



Experimental evidence for stronger impacts of larval but not adult rearing temperature on female fertility and lifespan in a seed beetle

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Abstract

Temperature impacts behaviour, physiology and life-history of many life forms. In many ectotherms, phenotypic plasticity within reproductive traits could act as a buffer allowing adaptation to continued global warming within biological limits. But there could be costs involved, potentially affecting adult reproductive performance and population growth. Empirical data on the expression of reproductive plasticity when different life stages are exposed is still lacking. Plasticity in key components of fitness (e.g., reproduction) can impose life-history trade-offs. Ectotherms are sensitive to temperature variation and the resulting thermal stress is known to impact reproduction. So far, research on reproductive plasticity to temperature variation in this species has focused on males. Here, I explore how rearing temperature impacted female reproduction and lifespan in the bruchid beetle *Callosobruchus maculatus* by exposing them to four constant temperatures (17 °C, 25 °C, 27 °C and 33 °C) during larval or adult stages. In these experiments, larval rearing cohorts (exposed to 17 °C, 25 °C, 27 °C and 33 °C, from egg to adulthood) were tested in a common garden setting at 27 °C and adult rearing cohorts, after having developed entirely at 27 °C, were exposed to four constant rearing temperatures (17 °C, 25 °C, 27 °C and 33 °C). I found stage-specific plasticity in all the traits measured here: fecundity, egg morphological dimensions (length and width), lifespan and egg hatching success (female fertility). Under different larval rearing conditions, fecundity and fertility was drastically reduced (by 51% and 42%) at 17 °C compared to controls (27 °C). Female lifespan was longest at 17 °C across both larval and adult rearing: by 36% and 55% compared to controls. Collectively, these results indicate that larval rearing temperature had greater reproductive impacts. Integrating both larval and adult rearing effects, I present evidence that female fertility is more sensitive during larval development compared to adult rearing temperature in this system.

Keywords Female fertility · Global climate change · Lifespan · Ova · Plasticity · Reproduction

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Introduction

Environmental temperature has profound effects on all aspects of biology (Colinet et al. 2015). With the current global climate change models predicting definitive changes to rising average temperatures, it is likely that biological processes and functions will diverge drastically for some ectothermic species such as insects (Colinet et al. 2015; IPCC 2022). Insects are found across many regions of our planet, delivering key ecosystem services, and their biology (e.g., reproduction) is closely linked to their environmental temperature (Colinet et al. 2015). Therefore, increasing environmental temperatures (averages, maxima and variation) can influence the abundance and distribution of insects (Angilletta 2009). Exciting recent work on *Drosophilids* (across 43 species) showed that sub-lethal thermal limits on insect fertility (TFL) explain species distribution better than lethal limits (CT_{max}), these CT_{max} models have been extensively used to map global insect distributions (Parratt et al. 2021). Other examples across taxa document how thermal variation induces adaptive plasticity for mitochondrial function in a fly and a copepod (Pichaud et al. 2010; Harada et al. 2019), behaviour in a butterfly (Pateman et al. 2012), phenology across terrestrial and marine ecosystems (Walther et al. 2002) and sex determination in a reptile (Warner and Shine 2008), population persistence across plant and animal populations (Reed et al. 2011), and invasion ecology in taxonomically distinct species (Kelley 2014; Chown et al. 2015).

Phenotypic plasticity is a well-known process that allows individuals and populations to express different environment-specific phenotypes in response to the changing conditions they experience (Stillwell and Fox 2005; Hallsson and Björklund 2012; Rodrigues and Beldade 2020). Established examples of environment-specific phenotypic plasticity can reflect the underlying physiological, biochemical state of the organism (Stearns 1989) and that plasticity can either have a genetic (past selection, longer term) or a non-genetic basis (within- and across-generation plasticity) in phenotypic modification (Stillwell and Fox 2005). Adaptive genetic mechanisms can be slow to attain compared to rapid acclimatory plastic responses (West-Eberhard 2005). Although such rapid acclimatory plastic responses can be energetically costly, it may provide the organism with a coping mechanism in shorter timescales, as evolutionary responses operate on much longer timescales (Angilletta 2009; Sgrò et al. 2016). Studies investigating the significance of plasticity usually focus on a single environmental factor of interest (Stillwell et al. 2007) and conclude that the within-generation plastic responses are adaptive without explicit tests for its adaptive value. However, some organisms such as insects live in ecologically complex environments where conditions fluctuate drastically and continuously in shorter- and longer-timescales with varied consequences on the different stages (Stillwell and Fox 2005; Stillwell et al. 2007). As a result, selection pressures on different life stages (e.g., larvae, pupae, adults) may respond continuously to the environment giving rise to environment specific phenotypic expression (Colinet et al. 2015; Sales et al. 2021). For example, in Sales et al. (2021) *Tribolium castaneum* late instar larvae were less sensitive to a 40 °C or 42 °C heat stress compared to pupae or immature adults for survival and reproductive output.

Plasticity in reproductive form and function within some taxa has been extensively studied as it is often thought to be more strongly affected by temperature (Blanckenhorn and Hellriegel 2002; Sales et al. 2018; Parratt et al. 2021; van Heerwaarden and Sgrò 2021; Schou et al. 2021; Rodrigues et al. 2022a). Also, it has been known that gamete form and function shows remarkable plasticity to developmental temperature (Blanckenhorn and Hellriegel 2002; Minoretti et al. 2013; Breckels and Neff 2014; Vasudeva et al. 2014, 2019; Iossa et al. 2019). Further, the evolutionary consequences

of plasticity within gametic investment has broader consequences for individual fitness and the evolution of polyandry (Vasudeva et al. 2021b). Thermal plasticity within male reproductive form and function (e.g., reproductive output) is also environment-specific and can express different phenotypes (~7% shorter sperm) within a single generation (Vasudeva et al. 2019). In *T. castaneum*, thermal plasticity within gametes was adaptive in a manner that gamete form and function operating within matched developmental environments showed better reproductive outcomes (Vasudeva et al. 2019). This directional plasticity could be due to physiological optimisations occurring during development that enhance gametic function, providing overall better reproductive performance within a permissible thermal range for a recent review, (see Wang and Gunderson 2022). Such developmental optimisations within a male's ejaculate can have evolutionary consequences under global warming scenarios, as sperm almost always function in a non-self environment such as the female reproductive tract (Pitnick et al. 2020).

Investment into the production of eggs (ova) remain poorly studied in response to different developmental temperatures. Ova too face many challenges, as in many oviparous invertebrates, females oviposit in a natural environment where eggs hatch and the zygotes and embryos are further subject to continuous stage-specific developmental variation (Hinton 1981; Vasudeva et al. 2019). Such stage-specific plasticity can be important and needs to be captured, as selection might operate differently on ova (see, Stillwell et al. 2007). A few studies have shown that rearing environment affects female investment into egg production and its fitness consequences (e.g., egg size, egg number and hatching success); cooler oviposition temperatures resulted in females producing larger eggs in a tropical butterfly (*Bicyclus anynana*) and that the patterns were determined by the prevailing environmental conditions (Fischer et al. 2003a, b; Geister et al. 2009). In another butterfly species, the migrant skipper (*Parnara guttata guttata*), rearing temperature of immature stages (first instar) resulted in plastic egg sizes, with larger eggs at 25 °C and 27 °C than those reared at 20 °C and 22.5 °C (Seko and Nakasuji 2006). Developmental optimisation within the ova form and function could further select for specific phenotypes that respond in a manner optimising fitness as egg size is closely linked to offspring fitness (Azevedo et al. 1996; also see, Vasudeva et al. 2019). Therefore, the environmental conditions under which females' adjust investment into eggs is of broad interest in ecology and evolution, especially studying the mechanisms that enhance female and offspring fitness (e.g., Azevedo et al. 1996).

Previous work using *Callosobruchus maculatus* beetles showed that rearing temperatures affected male investment into reproductive traits and its function, mating behaviour and the timing of sensitivities into male gametic expression (Vasudeva et al. 2014, 2018, 2021a). Here, I focus on adult female reproductive investment either when larval or adult stages have been exposed to four (species-specific) biologically relevant constant temperatures (17 °C, 25 °C, 27 °C and 33 °C). These temperature environments were chosen as they occur naturally within the range of these beetles (Germain et al. 1987). I assessed the impacts of larval versus adult rearing on female reproduction and lifespan. I hypothesised that the stages exposed here (larvae or adults) will have trait specific responses to temperature. Using this set up I investigate, a) the impacts larval and adult rearing on female reproduction (fecundity) and the extent of temperature-induced plasticity overall and through time, b) the nature of plasticity within egg morphology (dimensions: length and width), c) plasticity in lifespan (intrinsic ageing) and finally, d) plasticity in egg hatchability (female fertility).

Materials and methods

Study species and maintenance

The strain of bruchid beetle (*C. maculatus*) used in this study were from a stock sourced from Naimey, Niger reared and then maintained in the lab on black-eyed beans (*Vigna unguiculata*) in a controlled environment [standard conditions: 27 °C (± 0.5 °C), 32% relative humidity and 16L:8D (Light:Dark) photoperiod] under nonoverlapping generations for > 10 years (each year ~10–12 generations are attained under these conditions). From this, ~5000 randomly selected adults (estimated by mass, Vasudeva 2014; Vasudeva et al. 2021a), aged between 3–5 days old post eclosion, were sourced at random to establish the moth bean stocks (MB hereafter). The MB stock was created by housing ~5000 adults on 200 g (7000–7500 bean pods) of moth beans (*Vigna aconitifoli*) for an hour to mate randomly and oviposit within Petri dishes (dimensions: 150 mm diameter \times 15 mm depth, Thermofisher, Triple vent) under standard rearing conditions [27 °C (± 0.5 °C), 32% relative humidity and 16L:8D (Light:Dark)]. This MB stock was maintained this way for 24 generations (~2 years of laboratory selection) prior to experimental tests. After 3–5 days of oviposition on fresh beans, the ovipositing adults were sieved using a mechanical sieve (Endecotts, London, UK; stainless steel sieve, aperture size: 1.5 mm) and frozen at -5 °C. Egg-laden moth beans were returned to standard conditions for completion of further development (Vasudeva 2014).

Moth beans was selected as the host seed to control for adult density, described previously (see, Vasudeva et al. 2014, 2018 and 2021). Egg-laden beans were placed into eight triple vent petri dishes (110 mm diameter) and prior to adult eclosion.

Temperature exposures and set up

Experimental set up

The biological replication was carried out in temperature-controlled incubators across four constant temperatures: 17 °C, 25 °C, 27 °C and 33 °C (± 0.5 °C, Lucky Reptile Herp Nursery II, The Reptile Shop, UK). Control temperature for these assays were 27 °C (Vasudeva 2014; Vasudeva et al. 2021a, b). The incubators were housed within the insectary, maintained under standard conditions (see previous section) and were assigned randomly to the different temperature environments between experimental exposures studied here. Only females were exposed to larval or adult rearing temperatures, males paired with the treated females were acquired from the MB stock population. The study animals were maintained as per the principles and standards for good ethical practise in research (Vasudeva et al. 2021a).

Experiment 1: Larval rearing temperatures

Approximately 1000 randomly chosen sexually mature adults (3–5 days old) were housed in with 300 g of fresh moth beans for an hour in Petri dishes. The adults were sieved out after an hour and the egg-laden beans were split into two roughly equal parts. One part of the egg-laden beans was distributed among the four different larval rearing temperatures; 17 °C, 25 °C, 27 °C (control) and 33 °C (Fig. 1A) and the other

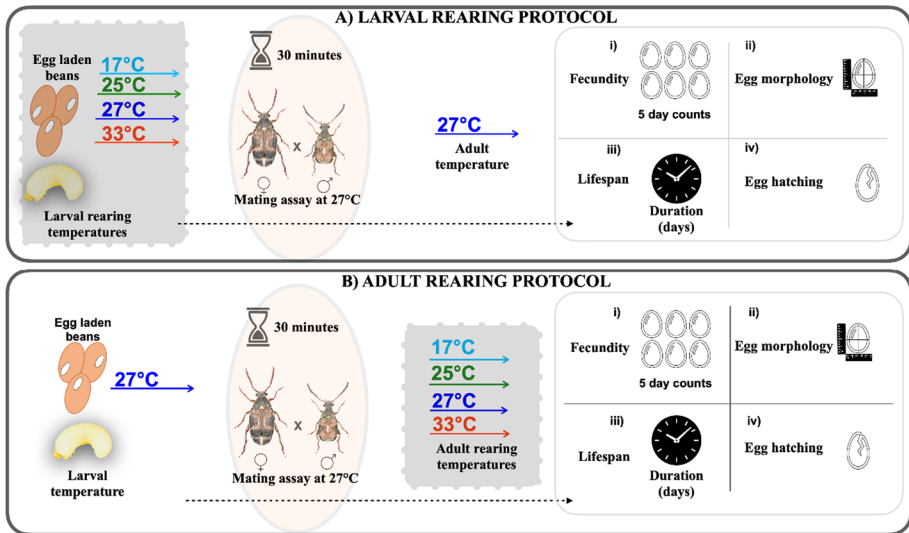


Fig. 1 Describes the scheme for larval and adult rearing thermal environments. Shaded boxes in grey within each protocol (A=larval, B=adult rearing) indicate when during the beetle's life cycle the temperature exposures took place and the traits measured (in boxes). The larval and adult temperatures were 17 °C, 25 °C, 27 °C (control) and 33 °C. Within each protocol, fecundity (egg counts) through time and total, egg morphology (length and width in mm), lifespan (intrinsic ageing rates measured as days until death) and hatching success (proportion of eggs that hatched) were recorded after females were allowed to pair up with a male (for 30 min) from the control stock (reared on Moth beans, maintained at 27 °C, 32% RH of a standard age). Dotted arrows within both assays for lifespan (iii) were conducted on virgin age-matched females, for other assays (i, ii & iv) singly mated females were used

part of egg-laden beans was allowed to continue developing at 27 °C for studying adult rearing temperatures (see next section). Developing larvae exposed to these rearing temperatures were allowed to complete development (egg to adulthood). Prior to adult eclosion from the beans, dark windows appear just under the seed coat and the seeds are then isolated to collect virgin adult beetles of a standard age for the assays. To ensure virginity, a single egg-laden bean was placed within a single cell of a 25-cell square petri dish (Thermofisher, UK) and sealed with a glass lid. If more than one adult beetle was present within a cell, these were discarded. All adults sourced for this study were 24–48 h post-eclosion at the time of the mating assays (took place at 27 °C) and treated females were paired with a control male from the MB population of a similar age (maintained at 27 °C).

Post-eclosion, the females remained in their larval rearing environment in single sex groups within the cells of the 25-cell square petri dish. Mature females were (aged 24–48 h) moved to 27 °C for an hour within an insectary to attain thermal equilibrium before being paired with a male in a clear Perspex petri dish (50 mm diameter) that was sourced from the MB stock population. Copulatory behaviour of the temperature groups was observed for 30 min, and the outcome of behaviours have been reported elsewhere (Vasudeva et al. 2018). After successful copulations, the males were discarded, and the temperature treated females were allowed to oviposit in 1 g of fresh moth beans (50–75 bean pods in a petri dish) at 27 °C through five consecutive one-day blocks (5 × one-day blocks). Females were provided a fresh set of (1 g) moth beans to oviposit in a clean Petri dish every 24 h. Females

were randomised within each of the temperature treatments across both 'larval' and 'adult' rearing experiments to allow for standardisation of egg laying day and age at reproduction so that all individuals were treated similarly before data collection. This allowed me to collect egg-laden beans every 24 h for each temperature treatment group; these egg batches were labelled and returned to the insectary. As most eggs are laid within the first 5 days (where no rematings took place), females were removed from the petri dishes and were frozen at -5°C for measuring body size. The egg-laden beans were frozen to record egg counts after about one to two weeks depending on the rearing temperature. I collected female fecundity (egg output), egg morphology over five consecutive days as this was logistically feasible and output declined naturally as the females aged. Successful eclosion is indicated by the eggs appearing white as the larvae burrow into the pod (or the eggs appears to be clearly turbid if failed to hatch which is visible on the pod it is laid on, the duration for this to occur depends on rearing temperature, it is longest at 17°C appearing within two weeks and shortest for 33°C about three days after oviposition). Under standard insectary conditions at 27°C , $\sim 96\%$ (115/120) of the eggs that turned white resulted in an adult beetle when reared on moth beans (unpublished data).

Experiment 2: Adult rearing temperatures

Individual egg-laden beans maintained under the standard insectary conditions from egg to adulthood at 27°C were placed into cells within 25-cell square petri dishes as described above (one egg-laden bean per cell). Adult eclosion was checked every few hours and upon attaining 24–48 h old post eclosion, the females were paired with a male from the stock population at 27°C prior to being placed in the different rearing temperatures. Post mating, males were removed from the mating arena and the females were randomly assigned to one of the four adult rearing temperatures, similar to the set up described above.

Trait measurements

- (a) Fecundity, eggs were laid on the seed coat of moth beans and became clearly visible after 3–7 days at 25°C , 27°C and 33°C (10–14 days at 17°C) with a characteristic oblate spheroid in shape (Messina 1991). Eggs were counted across the five consecutive days (five-day blocks) allowing me to track individual females across the temperatures from both rearing experiments. Additionally, overall female egg outputs across all four larval and adult rearing temperatures were quantified ($N = 20\text{--}22$ per larval; $N = 15$ per adult rearing temperature). Unhatched eggs appear transparent on the surface of the seed coat, housing a dead first-instar larva within it (seed under a dissecting microscope, personal observation) which was excluded from fecundity counts. Successfully hatched eggs were counted across the five days for each female, within each treatment group, to obtain total eggs laid and an average of this was used to calculate total fecundity (female reproductive output) for each treatment group.
- (b) Egg morphology (length and width, mm) was measured using an Olympus SZH10 dissecting microscope, mounted with a digital camera to capture the images (Moticam 2000 camera at 10X magnification). Two independent eggs were measured per female ($N = 16$ females/larval, adult rearing temperature) for each time point (days 1–5) equating to 160 eggs per larval or adult rearing temperature. Measurements were carried out using ImageJ (Schneider et al. 2012) using the linear segmented tool, calibrated with a standard 1 mm graticule slide. Individual eggs were aligned to the

same plane each time prior to measuring and the longest axis between the two points was determined for egg length and the widest plane to measure egg width (Fig. 1). Egg length and width was the perpendicular axis to each other in the plane of measurement and eggs that successfully hatched (appearing a distinct white colour on the bean) were randomly selected for quantifying egg morphology without being scraped from the seed.

- (c) Female lifespan in this study was measured on virgin females that were 0–24 h when they were sourced. They were left singly in a Petri dish with ad libitum moth beans and survival checks were done twice daily (morning/evening). Virgin females from the larval rearing temperatures were assayed for survival under standard insectary conditions (at 27 °C) while those from the adult rearing environments were checked at 17 °C, 25 °C, 27 °C or 33 °C. Within these lifespan assays, virgin females allowed me to control for the ‘cost of reproduction’. Male lifespan was measured here across both experimental exposures using the same methodology (see supplementary materials).
- (d) Hatching success (female fertility, see Arnqvist and Nilsson 2000) was measured on a subset of randomly collected egg-laden beans after 1000 adult females oviposited for an hour (see section, larval rearing temperature) across experimental temperatures. Seed pods with a single egg glued on it was used for this assay to ensure control over larval density, although in some instances more than a single egg was laid, these were excluded from hatching success assays. A single seed pod was placed within a cell of a 25-cell square petri dish (ThermoFisher, UK) and sealed with a glass lid. Successful eclosion of an adult beetle was checked every day and the sex of the emerging adult noted. Egg-laden beans that failed to produce adults were recorded. Proportion was calculated using eclosion success versus failures to eclose.
- (e) Female body size was measured by dissecting a single elytron (as a proxy for body size) using the line tool on ImageJ (length, mm; described previously in Vasudeva et al. 2021a, b) to compare the differences only within larval rearing temperatures. Females from adult rearing temperatures were all reared under standard insectary conditions at 27 °C (N = 20–22 per larval; N = 15 per adult rearing temperature).

Statistical analysis

All replication presented here is biological data and were analysed using R version (v 4.2.0) (R Development Core Team 2022) in RStudio (v 2022.02.03.492) (R Studio Team 2022). All the plots were produced using “ggplot2” package (Wickham 2016). Mixed effects models were built using ‘glmmTMB’ (Brooks et al. 2017) package v 1.1.3 and further tests were carried out using extensions within ‘lmerTest’ (Zeileis and Hothorn 2002), ‘lmerTest’ (Kuznetsova et al. 2017), ‘car’ and ‘MASS’ packages (Fox and Weisberg 2019; Venables and Ripley 2002). Data summaries and wrangling (e.g., treatment averages) were performed using ‘Rmisc’, v 1.5.1, “tidyverse” and ‘dplyr’, v 1.0.9 packages (Hope 2022; Wickham et al. 2019, 2022). A fully fitted saturated model (with interactions; ‘Treatment*Day’) was then simplified by removing the non-significant terms for further model simplification with assumptions for data distributions, appropriate error distributions and the relationship between mean and variance of the response variable taken into consideration (Crawley 2012). Both ‘treatment’ and ‘day’ were treated as categorical variables, and I had apriori reasons to use days 1–5 (‘day’) as categorical rather than continuous variable in these assays. My sampling methods focused on ‘days one to five since mating,’ with day ‘one’ resulting in high reproductive output (adequate ‘sperm

present') and day 'five' being relatively low output (potentially 'sperm limited' and other factors such as 'female age'). Female replicate ID within each experiment was entered as a random factor (Thomas et al. 2015). Violations of model residuals to assumptions were checked using 'DHARMA' package, v 0.4.5 (Hartig 2022) and 'emmeans' to perform meaningful contrasts (default = "Tukey") using v 1.7.4-1 (Lenth 2022). A random intercept model was employed with 'temperature treatment' as fixed effect to test the response variable of interest with no unmeasured treatment groups in this design (Harrison et al. 2018).

Fecundity

A negative binomial mixed effect model was fit in 'glmmTMB' to account for overdispersion (family: "nbinom2") (Brooks et al. 2017). In the full model, "treatment" (larval rearing or adult rearing temperature) along with the interaction between 'day' (block) were entered as predictor variables. 'Replicate ID' (female #) was entered as random effect. The sample size for larval rearing temperature was (N=20, 22, 21 and 21 for 17 °C, 25 °C, 27 °C and 33 °C) and N=15 per adult rearing temperature. The influence of female body size on overall fecundity was included for larval rearing temperatures. Females for the adult rearing temperatures experiment came from the 27 °C control temperature prior to being exposed, experiencing standard rearing until adulthood so body size effects were not considered.

Egg morphology

Both egg length (longest vertical axis along the centre, see Fig. 1) and width (perpendicular axis to length along the broadest part of the egg, 'mm', see Fig. 1) were normally distributed (parametric data) was analysed by building a LMM (linear mixed model). Data distribution was checked by plotting histogram of the response variables (separately for egg 'length' and 'width') in R. 'Treatment' (larval or adult rearing temperature) with 'Day' (temporal measurement in one-day blocks) were entered as interacting predictor variables. To estimate the variation in egg shape within rearing temperatures, egg length over width was assessed using a simple linear model to evaluate the correlation between the two traits. 'Egg width' was entered as a response variable and 'egg length', 'rearing temperature' and the interaction between them as predictor variables (See supplementary materials, Figures S2 and S3). Finally, I checked whether female body size influenced egg morphology by building a linear model with 'egg width' entered as response and 'egg length', 'treatment' and 'female body size' and their three-way interactions as predictor variables. Residuals of the model were checked for violations.

Female lifespan

Virgin females (aged 24–48 h) from both experimental exposures were left on their own in ad libitum beans in a standard petri dish, were tracked through time to record their lifespan; survival (1 = alive, 0 = dead) until death. Lifespan was analysed using cox's regression method using the 'survminer', v 0.4.9 and 'survival', v 3.3-1 packages in 'ggplot2' (Kassambara et al. 2021; Therneau 2022). The sample sizes for larval rearing temperature was N=20, 24, 24 and 21 for 17 °C, 25 °C, 27 °C and 33 °C) and N = 15

females per adult rearing temperature. Male lifespan was measured in these assays and the results are in the supplementary materials.

Hatching success

Hatching success was analysed using a binomial ‘glm’ with “link=logit”. Eggs were randomly collected from the assays and assessed for hatching success (1 = success/0 = failure) across the rearing temperatures through five days of oviposition. The sample sizes for individual egg hatching success within larval and adult rearing temperature was N = 60, 62, 109 and 95 for 17 °C, 25 °C, 27 °C and 33 °C and N = 69, 66, 147 and 60 for 17 °C, 25 °C, 27 °C and 33 °C.

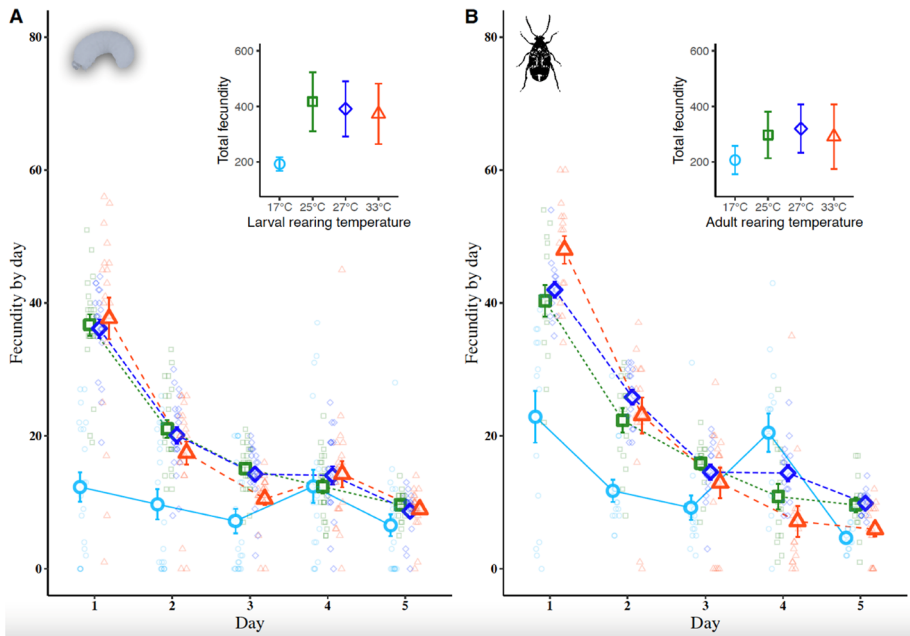


Fig. 2 Fecundity (mean, \pm SE) measured as a function of larval (panel **A**) or adult (panel **B**) rearing temperatures through time (five consecutive days). In panel **A** fecundity was measured when larvae were reared at 17 °C-light blue (circle), 25 °C-green (square), 27 °C-dark blue (diamond), 33 °C-red (triangle) and oviposited at 27 °C. In panel **B**, fecundity is measured when mated females were reared in a common environment at 27 °C and then exposed to oviposit at 17 °C, 25 °C, 27 °C and 33 °C rearing temperatures. Smaller jittered points indicate the raw data around the mean (larger point) and the averages are connected through time by lines to highlight fitness trajectories. Inset plots indicate total fecundity (\pm SE) across the five days. Total fecundity was calculated by adding egg outputs for each day and then averaging this for each treatment group resulting in four treatment totals per temperature exposure

Results

Larval rearing temperature

Fecundity

I found that there was a strong overall negative effect ($\chi^2_3=61.48$, $P<0.0001$, Fig. 2A) and a similar effect of rearing temperature through time within the temporal assays (five one-day blocks) ($\chi^2_4=19.37$, $P<0.0001$, Fig. 2A). The effect was driven by reduced reproductive outputs at 17 °C to those experiencing 25 °C, 27 °C or 33 °C (Fig. 2A). When compared to females from the control group (27 °C), fecundity at 17 °C decreased by 51%, no such dramatic decline in reproductive output were observed in the other two temperature treatments at 25 °C (7% increase) and 33 °C (5% decrease). Further, the overall interaction between treatment temperature and time (five one-day blocks) was highly statistically significant ($\chi^2_{12}=33.06$, $P<0.0001$, Fig. 2A). Post hoc tests confirmed these effects shown through time. Females from 17 °C produced significantly fewer offspring by comparison to the three other rearing temperatures on day one (25 °C: $t=-6.68$, $P<0.0001$; 27 °C: $t=-6.47$, $P<0.0001$ and 33 °C: $t=-6.66$, $P<0.0001$). On day two, 17 °C fecundity was different to 25 °C ($t=-4.62$, $P<0.001$) and 27 °C ($t=-4.23$, $P<0.01$), but not at 33 °C ($t=-3.52$, $P>0.05$). A similar trend to 'day two' was observed on 'day three' in terms of treatment differences, however by 'days four and five' all treatments reached similar levels of fecundity ($P>0.05$). This could be due to limitation of viable gametes in the absence of female remating. There was no evidence for female body size effects on total fecundity ($z=1.45$, $P>0.05$, Table S2a).

Egg morphology

Both egg length ($\chi^2_3=224.8$, $P<0.0001$, Fig. 3A) and egg width ($\chi^2_3=206.04$, $P<0.0001$, Fig. 3B) were significantly greater at 17 °C and 33 °C compared to eggs oviposited at 27 °C (egg length, 17 °C–27 °C: $t=6.24$, $P<0.0001$; 27 °C–33 °C: $t=-6.71$, $P<0.0001$ and egg width, 17 °C–27 °C: $t=5.97$, $P<0.0001$ and 27 °C–33 °C: $t=-5.23$, $P<0.0001$). Similarly, temporal variation (five days) in egg morphology (length/width, mm) was statistically significant overall (length: $\chi^2_4=186.58$, $P<0.0001$, Fig. 3A and width: $\chi^2_4=263.41$, $P<0.001$, Fig. 3B). Egg morphology interacted significantly with the treatment applied over the five days (length: $\chi^2_{12}=43$, $P<0.0001$, Fig. 3A, width: $\chi^2_{12}=67.93$, $P<0.0001$, Fig. 3B). But, within the 17 °C treatment, egg morphology (both length and width) did not vary much through time relative to the other temperatures (see, Fig. 3A, B). At 17 °C and 33 °C, egg morphology (length, width) increased by no greater than 4% approximately and 4% decrease at 25 °C compared to those at 27 °C. The differences in egg morphology (length, width) however between 25 °C and the two temperature extremes (17 °C & 33 °C) were no greater than 7%. Changes to egg morphology (length and width) was not consistent across the rearing temperature treatments within these assays. There was no evidence for body size effects on egg morphology; length ($t=-0.544$, $P>0.05$) and width ($t=0.065$, $P>0.05$).

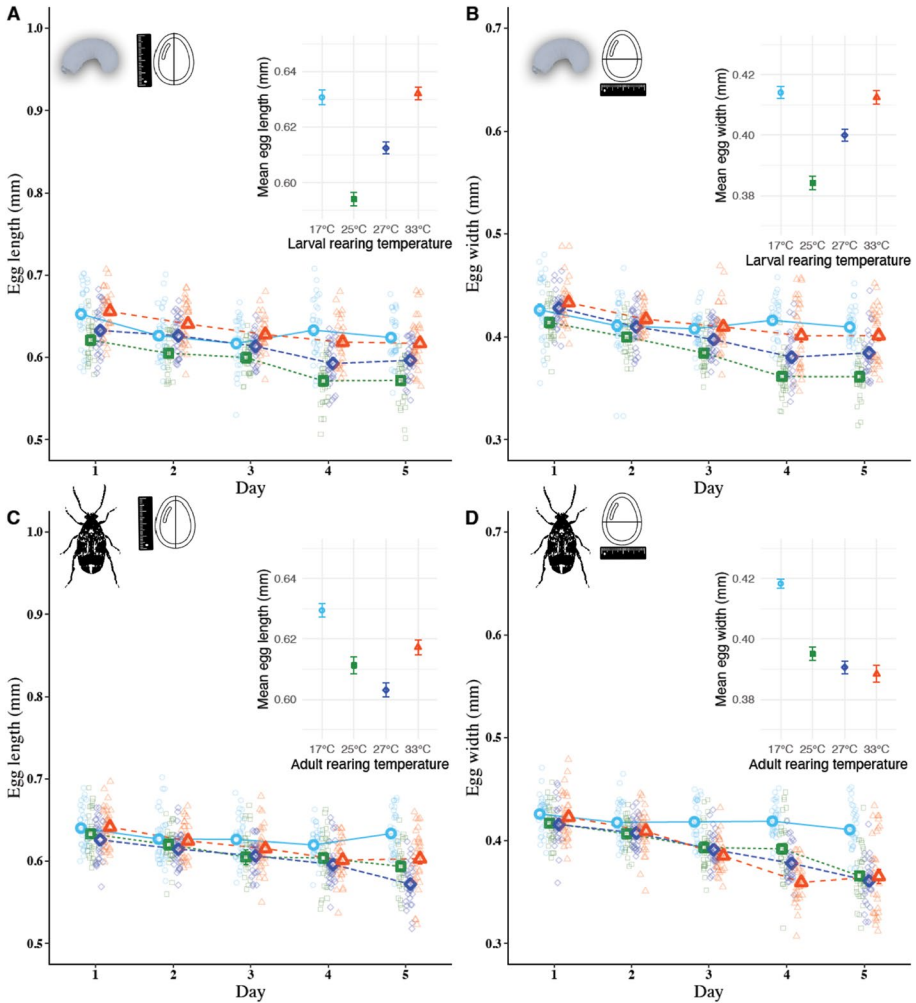


Fig. 3 Egg size dimensions (mean \pm SE) through time (across five one-day blocks) during larval rearing (panels **A**, **B**) at 17 °C-light blue, 25 °C-green, 27 °C-dark blue and 33 °C-red and oviposited at a 27 °C. Inset plots show the mean values (\pm SE). Panels **C**, **D** show egg dimensions (mean \pm SE) when adults were exposed to different adult environments for oviposition. Development from egg-to-adulthood took place at 27 °C, adults were then exposed to four different adult rearing environments. Smaller points (jitters) represent the raw data and the larger points indicate mean (\pm SE, y-axis) value for each time point measured (day, x-axis)

Female lifespan

Larval rearing temperature influenced female lifespan overall (log-rank test: 20.82 on $df=3$, $P < 0.0001$, Fig. 4A). Females reared at 17 °C (mean \pm SE: 14.1 ± 0.84 days) showed a 26–27% increase in lifespan compared to those reared at 25 °C (mean \pm SE: 10.7 ± 0.42 days) or 27 °C (mean \pm SE: 9.9 ± 0.50 days); 17 °C and 25 °C (Hazards ratio (HR): 0.313, 95% CI[0.135–0.727], $z = -3.54$, $P = 0.002$) and 17 °C and 27 °C (HR

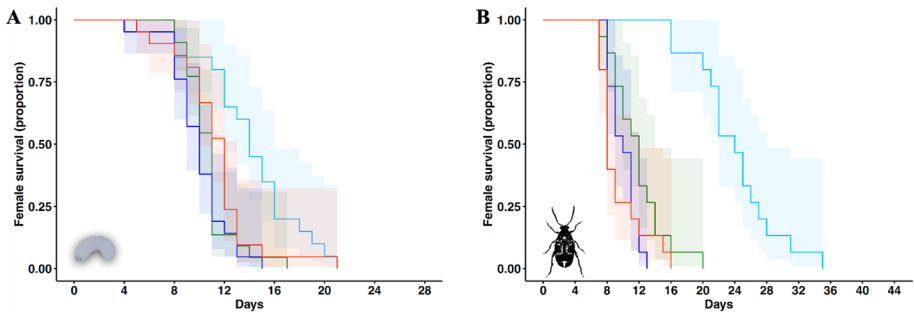


Fig. 4 Plasticity in female lifespan when experiencing larval (A) or adult rearing temperatures (B, 17 °C–light blue, 25 °C–green, 27 °C–dark blue and 33 °C–red). Female survival (proportion, y-axis) as a function of rearing environment is measured through time (days, x-axis). Shaded area around individual lines indicate the confidence intervals

0.251, 95% CI [0.106–0.594], $z = -4.12$, $P = 0.0002$). Overall lifespan differences at the temperature extremes were not significantly different; 17 °C (mean \pm SE: 14.1 ± 0.84 days) and 33 °C (mean \pm SE: 11.3 ± 0.70 days; HR 0.495, 95% CI [0.216–1.131], $z = -2.18$, $P = 0.126$). Similarly, lifespan compared within 25 °C and 33 °C (HR 1.578, 95% CI 0.704–3.538, $z = 1.451$, $P = 0.467$) and 27 °C and 33 °C (HR 1.971, 95% CI 0.871–4.461, $z = 2.13$, $P = 0.142$) cohorts were not statistically different (Fig. 4A).

Hatching success (female fertility)

Females reared at 17 °C had lower hatching success with a 42% decline in their fertility compared to those experiencing 27 °C control ($z = -2.137$, $P = 0.03$) or 33 °C ($z = -2.093$, $P = 0.03$). Overall, there were no differences in female fertility between 17 °C and 25 °C ($z = -1.279$, $P = 0.57$). Despite a 16% drop in fertility for the 25 °C females during larval development, there were no statistically significant differences compared to females experiencing 27 °C (control) or 33 °C during larval development; (25 °C–27 °C: $z = -0.759$, $P = 0.87$ and 25 °C–33 °C: $z = -0.741$, $P = 0.87$).

Adult rearing temperature

Fecundity

Overall, there was a significant negative effect of adult temperature ($\chi^2_3 = 38.30$, $P < 0.0001$, Fig. 2B) with adults experiencing 17 °C producing 35% fewer offspring (in total) than those at 27 °C, and through time, these effects persisted over the five one-day blocks ($\chi^2_4 = 407.82$, $P < 0.0001$, Fig. 2B). Fecundity levels interacted significantly through time overall, this was accentuated by low fecundity at 17 °C compared to females at 27 °C ($\chi^2_{12} = 89.07$, $P < 0.0001$, Fig. 2B). On day one, fecundity was significantly different between 17 °C and 33 °C ($t = -4.28$, $P < 0.01$), but no differences in fecundity between 17 °C and 25 °C or 17 °C and 27 °C for day one (25 °C: $t = -3.26$, $P > 0.05$; 27 °C: $t = -3.5$, $p > 0.05$). On day two, fecundity was significantly different at 17 °C and 27 °C ($t = -4.27$, $P < 0.01$) and 17 °C and 33 °C ($t = -3.64$, $P < 0.05$), but no apparent differences between 17 °C and 25 °C treatments ($t = -3.47$, $P > 0.05$). On day four,

fecundity was greater at 17 °C than 33 °C ($t=5.38$, $P<0.0001$), with no other apparent treatment differences (on days three, four or five) for the remainder of the sampling period across the treatments.

Egg morphology

Egg length ($\chi^2_3=82.76$, $P<0.0001$, Fig. 3C) and egg width ($\chi^2_3=216.85$, $P<0.0001$, Fig. 3D) were significantly different; egg length: 17 °C females laying 4% longer eggs than 27 °C females and egg width: 7% wider eggs at 17 °C than at 27 °C. Investment into eggs through time (length and width, mm), measured across five one-day blocks was highly significant (length: $\chi^2_4=135.98$, $P<0.0001$, Fig. 3C and width: $\chi^2_4=389.69$, $P<0.0001$, Fig. 3D). Egg morphology interacted significantly between rearing temperature and day (five one-day blocks) (length: $\chi^2_{12}=42.97$, $P<0.0001$, Fig. 3C, width: $\chi^2_{12}=113.68$, $P<0.0001$, Fig. 3D). However, within the 17 °C treatment, both egg morphology (both length and width) remained similar through the five days of sampling ($P>0.05$), but for the other treatment temperatures; 25 °C, 27 °C and 33 °C, egg morphology drastically changed through the sampling time ($P<0.05$) (see, Fig. 3C, D).

Female lifespan

Lifespan of females was significantly affected (log-rank test: 47.75 on $df=3$, $P<0.0001$, Fig. 4B), with females experiencing 17 °C living significantly longer (mean \pm SE: 24 ± 1.32 days) than females experiencing 25 °C (mean \pm SE: 11.8 ± 0.86 days; HR: 0.050, 95% CI 0.008–0.289, $z=-4.402$, $P<0.0001$), 27 °C (mean \pm SE: 10.0 ± 0.43 days; HR: 0.024, 95% CI 0.003–0.160, $z=-5.104$, $P<0.0001$) or 33 °C (mean \pm SE: 9.4 ± 0.73 days; HR: 0.023, 95% CI 0.003–0.142, $z=-5.350$, $P<0.0001$). I observed doubling in female lifespan at 17 °C as opposed to 27 °C. There was no evidence that adult rearing temperatures influenced female lifespan at the other three temperatures exposed 25 °C, 27 °C or 33 °C ($P>0.05$, Fig. 4B).

Hatching success

Overall there were no statistical differences among the cohorts within this assay ($\chi^2_3=0.09$, $P>0.05$, Fig. 5B).

Discussion

Manipulation of larval rearing temperatures resulted in greater changes to traits associated with reproduction than alterations in adult temperatures. This is presumably due to lower temperatures being physiologically stressful and affecting female gametic development. Here, I found that (a) temperature-induced plasticity in egg morphology expressed stage-specific differences to the temperatures exposed, (b) female lifespan increased at 17 °C across both experiments, but males consistently outlived females in this strain regardless of the rearing temperature (see, supplementary materials for male-specific responses), (c) female fertility was affected by low temperatures (17 °C) and remained fertile at relatively higher temperatures, 33 °C. Also, it becomes clear that female fertility limits are not identified with my results studied at these larval and adult rearing temperatures.

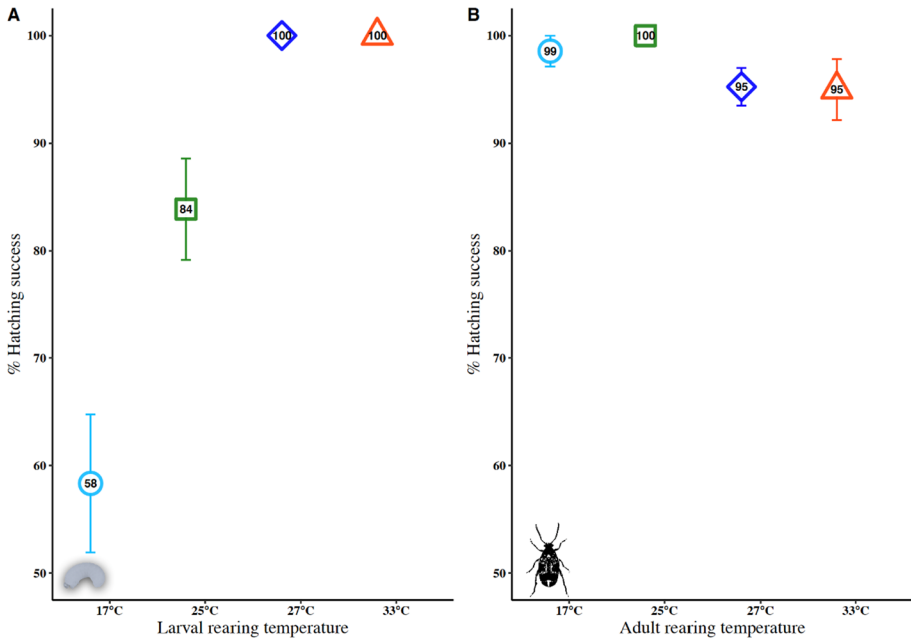


Fig. 5 Proportion egg hatching success as a percentage (female fertility) in response to larval (panel **A**) or adult (panel **B**) rearing temperatures; (17 °C–light blue, 25 °C–green, 27 °C–dark blue and 33 °C–red). Filled symbols indicate treatment mean (\pm SE) for percentage of hatching eggs

I speculate that a 2 °C–3 °C increase in temperature, which is still relevant within the ecology of these beetles, should be able to shed light on female fertility limits. But these results provide some insights on how selection might operate on females functioning across different ecological contexts, favouring flexibility within females to rapid changes to the environment (e.g., maternal effects). This flexible phenotypic expression and their functionality can aid in shorter- versus longer-term responses (e.g., Kuijper et al. 2014). Further experiments are required to unravel the adaptive significance of egg morphology and offspring quality. Both larval and adult rearing temperatures showed context-specific phenotypic expression in the fitness traits measured here (Maynard Smith 1957). More recently, using the same species Baur et al. (2022) showed that females developing at 35 °C showed a 32% reduction in fertility compared to unexposed females and this was clearly associated with mating system which in the current study was not manipulated.

Larval rearing enforced stronger selection on fecundity than adult rearing temperatures

Total fecundity across both larval and adult rearing temperatures differed in magnitude (Fig. 2A and B inset). At 17 °C females produced fewer eggs overall across both experiments and similar responses in other insects have been recorded previously (see, Huey et al. 1995; Fischer et al. 2003c). Here, females exposed to 33 °C were equally fecund and produced relatively larger eggs as females from control conditions at 27 °C across both larval and adult rearing temperatures, but male reproductive function in a

previous study was highly compromised at 17 °C and 33 °C larval rearing temperatures (Vasudeva et al. 2014, 2021a). Interestingly, male sperm competitiveness (both P_1 and P_2) and sperm length were affected at 17 °C and 33 °C compared to 27 °C controls—this opposing response in reproductive trait form and function to larval rearing temperature between the sexes requires further experimental testing. In fruit flies, it has been shown that male fertility limit is more sensitive than females (Fertility Thermal Limit was 1.3 °C lower for males; van Heerwaarden and Sgrò 2021) and developmental and adult acclimatory responses for CT_{max} ranges between 35 °C and 37 °C (Kellermann et al. 2017). Therefore, future work on this strain should incorporate a wider range of temperatures to unravel the sex-specific sensitivities to reproductive decline to understand thermal fertility limits. However, experimental manipulation of (female) larval temperature in a mosquito (*Anopheles gambiae* s.s.) revealed the opposite effect to the results reported in this study, where fecundity was lower by about half at 31 °C compared to females at 23 °C or 27 °C (Christiansen-Jucht et al. 2015).

Female exposures at 17 °C as larvae followed by oviposition at 27 °C had total fecundity reduce by 51% or by 35% (as adults) compared to controls (Fig. 2A, B, inset). Though females experiencing 33 °C had a less dramatic decline in total fecundity; 5% (larval) or 9% (adult) decline compared to the control groups reared at 27 °C. Contrastingly, recent experiments in this beetle revealed that female reproductive anatomy (the bursa copulatrix) was shortest at 17 °C and 33 °C (compared to 27 °C) (Farrow et al. 2022) which also seemed to affect their likelihood to copulate, indicating a complex trade-off between mating likelihood and reproductive trait morphology. Thus, further tests are required to study the interactions between female oviposition strategies and developmental temperature by incorporating reproductive trait morphology. Experiments using the fruit fly (*D. melanogaster*) demonstrated that higher adult temperatures saw only a slight decline in female fitness (relative to males') (Rodrigues et al. 2022b), concordant with the findings reported here. Also, from the current data female fecundity is unaffected when exposed to high adult rearing temperatures post mating (at 33 °C), producing viable eggs through five days of oviposition. The sperm load received remains protected within female reproductive tracts allowing further oviposition (Fig. 2B, inset plot). Conclusive follow-up experiments are required to tease apart the female role in sperm protection and their capacity to buffer thermal stress through various mechanisms (e.g., plasticity). Further empirical tests should account for the nature of male versus female exposures to get a clearer picture on vulnerability of stored sperm in the female reproductive tract with physiology and how that affects fertility. It could be that the temperature thresholds for sperm damage during storage might be higher than what has been applied on females here, as I clearly do not find a female fertility limit within these experimental assays.

Two independent empirical studies shed some light on this phenomenon; in *Tribolium* flour beetles when control mated females were exposed to heatwaves (42 °C for five consecutive days), a highly significant drop in reproductive output was observed compared to experimental controls (Sales et al. 2018). In two fruit fly species, stored sperm within female tracts remain vulnerable to heat stress and females were unable to protect them (Walsh et al. 2022). As *C. maculatus* females can maintain higher fitness through polyandry (Wilson and Tomkins 2015), it seems plausible that females can filter viable sperm within temperature damaged ejaculates through postcopulatory intraspecific filters protecting fertility from high temperature damage. Baur et al. (2022), Sutter et al. (2019) and Vasudeva et al. (2021a, b) showed that (facultative) polyandry can help females recover fertility through multiple mating (with normal males) when fertility is damaged by high temperatures, clearly linking the benefits of multiple mating with fertility recovery.

Polyandry and fertility recovery needs to be properly tested in *C. maculatus* in response to heatwave damage within an ecological and evolutionary framework to capture the extent of plasticity and evolvability across thermal maxima, variation and averages within populations.

Overall, 17 °C larval and adult rearing temperatures were stressful to females resulting in low reproductive output (Fig. 2A, B), but not at 33 °C. These tropical beetles can adjust their egg output when facing unfavourable thermal conditions under natural settings and different life stages show varied tolerance to temperatures (see, Germain et al. 1987 and Loganathan et al. 2011). However, if females positively reproduce at warmer average temperatures, continued global warming and prolonged exposure to thermal stress can impact reproduction via breakdown in physiology, development and behaviour (Sheridan and Bickford 2011; González-Tokman et al. 2020). It is predicted that many ectothermic organisms, including shorter-lived insects, might face warmer, fluctuating conditions as the frequency and duration of hotter days increases under global warming (Zhang et al. 2015). The evolutionary consequences of continued warming on female fertility without additional resources such as high calorie food intake could affect fitness; as organisms could face finite resources to budget energy demands between physiological maintenance, growth and reproduction (Sheridan and Bickford 2011). Fluctuating temperature conditions can also result in lower genetic variation, affecting plastic responses to global warming (Hallsson and Björklund 2012). In some cases, this could affect the abundance of some insect groups (Bowler and Terblanche 2008), altering their distributions (Sharma 2014).

Temperature-mediated plasticity in egg morphological dimensions is context specific

Both larval and adult rearing temperatures had varied effects on egg morphology. The responses reported here for eggs contrast with the phenotypic expression of mature sperm in a different study, when males were exposed to different larval rearing temperatures (Vasudeva et al. 2014). Both egg length and width were greatest at 17 °C and 33 °C larval rearing temperatures, where sperm remained morphologically shorter (Vasudeva et al. 2014, also see, Vasudeva et al. 2019 for sperm and egg thermally induced plasticity in *Tribolium* beetles). Such temperature mediated gamete optimisation could be a response mechanism to match thermal reproductive environments to cope functionally, but phenotypic variability alone is unlikely to be responsible (see, Vasudeva et al. 2019). It has been shown that females producing larger eggs in the tropical butterfly *B. anynana* provision more water, lipids, protein and glycogen within each egg, whether these provisioning mechanisms operate similarly across temperature gradients is unknown (Geister et al. 2009). Additionally, investment into somatic traits such as female body size did not explain the current results and is concordant with another experimental study on a tropical butterfly (Fischer et al. 2003b) and others have shown that larval density (Credland et al. 1986) and rearing host and temperature during development (Stillwell et al. 2007) affected body size in different strains of this species. By contrast, at adult rearing temperatures, investment into larger eggs at 17 °C during could be an acclimatory response mediated by temperature, as has been shown in other arthropods (Azevedo et al. 1996; Fischer et al. 2003c; Seko and Nakasuji 2006). Larger eggs produced at 17 °C during AR conforms to temperature-size rule, also see (Fischer et al. 2003a, c). In arthropods, smaller eggs are usually the norm with increasing temperature (Fox and Czesak 2000) and egg size variation is known in response to

other biotic factors such as quality of host plants in the seed beetle *Stator limbatus* (Fox et al. 1997) and in line with their findings, females within the current experiments laid different shaped eggs across rearing temperatures. At the moment, the adaptive nature of these changes to egg morphology due to larval and adult rearing temperatures remain unknown. However, in the global pest *T. castaneum*, thermally induced plasticity in ova form and function was adaptive after a single generation of being exposed to different thermal environments, where ova produced at 38 °C were larger and hatched more successfully at 40 °C compared to smaller ova produced at 30 °C (Vasudeva et al. 2019). Therefore, it is possible that females can match eggs to the rearing temperature, within a permissible range, to enable optimal offspring production. Although during development (at 17 °C), females produced larger (but fewer) eggs, they were poor at hatching. This could be indicative of a mismatch between form and function (female fertility). Functional impairment was studied previously in males of this strain using a similar design. It highlighted that male reproductive function during sperm competition was compromised when sperm was produced through development at 17 °C, much lower when compared to 33 °C (within both P₁ and P₂ assays; see, Vasudeva et al. 2014).

Plasticity in lifespan is directional to larval and adult rearing temperatures

Female lifespan was longest at 17 °C across both larval and adult rearing temperatures. Under larval rearing, female lifespan increased by 26% at 17 °C or by 4% at 33 °C compared to the 27 °C controls. The small statistically non-significant increase in lifespan at 33 °C (larval rearing) compared to 27 °C controls could be due to pathways associated with genetic stability linked to hormesis, here it is possible that the up-regulation of cellular processes linked to high temperature responses might be involved (for a review, see Keil et al. 2015). Interestingly, female lifespan under adult rearing more than doubled (55% longer life) at 17 °C and decreased lifespan by 17.5% at 33 °C compared to controls at 27 °C. No discernible effects in terms of lifespan were observed between 25 °C and 27 °C across both experiments. Although females of this species usually live longer than males (see, Fox et al. 2004) nongenetic parental effects can affect lifespan of the sexes differently as the combined effects of egg provisioning, ovarian dynamics and reproductive output can interact with adult lifespan (Gibbs et al. 2010). Also, the accrued effects of the environment experienced during early development can have varied effects on adulthood through genetic variation and covariation, explaining the variation in lifespan (Mousseau and Dingle 1991). Collectively, these results indicate that the mechanisms operating in increased lifespan might have different effects on female reproduction. Male intrinsic lifespan was also measured and follows a similar pattern to females, but males were observed to live longer than females under both experimental temperatures. These differences in lifespan demonstrated between the sexes could be due to differential rates of increase in mortality as a result of the rearing temperature enforced on the different life stages affecting resource allocation during ontogeny (see, Fox et al. 2004; for differential larval development, see Vasudeva et al. 2018). In the mosquito *A. gambiae* s.s., a change in the larval temperature with subsequent tests on adult lifespan (by 8 °C; reared at 23 °C and tested at 31 °C) resulted in a similar extension of lifespan (Fig. 3C in Christiansen-Jucht et al. 2014). Adult survival was attributed to the age-dependent effects of temperature on larval stages (e.g., developmental rates, life history parameters).

Larval rearing had stronger impacts on egg hatching (female fertility) than adult rearing temperatures

Hatching success was severely affected under larval rearing temperatures despite producing relatively (~4%) larger eggs at 17 °C compared to controls (also see, Hallsson and Björklund 2012 for hatching success). Egg morphological attributes were similar between females exposed at the extreme larval rearing temperatures (17 °C and 33 °C), but still differed in their hatchability. This suggests that the relationship between egg size and hatchability is not straight forward (and possibly not directly linked), while other factors could be important for the overall hatching success. In *B. anynana* butterflies, larger eggs produced at lower temperatures showed greater (~10% increase) hatching success (Fischer et al. 2003b), this is in contrast to the responses observed here at 17 °C during larval rearing (~42% decline compared to 27 °C & 33 °C). In the current study, egg hatchability under adult rearing temperatures was all comparable: females producing larger (and fewer eggs at 17 °C) survived equally well to those that hatched at 25 °C, 27 °C or 33 °C (Fig. 5B). It is clear that egg size alone might not be insightful to explore the inherent costs of thermally induced plasticity in this species and that other qualitative changes (e.g., physiology, nutrient load, water content) to low temperature exposures may need to be accounted for. Other possible mechanisms that might operate are physiological acclimation, regulatory plasticity linked to anticipatory environmental responses, haploid selection and/or epigenetic modifications (see discussion, Vasudeva et al. 2019). Sex ratios remained unaffected by larval rearing temperatures (male:female-17 °C, N=60; 0.25:0.33), (25 °C, N=62; 0.45:0.38), (27 °C, N=109; 0.52:0.48) and (33 °C, N=95; 0.54:0.46). Similarly, in the fruit fly *Drosophila melanogaster*, developmental thermal stress had little impact on sex ratios of the offspring (Rodrigues et al. 2022b).

Conclusion

By comparing the larval and adult stages in the same study, I have shown that there is considerable stage-specific reproductive plasticity in females to thermal stress from rearing temperatures, adding to a growing body of studies within evolutionary ecology, studying insect fertility limits. Here, females with stored sperm can withstand higher temperatures maintaining fertility through time and be reproductively functional, although the impacts vary within life stages. Future work is needed to account how temperature fluctuations around averages (taking in account both daytime and nighttime variations) affect female responses into reproductive investment, trade-offs (e.g., egg volume, fertility limits) at various ecologically relevant conditions. Also, through translocation experiments using factorial designs, tests for adaptive significance of temperature-induced plasticity should also be considered in both the shorter- versus longer-term across other insect groups. This will help predict more precisely the effects of temperature variation on biological functions such as 'fertility limits.' Female fertility responses to temperature remains understudied in an ecological and evolutionary biology framework and could be a potential tool in integrated pest management. By accounting for it, we can better understand how thermal stress from continued global warming impacts female reproductive function and the processes involved in biodiversity declines.

Data and code availability

All data generated as part of this study, fully annotated R code scripts, analysis details and packages used (with the version information) will be made openly available in line with the data transparency policy as an associated source file in my (Mendeley data repository with the DOI (<https://doi.org/10.17632/5h362x69dc.1>)). The complete dataset will be publicly accessible after the embargo period, from (to be confirmed) using the DOI mentioned above.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10682-022-10227-z>.

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Declarations

Competing interests The authors declare no competing interests.

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