favour the evolution of generalists with increased phenotypic plasticity (or reversible phenotypic plasticity, Ratikainen & Kokko, 2019) and enhanced genetic variation (Reboud & Bell, 1997; see Table 1 and 2 from Kassen, 2002). However, this plasticity might carry a cost, for example if individuals trade off increased thermal tolerance with reduced reproductive performance (e.g. the “Jack of all Trades, Master of None” hypothesis - Huey & Kingsolver, 1989; Berger *et al.*, 2014; Fig. 1). Therefore, we would predict that populations evolving in fluctuating environments should underperform (i.e., through reduced fitness), in comparison to those in more constant benign environments. Alternatively, If phenotypic plasticity is instead beneficial and can evolve without a cost to reproduction, then beetles evolving in fluctuating environments should have higher performance (i.e. evolve to become “Jack of all Trades, Master of All” - Huey & Hertz, 1984 or “Superior Thermal

Generalists", see Berger *et al.*, 2014; Fig. 1) than those evolving in constant benign environments. As a result, the relative comparison between benign and stressful environments will largely depend on the costs and benefits of phenotypic plasticity (see Fig. 1). Lastly, individuals from a benign environment that are then *switched* to a stressful environment should suffer reduced reproductive performance owing to a lack of evolved phenotypic plasticity.

The predictions for development time and body size were more straightforward. Increases in temperature have been shown to reduce development time in many insects (Hagstrum & Leach, 1973; Nylin & Gotthard, 1998; Hallsson & Björklund, 2012b; a; Czarnoleski *et al.*, 2013). Since the mean temperature of the fluctuating environment was higher in our experiment, we predicted that individuals that evolved in, or were later placed into, the fluctuating environment would experience shorter development times. Finally, increasing mean temperature whilst within fluctuating environments has been shown to decrease body size in insects (Nylin & Gotthard, 1998; Kingsolver & Huey, 2008; Hallsson & Björklund, 2012a; b; Czarnoleski *et al.*, 2013). Many organisms, particularly insects, experience faster growth at higher temperatures; under these conditions, decreasing cellular size and thus body size would allow for an optimum rate of resource exchange (Czarnoleski *et al.*, 2013). Therefore, we predicted that body size should be smaller in the populations that evolved in, or were placed into, the fluctuating environment.

To investigate potential population-level differences we used two different strains of *C. maculatus*, derived from USA and Leicester. 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 strains of *C. maculatus* (Rankin & Arnqvist, 2008). Lastly, we also investigated relative differences between the two sexes, as males and females in this species can vary in their response to

stressful environments (Stillwell & Fox, 2007; Rogell *et al.*, 2014). Previous research on this species has shown that sexual dimorphism in traits such as body size at emergence decreases when beetles are gradually exposed to novel, higher temperatures over 18 generations (Hallsson & Björklund, 2012b; 2012a). We therefore predicted that any differences between sexes would similarly decrease in populations exposed to more stressful, fluctuating temperatures.

Figure 1. Hypotheses regarding reproductive performance and phenotypic plasticity and the evolution of generalist or specialist strategies.

Methods

Study system

C. maculatus is an agricultural pest that originates from Africa and Asia but is currently found throughout the world. Adult seed beetles are facultatively aphagous – that is, 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 two

weeks but adults with access to nutrients can live three 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).

Laboratory evolution lines and climatic conditions

In our experiment, we used two different strains of *C. maculatus*, South India (SI) USA and SI Leicester, both derived from a population of beetles collected in Tirunelveli, India, in 1979 (Mitchell, 1991) and then subsequently raised at the University of Kentucky, USA, and the University of Leicester, UK (from 1992), respectively (Fricke & Arnqvist, 2004). We obtained these strains from Uppsala University in Sweden and maintained them at the American University of Paris for approximately 25 generations before establishing the replicates for the current experiment. 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. They were housed at aphagy in 1L jars with 250g of beans, and approximately 250-350 newly hatched beetles were transferred to new jars with fresh beans every 24 days on a continual basis.

For 19 generations prior to the experiment, we subdivided SI USA and SI Leicester populations (Fig. 2A) into four replicates each. Two “Control” replicates of each were maintained at a constant temperature of 29°C, while two “Stress” replicates were subject to daily temperature cycle (Fig. 2B). The daily cycle consists of 12 separate 2hr periods of constant temperature T_i ,

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

where $T_{mean} = 33^{\circ}\text{C}$, $\Delta T = 7^{\circ}\text{C}$, and $i = 0, 1, \dots, 11$. This is a stepwise sinusoidal temperature cycle with $T_{max}=40$ and $T_{min}=26$ and which mimics typical late spring condition in Southern India, where this species evolved. Humidity and light cycles remained the same for all replicates: 50% relative humidity, and a 12:12 h light:dark cycle.

Prior to the assays of body size, development time, and reproductive fitness (see next section), we created two replicates of two populations (SI USA and SI Leicester; see Fig. 2A/B) for:

1. beetles adapted to and assayed at Control conditions (**USA-C** and **LEI-C**);
2. beetles adapted (for one year) to and assayed in Stress conditions (**USA-S** and **LEI-S**);
3. beetles adapted to Control conditions that were then acclimatized to Stress conditions for two generations without selection prior to being assayed (**USA-C-to-S** and **LEI-C-to-S**); and
4. beetles adapted to Stress conditions that were acclimatized to Control conditions for two generations without selection prior to being assayed (**USA-S-to-C**, **LEI-S-to-C**).

To separate genetic adaptation from phenotypic plasticity – that is, to control for the influence of parental effects – we created “acclimatized” treatments by allowing beetles from one set of conditions to adjust to the new set of conditions for two generations prior to assays (C-to-S and S-to-C; Fig. 2B). Critically, to remove the possible effects of natural selection and 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. That is, we removed the effects of assortative mating.

For the Stress-to-Control (USA S-to-C, LEI S-to-C) and the Control-to-Stress (USA C-to-S, LEI C-to-S) treatments, the following steps were performed. First, we

255 moved fertilized beans from jars into 48-well virgin chambers. When enough males
256 and females had hatched (from day 21-24 after hatch), we paired 60 males and 60
257 females in 60 60-mm petri dishes with 100 beans in each dish and placed these
258 dishes in the opposing chamber. We made sure that there were sufficient number of
259 beans in each dish for females to lay just one egg per bean, thereby eliminating
260 competition among multiple larvae within a bean. To further reduce larval
261 competition, we removed adult beetle pairs from the petri dishes after 72 hours.

262 After 19 days, we then choose the 50 petri dishes with the most fertilized
263 beans and put 48 fertilized beans from each petri dish into individual marked 48-well
264 virgin chambers. One day after eclosion, we randomly selected and paired 50
265 females and 50 males and put them into 50mm petri dishes with 80 beans. After 72
266 hours, the adult males and females were removed from the petri dishes. After 19
267 days, fertilized beans from each petri dish were transferred into 48-well virgin
268 chambers. We then marked the date of hatch and sex of all offspring and conducted
269 assays of development time, body mass, and reproductive fitness.

270 For the continuous C and S treatments, we skipped the acclimatization steps
271 and simply moved fertilized beans to virgin chambers prior to hatch, selecting one-
272 day old males and females for all subsequent assays.

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A

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B

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Figure 2. (A) A schematic diagram of the four temperature treatments (Control, Stress, Control-to-Stress, and Stress-to-Control) across both the USA (SI USA) and Leicester (SI LEI) populations. (B) A representation of the four different temperature treatments used. The black bar in the bottom two treatments, denotes a switch from one treatment to another in order for acclimatization to occur over two generations prior to assaying.

Body mass assay

For each of the 16 populations, we weighed male and female beetles separately in two batches of 12 beetles each using an Ohaus Pioneer Plus Analytical Balance (Model PA214C). All virgin chambers were weighed empty, prior to adding the beetles to be weighed. We then calculated the difference between the weight of the virgin chamber before the beetles were placed inside and the weight of the virgin chamber after the beetles were placed inside. 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. We paired 50 1-day old male and female beetles and placed them into 60mm petri dishes filled with 85 beans. After 24 hours, each pair was moved together into a new 60mm 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, all the fertilized beans 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+). To calculate individual fitness (or λ_{ind}), we took the dominant eigenvalue (using the popbio v. 2.7 package, (Stubben & Milligan, 2007) from an age-structured Leslie matrix (Leslie, 1945) where the top row denotes fertility and the sub-diagonal represents survival from age t to $t+1$. 24 days of development time were added prior to day 0-2+ fertility schedule.

Statistical analyses

All statistical analyses were performed in R v. 4.1.0 (R Core Team, 2016) using glmmTMB v. 1.0.2.9000 (Brooks *et al.*, 2017; Magnusson *et al.*, 2019) with pairwise interactions and slopes compared using emmeans v. 1.6.1 (Lenth *et al.*, 2019). Ggplot2 v. 3.3.4 (Wickham, 2009) was used for all graphical visualization. The overall effects of each variable were identified using the Type 3 Anova function from car v. 3.0-10 package (Fox *et al.*, 2012).

For both development time, body mass, and individual fitness, a Gaussian linear mixed effects model was fitted (after log-transforming development time to reduce right-skew) with the fixed effects of Population, Treatment, Sex (for development time and body mass), and all higher order interactions. In addition, the fixed factor of replicate number and, for development time, the random effect of parental ID was added to account for potential pseudoreplication. If the three-way interaction was non-significant (identified from the Anova function), it was removed in order to reduce model complexity. A generalized linear mixed effects model with Poisson distribution and similar fixed effect structure was fitted for LRS and age-specific reproduction, albeit without the variable Sex and all subsequent two/three-way interactions. In addition to a covariate of Day (and all higher-order interactions with Population and Treatment), a random effect of individual ID was added to the age-specific reproduction model to account for repeated measures. Both models were checked for overdispersion and zero-inflation (ZI) using DHARMa v. 0.4.1,

(Hartig, 2020) by simulating the residuals of a Poisson model. If significant, a variety of error distributions with/without zero-inflated parameters (if ZI was identified) were then fitted and the best model identified by Akaike's Information Criterion (AIC).

Results

Body mass

Individual body mass was significantly affected by both of the interactions involving Sex (Sex and Population: $\chi^2 = 25.95$, $p < 0.001$; Sex and Treatment: $\chi^2 = 19.18$, $p < 0.001$; Table S1A) but not by the remaining two-way interaction of Population and Treatment ($\chi^2 = 2.51$, $p = 0.47$) or the three-way interaction ($\chi^2 = 6.37$, $p = 0.09$). Females were generally heavier than males (Fig. 3) in both populations. The difference was more pronounced in the USA population (Average body mass (g): F-LEI = 0.087, M-LEI = 0.075, estimate = 0.013, $p < 0.001$; F-USA = 0.089, M-USA = 0.068, estimate = 0.021, $p < 0.001$; Fig. 3, Tables S1A-B). The females of both populations were similar in weight, but the USA males were smaller (F-LEI = 0.087, F-USA = 0.089, estimate = -0.002, $p = 0.35$; M-LEI = 0.075, M-USA = 0.068, estimate = 0.007, $p < 0.001$; Fig. 3, Tables S1A-B). The relative difference between male and female weight was largely dependent on temperature treatment (Fig. 3). Overall, beetles raised in the S-to-C regime were smaller for both sexes in comparison to other treatments (all comparisons significant to $p = 0.05$; Tables S1A-B). However, the difference between male and female weights was far less pronounced in the constant stress (S) treatment (albeit all differences were still significant to $p < 0.001$; F-S-to-C = 0.083, F-S = 0.089, F-C-to-S = 0.091, F-C = 0.089; M-S-to-C = 0.064, M-S = 0.079, M-C-to-S = 0.071, M-C = 0.071; Tables S1A-B).

Figure 3. Average body mass of individuals from the Leicester (Red) or USA (Blue) populations in the C (Control), C-to-S (Control-to-Stress), S (Stress), or S-to-C (Stress-to-Control) temperature treatments. Sex is denoted with either circle (Female) or triangle (Male) points. Points represent mean values with accompanying standard errors. Half violins show the distribution of raw data.

Development time

Development time was influenced by both two-way interactions involving

Temperature treatment (Treatment and Population: $\chi^2(3) = 105.94$, $p < 0.001$;

365 Treatment and Sex: $\chi^2(3) = 11.76$, $p = 0.008$). Similar to body mass, no detectable
366 three-way interaction was found ($\chi^2(3) = 1.71$, $p = 0.635$). The USA and Leicester
367 populations responded differently to the four temperature treatments, with beetles
368 from the Leicester population developing faster on average. Populations from USA
369 took significantly longer to develop in comparison to populations from Leicester in
370 both the Control and S-to-C Treatments (Average Development Time (Days): LEI-C
371 = 23.7, USA-C = 24.7, Ratio = 0.960, $p < 0.001$; LEI-S-to-C = 23.1, USA-S-to-C =
372 24.1, Ratio = 0.961, $p < 0.001$; Fig. 4) but not in C-to-S (LEI-C-to-S = 23.0, USA-C-to-
373 S = 23.0, Ratio = 0.999, $p = 1.00$) or S Treatments (LEI-S = 22.5, USA-S = 22.3,
374 Ratio = 1.01, $p = 0.38$; Fig. 4, Tables S2A-B). Despite these differences, both
375 populations in the S treatment developed faster compared to other temperature
376 treatments (LEI-S = 22.5, LEI-S-to-C = 23.1, LEI-C-to-S = 23.0, LEI-C = 23.7; USA-S
377 = 22.3, USA-S-to-C = 24.1, USA-C-to-S = 23.0, USA-C = 24.7). In addition, whilst
378 males typically developed faster in both populations (Fig. 4), only in the S-to-C
379 treatment was this difference significant (F-S-to-C = 23.7, M-S-to-C = 23.50, Ratio =
380 1.008, $p < 0.001$; Fig. 4, Tables S2A-B). However, both males and females in the S
381 treatment developed faster than other temperature treatments and showed no sex
382 difference in development time (F-S = 22.4, F-S-to-C = 23.7, F-C-to-S = 23.0, F-C =
383 24.2; M-S = 22.4, M-S-to-C = 23.5, M-C-to-S = 22.9, M-C = 24.1).

Figure 4. Average development time in days of individuals from the Leicester (Red) or USA (Blue) populations in the C (Control), C-to-S (Control-to-Stress), S (Stress), or S-to-C (Stress-to-Control) temperature treatments. Sex is denoted with either circle (Female) or triangle (Male) points. Points represent mean values with accompanying standard errors. Half violins with associated jittered points show the distribution of raw data.

Lifetime reproductive success

LRS was significantly influenced by the interaction between Population and Treatment ($\chi^2(1) = 12.61, p = 0.005$). Across all temperature treatments, LRS was

395 lower in the USA population than in the Leicester population (Average LRS: LEI =
396 53.2, USA = 40.2, Ratio = 1.32, $p < 0.001$; Fig. 5, Tables S3A-B). The largest
397 differences between populations occurred in the C-to-S and C treatments (LEI-C-to-S
398 = 50.2, USA-C-to-S = 33.6, Ratio = 1.49, $p < 0.001$; LEI-C = 52.6, USA-C = 37.2, Ratio
399 = 1.41, $p < 0.001$; Fig. 5, Tables S3A-B). Despite these differences, LRS was highest
400 in the S-to-C treatment for both populations (LEI-C = 52.6, LEI-S-to-C = 69.7, Ratio =
401 0.75, $p < 0.001$; USA-C = 37.2, USA-S-to-C = 60.9, Ratio = 0.61, $p < 0.001$; Fig. 5,
402 Tables S3A-B).

403
404 **Figure 5.** Average lifetime reproductive success (LRS) of individuals from the
405 Leicester (Red) or USA (Blue) populations in the C (Control), C-to-S (Control-to-

Stress), S (Stress), or S-to-C (Stress-to-Control) temperature treatments. Points represent mean values with accompanying standard errors. Half violins show the distribution of raw data.

Age-specific reproduction

The interaction between population, treatment and day significantly influenced age-specific reproduction ($\chi^2(3) = 203.54, p < 0.001$, Fig. 6). Whilst continuous C and S treatments exhibited similar age-specific patterns across both populations (Fig. 6), there were striking differences between S-to-C and C-to-S treatments. This was particularly apparent on Days 1 and 3 of reproduction (Fig. 6). Individuals from both LEI-S-to-C and LEI-C-to-S treatments exhibited far greater Day 1 reproduction than all other treatments from both populations (including the analogous S-to-C and C-to-S treatments). In stark contrast, USA-S-to-C exhibited increased reproduction *after* Day 1, with the result that Day 3 of reproduction was comparable with the Day 1 reproduction for LEI S-to-C (Fig. 6). This variation in reproductive schedule resulted in large differences in age-specific slopes between the USA S-to-C and LEI S-to-C treatments (Average change in average offspring produced per day: LEI S-to-C = -7.14, USA S-to-C = 8.78, estimate = -15.92, $p < 0.001$; Fig. 6, Tables S4A-B).

Figure 6. Age-specific reproduction (LRS) of individuals from the Leicester (dash-dot) or USA (solid) populations in the C (Control, Red), C-to-S (Control-to-Stress, Green), S (Stress, Blue), or S-to-C (Stress-to-Control, Purple) temperature treatments. Points with error bars represent mean values with accompanying standard errors. Jittered points represent raw data.

Individual fitness

Whilst the individual main effects of Population and Temperature treatment

significantly influenced λ_{ind} ($\chi^2(1) = 81.02, p < 0.001, \chi^2(3) = 111.23, p < 0.001$,

434 respectively; Fig. 7), the interaction between these two did not ($\chi^2(3) = 7.64, p =$
 435 0.054). In a similar manner to LRS, the USA population had reduced λ_{ind} over all
 436 temperature treatments (Average λ_{ind} : LEI = 1.164, USA = 1.146, estimate = 0.018,
 437 $p < 0.001$; Fig. 7, Tables S5A-B). Across both populations, individuals from the S-to-C
 438 treatment had the highest λ_{ind} (C = 1.154, C-to-S = 1.148, S = 1.146, S-to-C =
 439 1.173, all comparisons $p < 0.001$; Tables S5A-B). This was also consistent when
 440 looking within each population (all comparisons $p < 0.001$).
 441

442 **Figure 7.** Fitness (Lambda) of individuals from the Leicester (Red) or USA (Blue)
 443 populations in the C (Control), C-to-S (Control-to-Stress), S (Stress), or S-to-C

(Stress-to-Control) temperature treatments. Points represent mean values with accompanying standard errors. Half violins show the distribution of raw data.

Figure 8. Hypotheses and subsequent results (indicated with ticks and crosses) regarding reproductive performance and phenotypic plasticity and the evolution of generalist or specialist strategies.

Discussion

Previous hypotheses (Lewontin, 1974; Nevo, 1978) have suggested that evolution in fluctuating environments should select for genetically variable individuals that are robust to a wide range of thermal conditions, often termed generalists (Kassen, 2002). In this experiment, generalists exposed to novel benign conditions (S-to-C, Fig. 8) (although not those exposed to continuously stressful environments, S, see below) had higher reproductive fitness (higher LRS and λ_{ind}) than specialists that were exposed to novel stressful conditions (C-to-S) or kept continuously in a benign environment (C). These results are broadly in line with previous research conducted

in the opportunistic bacterial pathogen, *Serratia marcescens* (Ketola *et al.*, 2013). Heterogenous environments selected for bacterial clones that 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 benign environments (Ketola *et al.*, 2013). However, results from a digital evolution experiment have suggested that this may only allow for optimization in short-term rather than long-term evolvability (Canino-Koning *et al.*, 2019).

We found that individuals within stressful fluctuating environments evolved strategies similar to thermal-tolerance generalists as well as performance specialists (Gilchrist, 1995). More specifically, individuals were able to tolerate and survive at wide thermal breadth, presumably owing to the evolution of phenotypic plasticity, but they reached maximal performance and experienced increased LRS only when conditions were optimal (Gilchrist, 1995; Ketola *et al.*, 2013). This 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). Similarly, this release of cryptic genetic variation could explain why individuals that were predicted to perform poorly when switching from a benign control to stressful fluctuating environment (C-to-S), suffered no fitness cost. However, the relative merits of this form of variation have been queried and require additional empirical testing (McGuigan & Sgro, 2009; Edwards & Yang, 2021; Johansson *et al.*, 2021). Regardless, these results suggest that the evolution of phenotypic plasticity in experiment did not carry substantial fitness costs.

When comparing body mass across treatments, individuals from the S-to-C group were significantly smaller (across both populations and sexes), despite having similar development times to those of the constant control group. Smaller body size could therefore be a cost of adaptation (Kassen, 2002; Ketola *et al.*, 2013) to the

fluctuating conditions that is only detected in the benign environment and is manifested in reduced body size (albeit with no negative effects on fitness). However, whether this reduction in mass is a disadvantage is difficult to determine. Large size provides a fitness advantage due to sexual selection (Andersson & Iwasa, 1996), whereas small size decreases larval competition (Messina, 2004). Interestingly, similar to Hallsson & Björklund (2012a), we found that sexual dimorphism in adult body size decreased in the continuously stressful environment (S), although this could be considered population-specific as individuals from USA failed to show such a decrease.

Lastly, there were consistent population-level differences across all environmental treatments. In general, beetles from the USA population produced fewer offspring, took longer to develop (although this difference disappeared under stressful conditions), and had greater sexual dimorphism in body size compared to individuals from the Leicester population. Such genotypic variation between populations of the same species (particularly in *Callosobruchus 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 *et al.*, 2004a; b). However, in this experiment, one particular observation stood out. Whilst a switch from a stressful environment to a benign regime (S-to-C) was associated with an increase in LRS and λ in both USA and Leicester populations, there was a noticeable difference in the age-specific reproductive schedules between the USA and Leicester populations. Leicester beetles produced more offspring on the first day and fewer on days two and three, whilst in the USA population the opposite was observed. Why such a difference in reproductive schedule exists deserves deeper investigation. If genetic variance differed between populations, this may explain why Leicester individuals were able to respond more quickly, with greater Day 1 reproduction. This could also in part explain why development time was similar across all experimental

temperature regimes for the Leicester population. 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 population-specific.

Successfully mimicking natural conditions within the laboratory is key to understanding how species will react to climate change. As temperatures increase and become more variable, the incorporation of more realistic conditions into laboratory studies such as these will allow us not only to predict more accurately how wild species will respond to climate change, specifically thermal stress, but also to produce accurate species- and population-specific thermal reaction norms (Paaijmans *et al.*, 2013). This study highlights the need to include both constant and more variable conditions in laboratory studies and suggests that the evolution of generalist and specialist strategies will be vital in explaining species-specific responses to climate change.

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