The role of recent evolutionary history in resilience to environmental change: social evolution effects versus founder effects

Abstract

Principles of social evolution have long been used retrospectively to interpret social interactions, but have less commonly been applied predictively to inform conservation and animal husbandry strategies. We investigate whether past social selection can predict resilience to environmental change. Upon exposure to harsh novel environments, populations that previously experienced more benign social environments are predicted either to suffer fitness losses (the "mutation load hypothesis" and "selection filter hypothesis") or maintain fitness (the "beneficial mutation hypothesis"). We tested these contrasting predictions using populations of burying beetles Nicrophorus vespilloides evolved experimentally for 48 generations under harsh versus benign social environments through manipulation of parental care. We exposed sexually immature adults from each population to varying heat stress and measured the effect on survival and reproduction. The more benign the social environment previously experienced by a population, the better its survival under heat stress during sexual maturation. Although consistent with the "beneficial mutation hypothesis", it is also possible that populations evolved under the harsher social environment were more prone to dying during maturation, regardless of their thermal environment. Overall, stochastic genetic variation, probably due to founder effects, was a better predictor of resilience. We discuss the implications for translocation and captive breeding programmes.

Introduction

In a world changing rapidly through the effects of climate change (Pörtner et al., 2022), habitat degradation (Millenium Ecosystem Assessment, 2005) and excessive use of antibiotics (Graham et al., 2019) and pesticides (Hawkins et al., 2019), our ability to predict how effectively organisms will adapt to new environments is more important than ever. The principles of social evolution have long been used retrospectively to understand social interactions, however they have been more rarely used predictively to inform the development of practical strategies to address global problems (Carroll et al., 2014). Yet an understanding of a population's evolutionary history, by which we mean the type of selective environment it has experienced, could predict its resilience to future environmental perturbation (Lumley et al., 2015). This has practical value in its potential to address several major economic and societal problems, including food security (pest/crop interactions, crop development), human and animal medicine (antibiotic development, anti-parasite interventions) and wild animal conservation (choice of populations for translocation schemes, identification of populations vulnerable to disease and environmental change) (Carroll et al., 2014).

Few studies have tested experimentally how past exposure to different social selective environments affects a population's future response to environmental change (but see Lumley et al., 2015). In theory, the effect on resilience depends on whether the social environment experienced by the population is benign or harsh - because this influences the extent of standing genetic variation available.

Benign social interactions, which are usually due to some form of cooperative interaction, are known to buffer individuals from environmental stress and therefore to reduce the extent to

which mildly deleterious alleles are exposed to natural selection (Linksvayer & Wade, 2009; Pascoal et al., 2022). Elevated mutation loads resulting from relaxed selection have been shown to reduce reproductive success in animals kept in captive breeding programmes (Araki et al., 2007; McPhee, 2004; Robert, 2009) and have been linked to a higher prevalence of genetic disorders in human societies where selection is buffered by widely available healthcare (Lynch, 2016; Saniotis et al., 2021; You & Henneberg, 2018). The "mutation load hypothesis" therefore predicts that individuals previously exposed to more benign social environments should be less resilient to new environmental stresses. However, whilst most of the mutations that accumulate under relaxed selection are likely to be (mildly) harmful, some could potentially be beneficial and might even facilitate greater resilience to environmental change at the population level. The "beneficial mutation hypothesis" therefore conversely predicts that individuals previously exposed to more benign social environments could be more resilient to new environmental stresses, if by chance they have accumulated cryptic genetic variation that is beneficial when expressed in these new environments (Barrett & Schluter, 2008; Paaby & Rockman, 2014). At the other end of the spectrum, a harsh social environment potentially imposes a strong selection filter, which is likely to reduce the population's mutation load, causing individuals generally to be fitter (Balmford, 1996). The "selection filter hypothesis" predicts that a history of exposure to harsher social environments means that populations are more likely to be resilient to environmental change.

Species in which offspring are facultatively dependent on parental care (Clutton-Brock, 1991) offer a way to test these contrasting predictions. Offspring that receive care are exposed to a benign social environment during development which, if sustained over many generations relaxes selection (Snell-Rood et al., 2016). Conversely, offspring that receive no care over many generations experience a harsher environment during development and consequently

experience stronger selection against deleterious mutations. We exploited this natural history to test whether, and how, past social interactions influence future resilience to environmental change. Our experiments focus on the burying beetle *Nicrophorus vespilloides*, which exhibits elaborate biparental care upon which offspring are facultatively dependent (Capodeanu-Nägler et al., 2016).

Burying beetle adults locate the carcasses of small vertebrates using their highly sensitive antennae (Kalinová et al., 2009; Trumbo & Steiger, 2020) and then fight intrasexually for ownership (Otronen, 1988). The winning pair then tears the fur or feathers from the carcass, covers the flesh with antimicrobial oral and anal exudates, rolls it into a ball and buries it underground (Cotter & Kilner, 2010). The female then lays eggs in the soil surrounding the carcass nest. When the larvae hatch, 2-3 days later, they crawl to the nest (Müller & Eggert, 1989), where their parents defend them from predators and intra- and interspecific competitors and feed them trophallactically (Eggert et al., 1998). The duration of post-hatching care is highly variable and in some cases is non-existent due to the death or desertion by parents after preparation of the carcass nest (Schrader, Jarrett, et al., 2015a; Smiseth et al., 2003).

We evolved two replicate populations of burying beetles in the laboratory under contrasting regimes of parental care for 48 generations. In the Full Care populations, beetles were allowed to stay with their offspring throughout development. Individuals in these populations experienced a benign environment during development and relaxed selection (Pascoal et al., 2022). By contrast, in the No Care populations, parents were removed just prior to larval hatching and so were unable to supply any post-hatching care at all. Therefore individuals in these populations experienced a harsher social environment and stronger selection against deleterious mutations (Pascoal et al., 2022).

101

102 After 47 generations of experimental evolution, we tested whether there were differences in
103 the resilience of beetles from these two types of experimental population when they were
104 exposed to higher temperatures during their juvenile adult stage. We define resilience as the
105 ability of a population to maintain normal processes, in particular reproductive success, in
106 response to a harsh novel environment (Lowerre-Barbieri et al., 2017; Martin & Wiebe, 2004;
107 Wingfield et al., 2011). Temperature was chosen as the environmental stressor because of its
108 ecological relevance in the context of climate change. Insects, being poikilothermic, are
109 particularly vulnerable to heat stress, and research on burying beetles specifically has shown
110 that they have reduced breeding success at higher temperatures (Moss & Moore, 2021; Quinby
111 et al., 2020; Sun & Kilner, 2020). We chose to expose the beetles to the temperature treatments
112 during sexual maturation alone because this is the time when an adult beetle's gonads are
113 developing (e.g. David et al., 2005; Gibbs et al., 2010; Sales et al., 2018; Zizzari and Ellers,
114 2011). Furthermore, Sales et al. (2021) show that this is the developmental stage that is most
115 vulnerable to effects of thermal stress on fertility – perhaps because in nature, sexually
116 immature individuals cannot stay underground to buffer themselves from heat stress because
117 they must emerge to feed.

118

119 We exposed sexually immature adults to different degrees of heat stress and measured the
120 effect on the following correlates of fitness: survival to sexual maturity, anal exudate lytic
121 activity, carcass sphericity, fecundity, and hatching success (Cotter and Kilner, 2010, Arce et
122 al., 2012; Cotter and Kilner, 2010, Duarte et al., 2021). We predicted that the contrasting
123 regimes of selection imposed by these different social environments for the 47 preceding
124 generations should yield a contrasting response to heat stress during sexual maturation. The
125 "mutation load hypothesis" and "selection filter hypothesis" each predict that individuals

derived from the No Care populations should be more resilient to heat stress, whereas the "beneficial mutation hypothesis" predicts that individuals derived from the No Care populations should be less resilient to heat stress.

Methods

Burying beetle husbandry in the laboratory: general methods

Burying beetles were bred by placing them in a plastic breeding box (17 x 12 x 6 cm), partially filled with damp soil (Miracle-Gro Compost), furnished with a 10-15 g mouse carcass for breeding. Boxes were placed in a cupboard after pairing to simulate underground conditions. Larvae were counted and weighed 8 days after pairing and each one placed in its own cell (2 x 2 x 2 cm) within a plastic pupation box (10 x 10 x 2 cm), filled with damp peat. Sexually immature adults were eclosed around 21 days later and each was placed in its own individual box (12 x 8 x 2 cm). Adults were fed twice weekly with beef mince until breeding, which took place 15 days post-eclosion. At all life stages, individuals were kept at 21°C. During breeding, pairs were kept in a constantly dark environment, but for all other life stages they were kept on a 16L: 8D hour light cycle.

Experimental Evolution

The *N. vespilloides* populations used in this study were part of a long-term experimental evolution project that investigated how populations of burying beetles adapt to the loss of parental care. There were four experimental populations in all: Full Care (FC) (replicated

151 twice) and No Care (NC) (replicated twice). Their establishment and husbandry have been
152 described in detail before (Duarte et al., 2021; Jarrett, Evans, et al., 2018; Jarrett, Rebar, et al.,
153 2018; Rebar et al., 2020; Schrader et al., 2017; Schrader, Jarrett, et al., 2015a, 2015b). Briefly,
154 these populations were established in 2014 with wild-caught beetles from four woodland sites
155 across Cambridgeshire, UK (Byron's Pool, Gamlingay Woods, Waresley Woods and Overhall
156 Grove). FC populations were left undisturbed (as described in general methods above) and
157 allowed to stay with their offspring until dispersal. However, in the NC population, at 53 h
158 post-pairing, when the carrion nest was complete but before the larvae had hatched, both
159 parents were removed to prevent post-hatching care. This procedure was repeated at every
160 generation. The experimental populations were bred in two separate blocks (FC1/NC1 and
161 FC2/NC2), and the timing of the lifecycle was staggered between blocks to be 7 days apart.
162
163 *Testing for resilience to heat stress*
164
165 ***Experimental generation 1: Partitioning current care environment from evolutionary***
166 ***history of care***
167 Beetles were taken for analysis after 47 generations of experimental evolution. We partitioned
168 the effects of current care experienced by individuals (No Care or Full Care), from an
169 evolutionary history of care experienced by the source population (No Care or Full Care), by
170 breeding beetles for one generation using the following treatments:
171 1) Adults from Full Care populations allowed to provide full parental care (Full Care
172 parents with a Full Care regime) = FC_{FC}
173 2) Adults from Full Care populations removed at 53 hours after pairing (Full Care parents
174 with a No Care regime) = FC_{NC}

3) Adults from No Care populations removed at 53 hours after pairing (No Care parents with a No Care regime) = NC_{NC}
4) Adults from No Care populations allowed to provide full parental care (No Care parents with a Full Care regime) = NC_{FC}

When larval development was complete, and larvae were starting to disperse away from the remains of the carrion nest (8 days after pairing), larvae were put in pupation boxes to mature and reach eclosion, as described above.

Experimental generation 2: Exposure to heat stress

Immediately after eclosion, each experimental individual was placed alone in a small plastic box (12 x 8 x 6 cm) containing a fine layer of soil. The boxes were placed in incubators (Panasonic MLR-352H-PE) set to either 22 °C, 25 °C or 28 °C, 0% humidity and a 16L: 8D hour light cycle. The boxes were removed briefly once weekly so that the beetles could be fed with beef mince. We then measured the following correlates of fitness for this generation of beetles:

Survival

To measure the effect of heat stress on survival, after 14 days (at sexual maturity) all individuals were removed from their incubators and classified as alive or dead.

The surviving individuals were then assigned to one of the following treatments for breeding:

5) 22 °C male x 22 °C female
6) 25 °C male x 25 °C female
7) 28 °C male x 28 °C female

199 Individuals were only bred within their populations and temperatures, e.g. 22 °C males from
200 FC_{FC} were only bred with 22 °C females from FC_{FC}, resulting in 12 treatments (Figure S1).
201 Pairs were placed in breeding boxes containing 350 ml of moist soil and a 10-13 g mouse
202 carcass, and the box was sealed and kept in a dark environment, as described under 'general
203 methods' above. All pairs were kept at the standard laboratory temperature of 22 °C, regardless
204 of their temperature treatment, to isolate the effects of the thermal environment during sexual
205 maturation on subsequent reproductive success.
206
207 ***Lytic activity***
208
209 At 53 h after pairing, we collected anal exudates from parents from a subsample of broods from
210 each treatment (n = 27 FC_{FC}, 27 FC_{NC}, 22 NC_{NC}, 19 NC_{FC}) by holding each beetle by the
211 abdomen and pressing a 5 ul glass microcapillary tube (Drummond) to its anus. Each sample
212 was pipetted immediately into a 1.5 ml Eppendorf tube on dry ice and subsequently stored at -
213 80 °C until analysis. We quantified the lytic activity of each sample in an automated microplate
214 reader (BioTek ELx808) by running turbidity assays that measured the degradation of bacterial
215 cell walls (see Duarte et al., 2021 for full details). Briefly, 1 ul of each exudate sample was
216 diluted in 24 ul of 6.4 pH 0.02 M potassium phosphate buffer and 10 ul of this solution was
217 added to a well of a 96-well flat-bottomed microplate (Thermo Scientific Nunc MicroWell)
218 containing 100 ul of a 1.3 mg ml^{-1} solution of lyophilized *Micrococcus lysodeikticus* cells in
219 potassium phosphate buffer. The microplate reader was set to 25 °C and took an initial reading
220 at 450 nm. Plates then went through a cycle of being shaken at a medium setting every 10
221 minutes, followed immediately by a 450 nm reading, for 60 minutes.
222
223 ***Carcass sphericity***

At 53 h, we also briefly removed the carcass from a subsample of broods (n = 33 FC_{FC}, 31 FC_{NC}, 33 NC_{NC}, 33 NC_{FC}) where the parents had experienced the 22 °C and 28 °C degree treatments. Each carcass was placed on a white background, alongside a scale for measurement, and photographed from perpendicular angles using two cameras (Canon DSLR, with 18-55mm lens) at 30 cm away from the carcass (Figure S2).

At 53 h after pairing, we removed the parents from all broods and counted the number of eggs laid, through inspection of the underside of the box (following Jarrett et al., 2017; Schrader et al., 2015c). At 70, 80, 94 and 104 h after pairing we checked the carcass and the surface of the soil for any first instar larvae, and counted them before discarding them.

Statistical analyses

All statistical tests were conducted in R version 3.6.1 (R Core Team, 2019) and a 0.05 significance threshold was used throughout. Data handling and visualisation were carried out using base R and the 'tidyverse' suite of R packages (Wickham et al., 2019). A stepwise deletion method using F and chi-squared tests (as appropriate), implemented in the base 'statistics' package in R, was used to determine significance of each term and remove non-significant terms sequentially (Crawley, 2007). For all analyses, temperature was modelled first as a continuous variable and then as a factor and the model fit examined. Model fit was best when temperature was treated as a continuous variable so it was treated as such in all the models presented here.

Survival

We ran a Gaussian linear model with survival status as the dependent variable ("0" = died in the incubator, "1" = survived to sexual maturity), and population evolutionary history (Full Care or No Care), generation 1 care regime (Full Care or No Care), temperature, sex, block and the two-way interactions of these terms as the independent variables.

Lytic activity

In some of the broods only one of the parents produced enough exudates to allow us to conduct the lytic activity analysis on their sample. The total useable sample size for exudates was 150 individuals, and for 110 of these we had a sufficient sample from their partner (55 pairs) and in the other 40 only one partner in the pair could be analysed (we could not collect enough exudate from the partners of 20 females and 20 males used in the analysis).

We used the percentage change between the 450 nm absorption at the first reading (0 minutes) and at 60 minutes as the dependent variable (as per Duarte et al., 2021). We first ran a paired t-test to determine whether there was a consistent sex difference within pairs in lytic activity. As the lytic activity of one individual in a pair may influence its partner's levels, we then ran separate binomial generalised linear models for males and females, with proportion of cells degraded (450 nm reading at 60 minutes/450 nm reading at 0 minutes) in either males' or females' anal exudate as the dependent variable, and population evolutionary history (Full Care or No Care), generation 1 care regime (Full Care or No Care), temperature, partner's lytic activity (if data were available), block, and the two-way interactions between temperature and evolutionary history, and sex and temperature as independent variables.

274 ***Carcass sphericity***

275

276 Carcass sphericity was calculated digitally in ImageJ (Schneider et al., 2012), using a macro
277 script adapted from De Gasperin et al (2016) (Script S1 and Figure S3). The ImageJ script
278 assumes that if a carcass were perfectly spherical, the two-dimensional images in the carcass
279 from the top and side would be exact circles. The script compares the actual images to a perfect
280 circle of the same perimeter and then calculates the difference between the two – such that a
281 perfectly spherical carcass would have a score of 1 to denote that it fills 100% of the space of
282 a perfect sphere (Figure S4).

283

284 We ran a binomial GLM with carcass sphericity as the dependent variable and population
285 evolutionary history (Full Care or No Care), generation 1 care regime (Full Care or No Care),
286 temperature, sex, block, and the two-way interactions of these terms as the independent
287 variables.

288

289 ***Failure to lay, clutch size, hatching failure and brood size***

290

291 To investigate predictors of fecundity, we first ran a binomial GLM with clutch failure ("0" =
292 no eggs laid and "1" = at least one egg laid) as the dependent variable and population
293 evolutionary history (Full Care or No Care), generation 1 care regime (Full Care or No Care),
294 temperature, carcass mass, block, and two-way interactions as listed in Table 4A as
295 independent variables.

296

297 Then to examine predictors of clutch size in the broods that did lay eggs, we ran a Gaussian
298 GLM with clutch size (the number of eggs counted from the underside of the breeding box) as

the dependent variable, and temperature, carcass mass, block, carcass roundness, and two-way interactions as listed in all two-way interactions as listed in Table 4B as independent variables.

We then ran two tests to investigate predictors of fertility. Firstly, we ran a binomial GLM with brood success ("0" = no larvae, "1" = at least one larva alive at dispersal) as the dependent variable and the same independent variables that were used in the binomial model analysing clutch failure vs success. Next, we ran a quasi-Poisson GLM with brood size (number of larvae counted on the carcass or soil) as the dependent variable and population evolutionary history (Full Care or No Care), generation 1 care regime (Full Care or No Care), temperature, carcass mass, block, clutch size, and two-way interactions as listed in all two-way interactions as listed in Table 5B as independent variables.

Results

Survival

Beetles from populations with an evolutionary history of No Care were more likely to die in the incubators ($\chi^2_{1, N=1218} = 20.244$, $p = <0.001$, Table 1), regardless of the temperature they experienced in the incubator or the care they received in generation 1 of the heat stress experiment. Mean survival of Full Care individuals across blocks was 94%, but 87% for No Care individuals. Beetles exposed to greater heat stress as they matured sexually were more likely to die, but only in block 2. Temperature had no equivalent effect on mortality in block 1 (block x temperature interaction: $\chi^2_{1, N=1218} = 17.284$, $p = <0.001$, Table 1; Figure 1).

324

325 *Table 1 – Results of binomial model investigating the effects on likelihood of a beetle surviving from*

326 *eclosion to sexual maturity in incubators set to 22 °C, 25 °C or 28 °C.*

Independent variable	**Estimate**	**SE**	χ^2	**df**	***p***
Intercept	7.628	2.716			
Evolutionary History (No Care)	**-1.008**	**0.231**	**20.244**	**1**	**<0.001**
Generation 1 Parental Care Regime (none)	-0.223	0.223	1.008	1	0.316
Temperature (°C)	**-0.137**	**0.104**	**109.86**	**1**	**<0.001**
Block (2)	**14.322**	**3.733**	**74.003**	**1**	**<0.001**
Sex (male)	0.076	0.222	0.117	1	0.733
Evolutionary History (No Care): Temperature (°C)	0.111	0.161	0.492	1	0.483
Gen 1 Parental Care Regime (none): Temperature (°C)	0.141	0.137	1.089	1	0.297
Temperature (°C):Sex (male)	0.113	0.133	0.735	1	0.391
Evolutionary History:Sex	-0.513	0.461	1.245	1	0.265
Gen 1 Parental Care Regime (none):Sex (male)	0.389	0.452	0.744	1	0.388
Gen 1 Parental Care Regime (none):Block (2)	0.160	0.589	0.074	1	0.785
Evolutionary History (No Care):Block (2)	-0.377	0.571	0.514	1	0.426

Temperature (°C):Block (2)	**-0.607**	**0.140**	**17.284**	**1**	**<0.001**
Sex (male):Block (2)	0.243	0.581	0.175	1	0.676

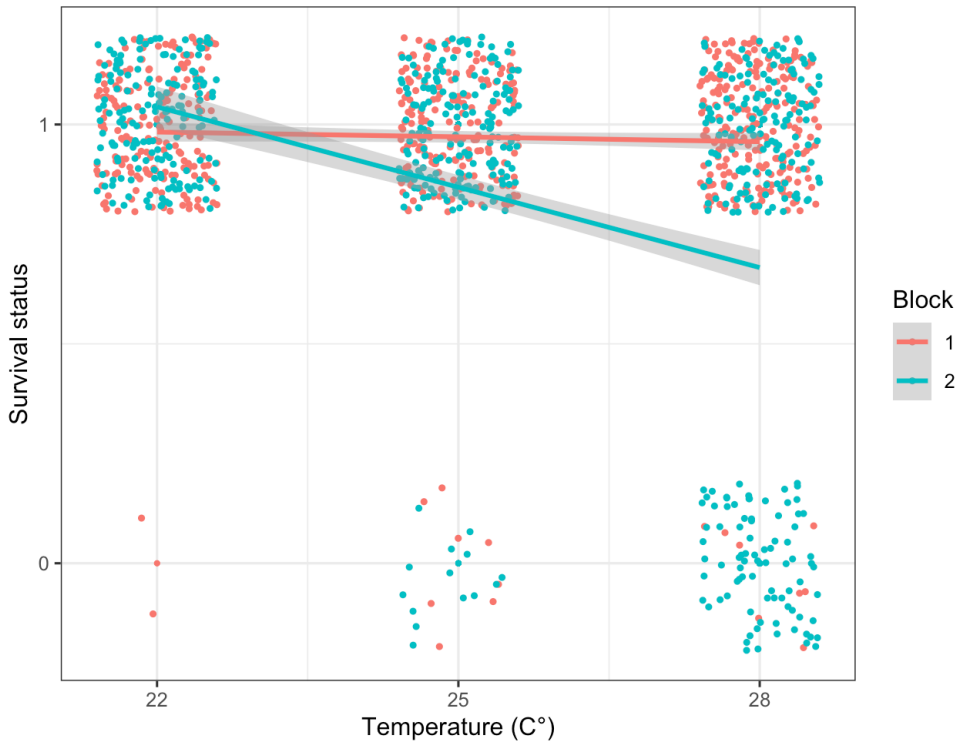

Figure 1 – The survival of beetles kept in incubators at different levels of heat stress throughout sexual maturation (1 = survived, 0 = died) in relation to block. Points represent data for individual pairs, lines represent the reaction norms for each block (1 = red, 2 = blue) and the shaded areas indicate the 95 % confidence intervals. N = 1,281 individuals.

To further investigate how block affected survival at higher temperatures we split the dataset by block and ran generalised linear models with survival probability as the dependent variable and temperature as the independent variable. In block 1 temperature did not significantly affect survival, but in block 2 there was a significant negative effect ($\chi^2_{1, N=600} = 122.19$, $p = <0.001$)

340

341 ***Lytic activity***

342

343 There was no difference between males and females in lytic activity strength: $t(54) = 0.446$, p

344 $= 0.658$). Lytic activity in both sexes was only significantly affected by block, with individuals

345 having stronger lytic activity in block 2 (Figure S5, Table 2).

346

347

348 *Table 2 – Results of binomial GLMs investigating the effects on A) male and B) female exudate lytic activity at 53h after pairing. We have defined lytic activity*

349 *as the proportion of cell degradation of* Micrococcus lysodeikticus *as recorded at 450 nm after 60 minutes*

	A) Male exudate					**B) Female exudate**				
Independent variable	**Estimate**	**SE**	χ^2	**df**	***p***	**Estimate**	**SE**	χ^2	**df**	***p***
Intercept	0.065	0.026				0.067	0.028			
Female Exudate Lytic Activity	-0.003	0.002	1.405	1	0.236	-0.003	0.003	1.391	1	0.238
Evolutionary History (No Care)	0.033	0.041	0.648	1	0.421	-0.023	0.041	0.300	1	0.584
Generation 1 Parental Care Regime (none)	-0.048	0.041	1.063	1	0.303	0.022	0.042	0.290	1	0.590
Temperature	-0.006	0.007	0.793	1	0.373	-0.006	0.007	0.633	1	0.426
Carcass mass (g)	-0.020	0.029	0.484	1	0.487	-0.008	0.029	0.075	1	0.785
Block (2)	**0.164**	**0.034**	**22.906**	**1**	**<0.001**	**0.165**	**0.036**	**21.588**	**1**	**<0.001**

Evolutionary History (No Care): Generation 1 Parental Care Regime (none)	-0.003	0.083	0.001	1	0.970	0.049	0.082	0.358	1	0.550
Temperature: Evolutionary History (No Care)	-0.010	0.014	0.568	1	0.451	-0.005	0.014	0.146	1	0.703
Temperature: Generation 1 Parental Care Regime (none)	0.010	0.014	00.537	1	0.464	-0.008	0.014	0.350	1	0.554
Evolutionary History (No Care): Block (2)	0.023	0.082	0.082	1	0.774	-0.048	0.082	0.339	1	0.561

352 ***Carcass sphericity***

353

354 No variables tested explained a significant amount of variation in carcass sphericity (all *p*

355 values = >0.05; Table 3).

356

357

358 *Table 2 – Results of binomial GLM investigating variation in carcass nest roundness as a proxy for*

359 *carcass preparation success*

Independent variable	**Estimate**	**S.E.**	χ^2	**d.f.**	***p***
Intercept	-0.075	0.176			
Female Exudate Lytic Activity	-0.008	0.02	0.083	1	0.773
Male Exudate Lytic Activity	0.012	0.037	0.095	1	0.758
Evolutionary History (No Care)	0.026	0.610	0.002	1	0.966
Generation 1 Parental Care Regime (none)	-0.208	0.692	0.091	1	0.763
Temperature	-0.084	0.059	2.029	1	0.154
Carcass mass (g)	-0.094	0.490	0.037	1	0.848
Block (2)	0.227	1.205	0.036	1	0.851
Evolutionary History (No Care): Generation	0.333	1.379	0.058	1	0.809

1 Parental Care Regime (none)					
Temperature: Evolutionary History (No Care)	-0.058	0.245	0.056	1	0.814
Temperature: Generation 1 Parental Care Regime (none)	0.018	0.243	0.006	1	0.941
Carcass mass: Evolutionary History (No Care)	0.310	0.966	0.103	1	0.749
Carcass mass: Generation 1 Parental Care Regime (none)	-0.056	1.106	0.003	1	0.960

Failure to lay eggs and clutch size

The population's evolutionary history of care (Full Care or No Care) did not explain significant variation in clutch size, and nor did the care regime experienced in the first generation of the heat stress experiment (Table 4A). Exposure to higher temperatures during sexual maturation were more likely to cause complete failure to lay, and there was a greater chance of complete laying failure in block 2 (Table 4A, Figure 2). In broods where some laying occurred, higher temperatures led to fewer eggs laid per clutch ($F_{1,304} = 166.8$, $p = <0.001$, Table 4B, Figure S6).

371

372 *Table 3 – Results of GLMs looking at variables affecting A) failure to lay: no eggs produced versus at*

373 *least one egg laid and B) clutch size for pairs that laid at least one egg*

A) Failure to lay					
Independent variable	**Estimate**	**S.E.**	χ^2	**d.f.**	***p***
Intercept	39.167	4.828			
Evolutionary History (No Care)	0.457	0.369	1.545	1	0.214
Generation 1 Parental Care Regime (none)	-0.041	0.364	0.013	1	0.910
Temperature	**-1.383**	**0.173**	**183.99**	**1**	**<0.001**
Carcass mass (g)	-0.140	0.269	0.270	1	0.603
Block (2)	**-2.082**	**0.422**	**30.439**	**1**	**<0.001**
Evolutionary History (No Care): Temperature	0.464	0.321	2.219	1	0.136
Gen 1 Parental Care Regime (none): Temperature	0.641	0.392	3.450	1	0.063
Evolutionary History (No Care): Gen 1 Parental Care Regime (none)	0.630	0.783	0.653	1	0.419
Block (2):Temperature	4.967	347.908	1.760	1	0.185
B) Clutch size					
Independent variable	**Estimate**	**S.E.**	**F**	**d.f.**	**p**
Intercept	86.468	5.153			

Evolutionary History (No Care)	2.234	2.734	0.668	1	0.421
Generation 1 Parental Care Regime (none)	-1.243	0.903	1.898	1	0.169
Temperature	**-2.717**	**0.210**	**166.8**	**1**	**<0.001**
Carcass mass (g)	-0.422	2.511	0.028	1	0.868
Carcass roundness	1.722	4.733	0.132	1	0.717
Block (2)	1.534	2.606	2.393	1	0.125
Male exudate lytic activity	0.205	0.154	1.774	1	0.193
Female exudate lytic activity	0.067	0.254	0.069	1	0.796
Evolutionary History (No Care): Temperature	-1.817	0.990	3.369	1	0.076
Gen 1 Parental Care Regime (none): Temperature	0.161	1.890	0.007	1	0.934
Evolutionary History (No Care): Gen 1 Parental Care Regime (none)	5.602	5.423	1.067	1	0.311
Evolutionary History (No Care): Carcass roundness	-12.378	27.842	0.001	1	0.972
Gen 1 Parental Care Regime (none): Carcass roundness	14.780	9.143	2.613	1	0.110
Block (2):Temperature	-0.776	0.999	0.604	1	0.444

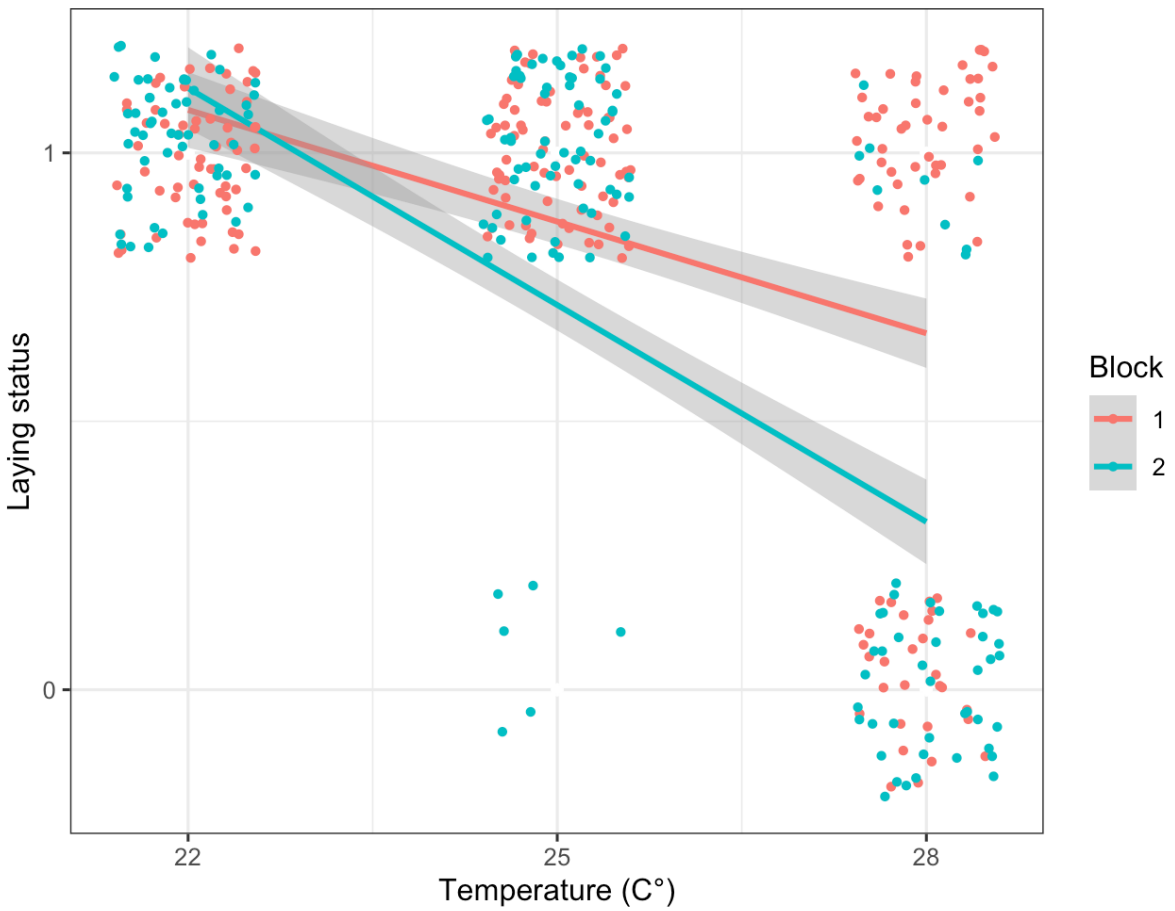

Figure 2 – The effect of temperature and block on laying status of pairs of beetles kept in incubators of 22 °C, 25 °C and 28 °C during sexual maturation (1 = at least 1 egg laid, 0 = no eggs laid). Each point represents a different clutch, lines represent the reaction norms for each block (1 = red, 2 = blue) and the shaded areas represent the 95 % confidence intervals. N = 383 clutches.

Hatching failure and brood size

There was no significant difference in hatching failure mediated by parents' evolutionary history (Table 5A). Parents kept at higher incubator temperatures between eclosion and sexual maturity were more likely to experience complete hatching failure (Figure 3, Table 5A) - with no eggs hatching in any of the broods where the parents had developed at 28 °C.

390 Fewer larvae hatched when parents had been kept at 25 °C than 22 °C ($F_{1, N=199} = 186.72$, $p =$
391 <0.001) (Figure S7), more larvae hatched in block 2, and more larvae hatched when there were
392 more eggs to start with ($F_{1, N=199} = 38.935$, $p =$ <0.001, Table 5B).
393
394
395 *Table 4 - Results of GLMs looking at variables affecting A) hatching success (at least one larva) or*
396 *failure (no larvae) and B) brood size for pairs where at least one larva hatched*

A) Hatching failure					
Independent variable	**Estimate**	**S.E.**	χ^2	**d.f.**	***p***
Intercept	46.570	8.380			
Evolutionary History (No Care)	-0.238	0.341	0.486	1	0.486
Generation 1 Parental Care Regime (none)	-0.330	0.343	0.931	1	0.335
Temperature	**-1.849**	**0.335**	**323.41**	**1**	**<0.001**
Carcass mass (g)	-0.338	0.233	2.138	1	0.144
Block (2)	0.579	0.340	2.946	1	0.086
Evolutionary History (No Care): Temperature	4.961	314.209	1.569	1	0.210
Gen 1 Parental Care Regime (none): Temperature	-5.158	482.152	1.298	1	0.255
Evolutionary History (No Care): Gen 1 Parental Care Regime (none)	-0.738	0.690	1.149	1	0.284

Block (2):Temperature	-5.420	766.035	1.152	1	0.283
B) Brood size					
Independent variable	**Estimate**	**S.E.**	**F**	**d.f.**	***p***
Intercept	10.392	0.685			
Evolutionary History (No Care)	0.084	0.070	1.428	1	0.234
Generation 1 Parental Care Regime (none)	-0.032	0.070	0.208	1	0.649
Temperature	**-0.366**	**0.030**	**186.72**	**1**	**<0.001**
Carcass mass (g)	-0.082	0.048	2.872	1	0.092
Block (2)	**0.289**	**0.071**	**16.917**	**1**	**<0.001**
Clutch size	**0.029**	**0.005**	**38.935**	**1**	**<0.001**
Evolutionary History (No Care): Temperature	0.035	0.060	0.349	1	0.555
Gen 1 Parental Care Regime (none): Temperature	-0.044	0.059	0.560	1	0.455
Evolutionary History (No Care): Gen 1 Parental Care Regime (none)	-0.133	0.140	0.901	1	0.344
Block (2):Temperature	0.065	0.060	1.163	1	0.282

397

398

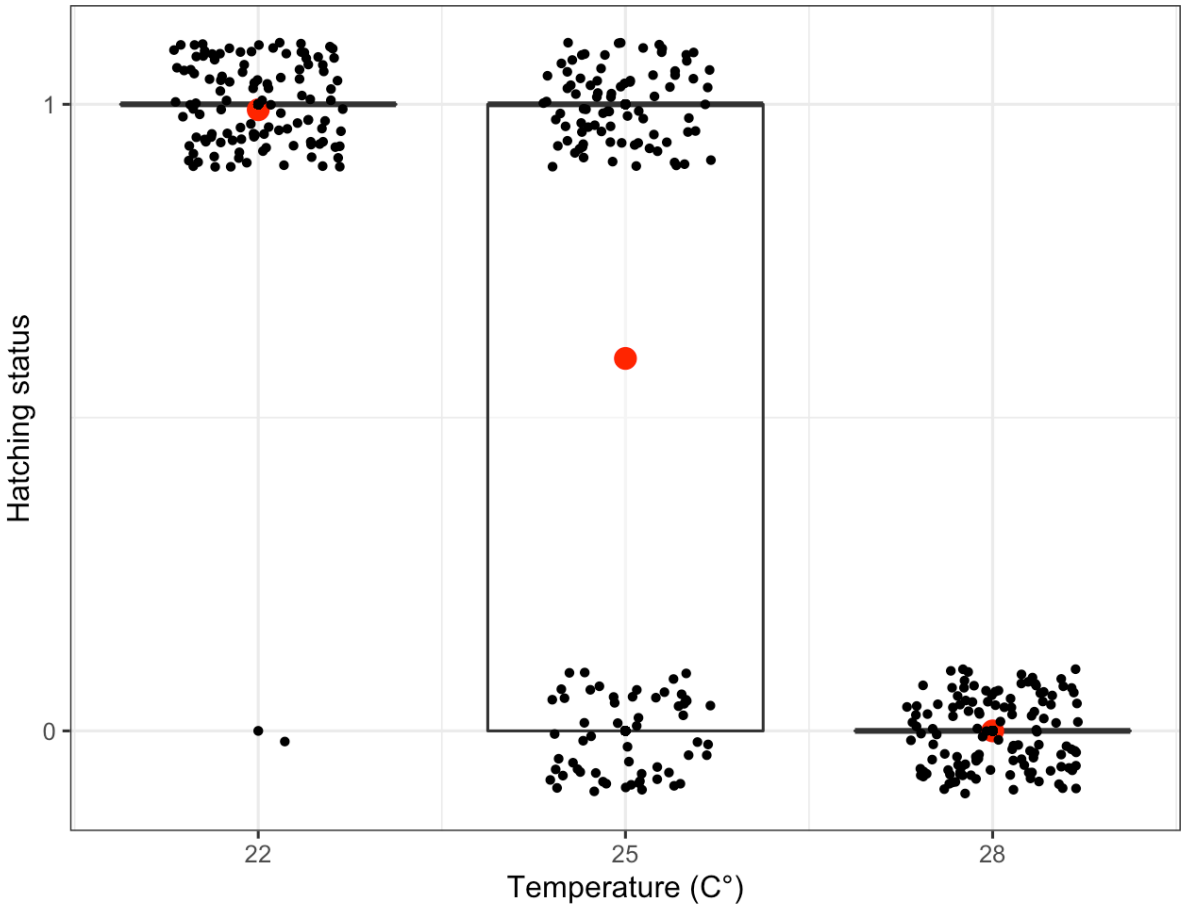

Figure 3 – The effect of incubator temperature during sexual maturation on hatching failure (1 = at least one larva hatched) or failure (0 = no larvae hatched). Each black datapoint represents one clutch, red points show the mean for each temperature treatment, the thick black lines represent the median for each treatment. N = 383 clutches.

Discussion

We tested experimentally whether a population's past sustained exposure to benign versus harsh social environments predicted its capacity to cope with heat stress during sexual maturation. The key findings of our experiments are summarised in Table 6, for each of the correlates of fitness we measured.

Table 6 – The key predictors tested, their effects on correlates of fitness measured, the slope estimates for each significant variable, and the hypothesis supported by the findings. "None" indicates that the predictor variable did not have a significant effect on the correlate of fitness. "Est" refers to the slope estimate for the predictor in the minimum adequate model. Full statistical results are presented in Tables 1-5.

Predictor variables tested	**Effect on correlates of fitness**							**Hypothesis supported**
	Survival	**Lytic activity**	**Carcass sphericity**	**Failure to lay**	**Clutch size**	**Hatching failure**	**Brood size**	
Population of origin (No Care or Full Care)	Lower survival in No Care (est: -1.008)	None	None	None	None	None	None	Beneficial mutation
Block	No effect in block 1, lower survival at higher temperature	Stronger lytic activity in block 2 (est for males:	None	Higher failure in block 2 (est: -2.082)	None	None	Larger broods in block 2 (est: 0.289)	Founder effect

	in block 2 (est for block 2: -0.723)	0.164; for females: 0.165)						
Temperature	Lower survival at higher temperature in block 2 (as cell above)	None	None	Higher failure at higher temperature (est: -1.383)	Smaller clutch size at higher temperature (est: -2.717)	Higher hatching failure at higher temperature (est: -1.849)	Smaller brood size at higher temperature (est: -0.366)	N/A

416

417

418 We found that the more benign the social environment previously experienced by a population
419 the better its survival during sexual maturation – which is consistent with the "beneficial
420 mutation hypothesis". Individuals derived from the No Care populations had poor survival in
421 the incubators, even in the most benign thermal environment, compared with those from the
422 Full Care populations. We have previously shown that individuals in No Care populations carry
423 a lower mutation load (Pascoal et al. 2022). Perhaps this means that, by chance, they lack the
424 cryptic genetic variation that enables them to cope with new levels of thermal stress during
425 sexual maturation (Paaby & Rockman, 2014). Alternatively, perhaps the No Care populations
426 were intrinsically inferior by this point, and more prone to dying between eclosion and sexual
427 maturity, regardless of the temperature to which they were exposed. For all other correlates of
428 fitness we could detect no differences between individuals drawn from the No Care versus Full
429 Care populations, which argues against this interpretation. Overall, our experimental data allow
430 us to reject the "mutation load hypothesis" and the "selection filter hypothesis". But perhaps
431 we have insufficient data to conclude we have found strong support for the "beneficial mutation
432 hypothesis".
433
434 Unexpectedly, we found that experimental block predicted some correlates of fitness (Table
435 1), which could be due stochastically to a founder effect. The four distinct evolving populations
436 were established from a single large stock population derived by interbreeding beetles from
437 four different wild populations. The size of the breeding population was identical for each
438 block throughout the experiment (n = 100 pairs), as was the maintenance regime, which implies
439 that any differences between blocks must be attributable to chance differences in genetic
440 variation when the population was founded. In analyses of earlier generations of the
441 experimentally evolving populations, we found that selection from social environment
442 explained divergence between populations in the traits we analysed to a greater extent than

443 block (e.g. Duarte et al., 2021; Jarrett et al., 2018a, 2018b; Schrader et al., 2015c, 2015a, 2015b,
444 2017, 2017). By generation 49, however, we find that founder effects are outweighing
445 differences due to selection from the social environment – at least on the response to heat stress
446 exposure during sexual maturation.
447
448 Founder effects have long been suggested to explain the success of translocation and captive
449 breeding in conservation programmes (e.g. Brown et al., 2009; Dolman et al., 2021; Miller et
450 al., 1999). However, in analyses of wild populations it is often hard to disentangle the scale of
451 founder effects from confounding factors of environmental differences (however subtle) in the
452 new location (Kolbe et al., 2012; Szűcs et al., 2017) especially when it is difficult to monitor
453 populations systematically after their release into the wild (Armstrong & Seddon, 2008; Mock
454 et al., 2004; Seddon et al., 2007; Weeks et al., 2011). Nevertheless, there is some evidence that
455 founder effects influence the response to selection in wild populations. When *Anolis sagrei*
456 individuals with long hindlimbs were translocated to seven Bahamian islands with a vegetation
457 structure known to favour short hindlimbed individuals, they evolved shorter hindlimbs over
458 four years. However the extent of morphological change was attributable to founder effects
459 (Kolbe et al., 2012). Perhaps a similar pattern explains the results we have found in this study.
460 More broadly our study indicates the importance of the initial population size in captive
461 breeding programmes. It also reinforces the wisdom of using genetic screening to determine
462 whether the founding population is representative of the source population and has high
463 heterozygosity (Bragg et al., 2021; Scott et al., 2020), especially in cases where there will no
464 migration into the population, such as on an isolated island.
465
466 For some of the traits we measured (failure to lay, clutch size, failure to hatch) temperature had
467 such a strong negative effect that it outweighed any effects that could be attributed to the

468 population's genetic background. This is consistent with previous work across different
469 species of burying beetle, which found that high temperatures during breeding led to reduced
470 clutch and brood sizes and a greater likelihood of complete brood failure (Keller et al., 2021;
471 Moss & Moore, 2021; Ney, 2021; Ong, 2019; Quinby et al., 2020; Sun & Kilner, 2020).
472 However, in all of these studies the extreme temperature treatment continued throughout
473 breeding, whereas in this study we elevated temperature only during sexual maturation to better
474 simulate the natural history of the burying beetle. Our results suggest that there are sensitive
475 windows in development which affect breeding success even when earlier stages of
476 reproduction have occurred under benign conditions. In a world with an increasing frequency
477 and intensity of heat waves (Perkins-Kirkpatrick & Lewis, 2020), this study shows that an
478 extreme temperature event can also reduce breeding success even if it is only brief, as long as
479 it coincides with these sensitive windows in development.
480
481 There are two likely causes for a reduction in egg laying and egg hatching at higher
482 temperatures: 1) behavioural – beetles maturing at higher temperatures mated less, and/or 2)
483 physiological – beetles maturing at higher temperatures had underdeveloped gonads.
484 Particularly in males, exposure to high temperatures prior to pairing leads to reduced courtship
485 behaviour in *Aphidius avenae* (Roux et al., 2010) and *Drosophila melanogaster, Drosophila*
486 *simulans and Drosophila mojavensis* (Patton & Krebs, 2001). There is also a great deal of
487 evidence to suggest that thermal stress during sexual maturation can have a physiological effect
488 on insects. In *Drosophila melanogaster* high temperatures during development cause a range
489 of spermatid cytological abnormalities (David et al., 2005; Rohmer et al., 2004) and in
490 *Tribolium castaneum* cause lower testis volume (Sales et al., 2021). In females, higher
491 temperatures during development cause smaller oocytes in *Pararge aegeria* (Gibbs et al., 2010)
492 and cause *Bicyclus anynana* to lay more, smaller eggs (Steigenga & Fischer, 2007). From this

study we are unable to say whether the effects were behavioural, physiological or both, and whether it was the males or females that drove the patterns of reduced reproductive fitness at higher temperatures. Further investigations tracking copulatory behaviour, mating thermally stressed males with control females and vice versa, and dissecting ovaries and testes following heat stress are needed to answer these questions.

It is widely accepted that a population's standing genetic variation will predict its response to new environmental challenges. Our study suggests that the social evolutionary history of burying beetle populations might predict the response to heat stress during sexual maturation to some extent, although further work is needed before a robust conclusion can be drawn. However, stochastic genetic variation, due to founder effects or genetic drift, was potentially a more powerful predictor of population resilience upon exposure to new environments. Translocation and captive breeding programmes should be designed accordingly to maximise their chance of success.

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Supplementary information

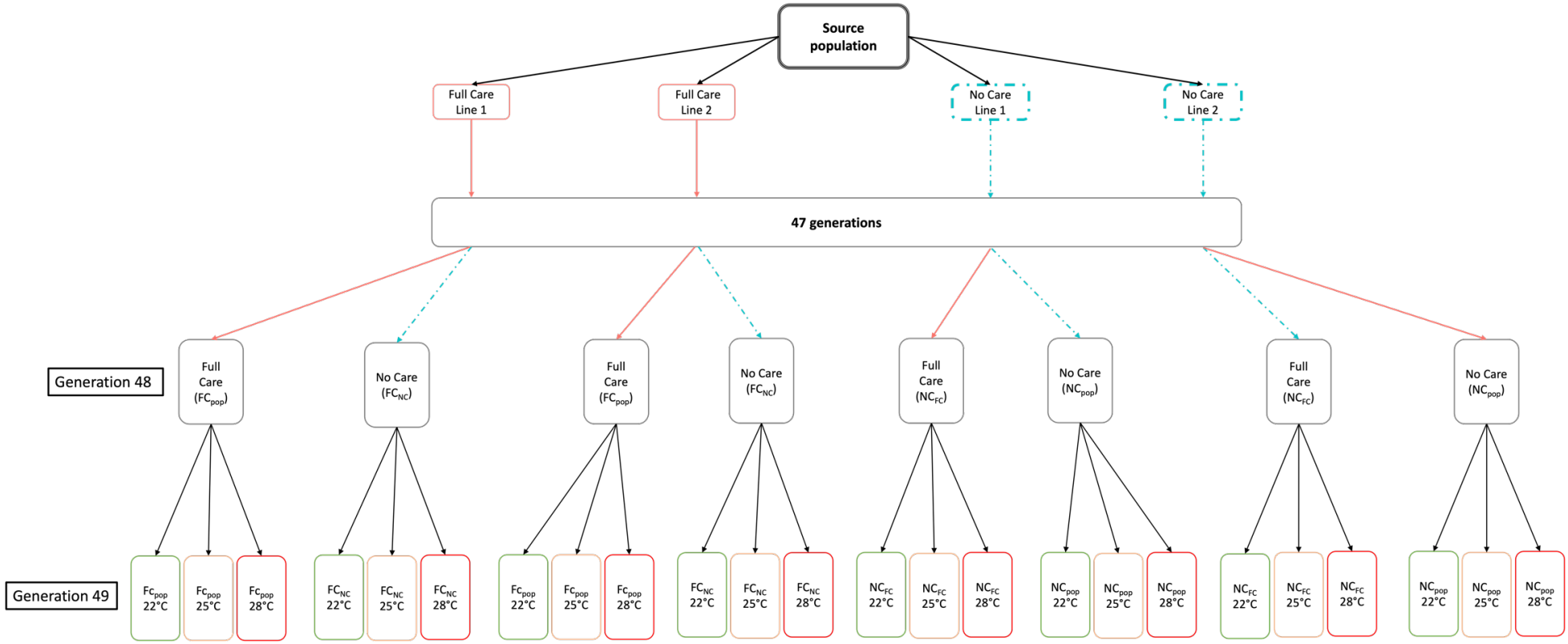


Figure S1 – Breeding protocol experimental design

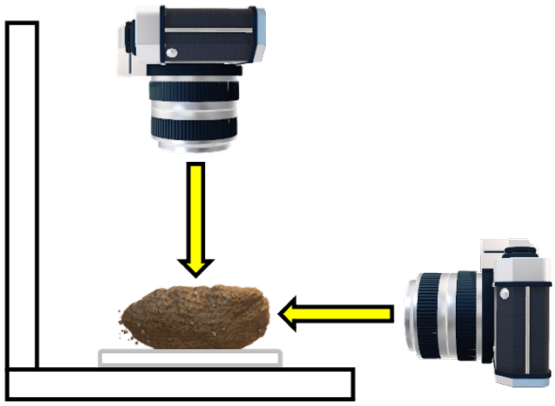


Figure S2 – Carcass photography protocol (not to scale). Two Canon DSLRs at a 90° angle to each other were used to photograph each carcass from 30 cm away against a white background. The carcass was placed on a clear plastic breeding individual box lid (12 x 8 x 1 cm) to avoid the white background getting contaminated with soil (the lid was wiped between carcasses to maintain a white background for the top image).

Script S1 – Carcass roundness script for calculating roundness in ImageJ. The script uses the blue channels from both the top and side photos (as this creates the greatest contrast between the carcass nest and the background), a threshold filter to separate the carcass from the background and a median filter to ignore the smallest black flecks of less than 25 pixels, in case any small soil marks remained.

```
//  topPath=File.openDialog("Select top-down image"); // get file location
 //  sidePath=File.openDialog("Select side image"); // get file location
Dialog.create("Settings");
Dialog.addMessage("Pixel/distance calibration:");
Dialog.addNumber("Top-down (pixels per mm)", 53.7523);
Dialog.addNumber("Side (pixels per mm)", 30.1806);
Dialog.addMessage("\nImage processing:");
Dialog.addNumber("Threshold", 70);
Dialog.addNumber("Smoothing radius (pixels)", 25);
Dialog.addMessage("\nCamera positioning:");
Dialog.addCheckbox("Flip image", true);
Dialog.show();
topPixelsMm = Dialog.getNumber();
sidePixelsMm = Dialog.getNumber();; thresholdVal = Dialog.getNumber();;; smoothingVal=
Dialog.getNumber();;;; flip = Dialog.getCheckbox();
// FILE LOCATIONS
```

```
topDir=getDirectory("Directory containing top-down images");
topFileList=getFileList(topDir);
sideDir=getDirectory("Directory containing side-on images");
sideFileList=getFileList(sideDir);
// select working directory // list of images in directory
// select working directory // list of images in directory
if(topFileList.length > sideFileList.length) // error if there are unequal numbers of files
exit("Error: unequal number of files in selected folders");
if(topFileList.length < sideFileList.length)
exit("Error: unequal number of files in selected folders");
print("\n_____");
print("SETTINGS");
print(" Threshold: " + thresholdVal);
print(" Smoothing Radius: " + smoothingVal);
// START OF BATCH PROCESSING LOOP
for(z=0; z<topFileList.length; z++1){ topPath = topDir+topFileList[z];
sidePath = sideDir+sideFileList[z];
// OPEN & PROCESS TOP IMAGE
open(topPath);
run("RGB Stack");
run("Delete Slice"); // only uses the blue channel (channel with highest contrast)
run("Delete Slice");
setTool(0); //Rectangle tool
waitForUser("Select the Target", "Draw a box to cover the mouse ball\nensure no other dark
objects are selected");
run("Crop");
if(flip==1); // if ticked, flips image so that both are the same way round
run("Flip Horizontally");
setThreshold(0, thresholdVal);
run("Convert to Mask");
medianScript = "radius="+smoothingVal+" slice";
run("Median...", medianScript); // threshold
w = getWidth(); // image dimensions
h = getHeight();
// SELECT CENTRE
doWand(w/2, h/2, 1, "4-connected");
List.setMeasurements; // save measurements of perimeter & area
topPerim = List.getValue("Perim.");
topArea = List.getValue("Area");
print("Top Perimeter: " + topPerim + " Top Area: " + topArea);
run("Make Inverse"); // invert selection & delete (gets rid of dirt spots etc..)
setForegroundColor(255, 255, 255);
run("Fill", "slice");
run("Make Inverse");
run("To Bounding Box");
run("Crop");
w = getWidth(); // image dimensions
h = getHeight();
topProfile = newArray(w);
for (x=0; x<w; x++){ // pixel column loop
```

```
topProfile[x] = 0;
for (y=0; y<h; y++){ // counts the number of white pixels in each column
} }
val = getPixel(x,y);
if(val==255)
topProfile[x] = topProfile[x] + 1;
topProfileCrop = newArray(0);
for(a=0; a<topProfile.length; a++){ if(topProfile[a]>0){
topProfileCrop = Array.concat(topProfileCrop, topProfile[a]);
} }
topProfileMm = newArray(topProfileCrop.length);
for(a=0; a<topProfileCrop.length; a++){
topProfileMm[a] = topProfileCrop[a] / topPixelsMm;
setResult("Top Pixels", a, topProfileCrop[a]);
setResult("Top Millimetres", a, topProfileMm[a]); }
close();
// OPEN & PROCESS SIDE IMAGE
// make array of millimetre values
open(sidePath);
run("RGB Stack");
run("Delete Slice"); // only uses the blue channel (channel with highest contrast)
run("Delete Slice");
setTool(0); //Rectangle tool
waitForUser("Select the Target", "Draw a box to cover the mouse ball\nensure no other dark
objects are selected");
run("Crop");
setThreshold(0, thresholdVal);
run("Convert to Mask");
medianScript = "radius="+smoothingVal+" slice";
run("Median...", medianScript); // threshold
w = getWidth(); // image dimensions
h = getHeight();
// SELECT CENTRE
doWand(w/2, h/2, 1, "4-connected");
List.setMeasurements; // save measurements of perimeter & area
sidePerim = List.getValue("Perim.");
sideArea = List.getValue("Area");
print("Side Perimeter: " + sidePerim + " Side Area: " + sideArea);
run("Make Inverse"); // invert selection & delete (gets rid of dirt spots etc..)
setForegroundColor(255, 255, 255);
run("Fill", "slice");
run("Make Inverse");
run("To Bounding Box");
run("Crop");
w = getWidth(); // image dimensions
h = getHeight();
sideProfile = newArray(w);
for (x=0; x<w; x++){ // pixel column loop
sideProfile[x] = 0;
for (y=0; y<h; y++){ // counts the number of white pixels in each column
```

```
888 } }
889 val = getPixel(x,y);
890 if(val==255)
891 sideProfile[x] = sideProfile[x] + 1;
892 sideProfileCrop = newArray(0);
893 for(a=0; a<sideProfile.length; a++){ if(sideProfile[a]>0){
894 sideProfileCrop = Array.concat(sideProfileCrop, sideProfile[a]);
895 } }
896 sideProfileMm = newArray(sideProfileCrop.length);
897 for(a=0; a<sideProfileCrop.length; a++){
898 sideProfileMm[a] = sideProfileCrop[a] / sidePixelsMm;
899 setResult("Side Pixels", a, sideProfileCrop[a]);
900 setResult("Side Millimetres", a, sideProfileMm[a]); }
901 // make array of millimetre values
902 close();
903 // CIRCUMFRENCE CALCULATION
904 arrayRatios = topProfileMm.length / sideProfileMm.length;
905 circumfrenceArray = newArray(sideProfileMm.length);
906 areaArray = newArray(sideProfileMm.length);
907 for(i=0; i<sideProfileMm.length; i++){
908 bLoc = round(arrayRatios*i);
909 b = topProfileMm[bLoc]/2;
910 a = sideProfileMm[i]/2;
911 circumfrenceArray[i] = PI * (a+b) * (1+(3*pow((a-b)/(a+b),2) / (10+pow(4-(3*pow((a-
912 b)/(a+b),2)),0.5))));
913 areaArray[i] = PI * a * b;
914 }
915 topLength = topProfileMm.length / topPixelsMm;
916 sideLength = sideProfileMm.length / sidePixelsMm;
917 aveLength = (topLength + sideLength)/2;
918 mmPerSlice = aveLength/sideProfileMm.length; // average length of mouse
919 VolumeSum = 0;
920 AreaSum = 0;
921 for(a=0; a<sideProfileCrop.length; a++){ // loop to count the area and volume of each slice
922 AreaSum = AreaSum + (circumfrenceArray[a]*mmPerSlice);
923 VolumeSum = VolumeSum + (areaArray[a]*mmPerSlice);
924 // setResult("Circumfrence", a, circumfrenceArray[a]);
925 }
926 EndArea = 0;
927 for(a=0; a<sideProfileCrop.length-1; a++)
928 EndArea = EndArea + (pow(pow(areaArray[a]-areaArray[a+1],2),0.5)); // sums end surface
929 values - always positive
930 AreaSum = AreaSum + EndArea;
931 // SPHERICITY CALCULATION - this takes the average value of the area to circumference
932 ratio of the top & side images
933 topCircleArea = PI*pow((topPerim/(2*PI)),2); // this calculates the area a circle would have of
934 the observed perimeter length
935 sideCircleArea = PI*pow((sidePerim/(2*PI)),2);
936 topRoundness = topArea / topCircleArea; // perfect circle area /observed area, so a perfect
937 circle would be 1, going down to zero with worse roundness
```

```
sideRoundness = sideArea / sideCircleArea;
sphericity = (topRoundness+sideRoundness)/2;
// PRINT RESULTS
print("\n.............................................................");
print("Top-down image: " + topFileList[z]);
print("Side-on image: " + sideFileList[z]);
print("Sphericity:\t"+ sphericity);
print("Volume (mm^3):\t"+VolumeSum);
} //end batch processing loop
// average roundness
```

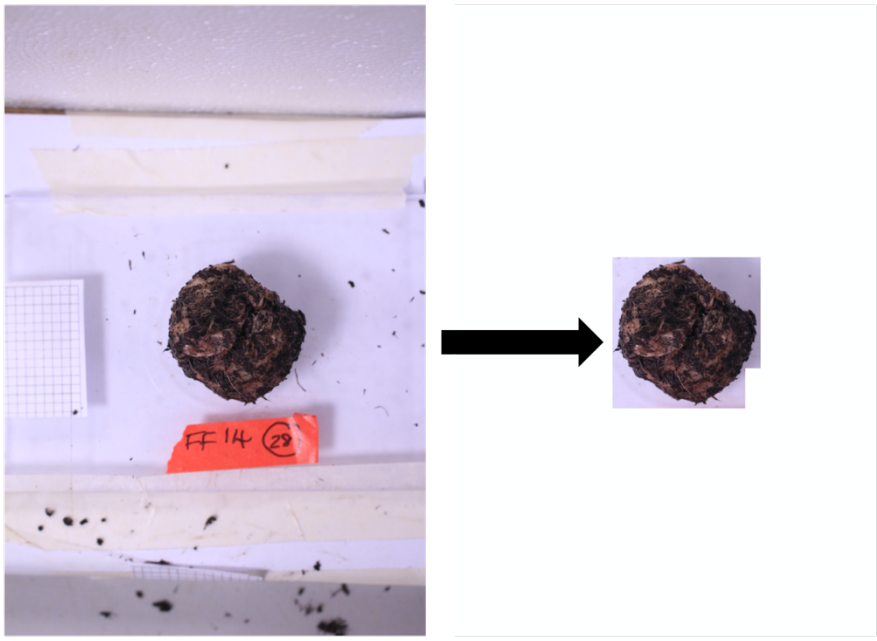


Figure S3 – The process of removing any dark background marks and identity labels, while ensuring that the size and proportion of the carcass ball remained the same, by digitally superimposing white rectangles over any dark objects in Apple Preview. Script S1 removes black flecks of less than 25 pixels but any larger dark shapes, such as clumps of soil, identity labels and unfurled tails, can influence the carcass roundness calculations so these were digitally removed using this process.

Figure S4 – An example of the variation in roundness between carcasses. The left image has a roundness value of 0.2957 and the right image has a value of 0.6031. In Script S1 both the top and side images were used to calculate roundness.

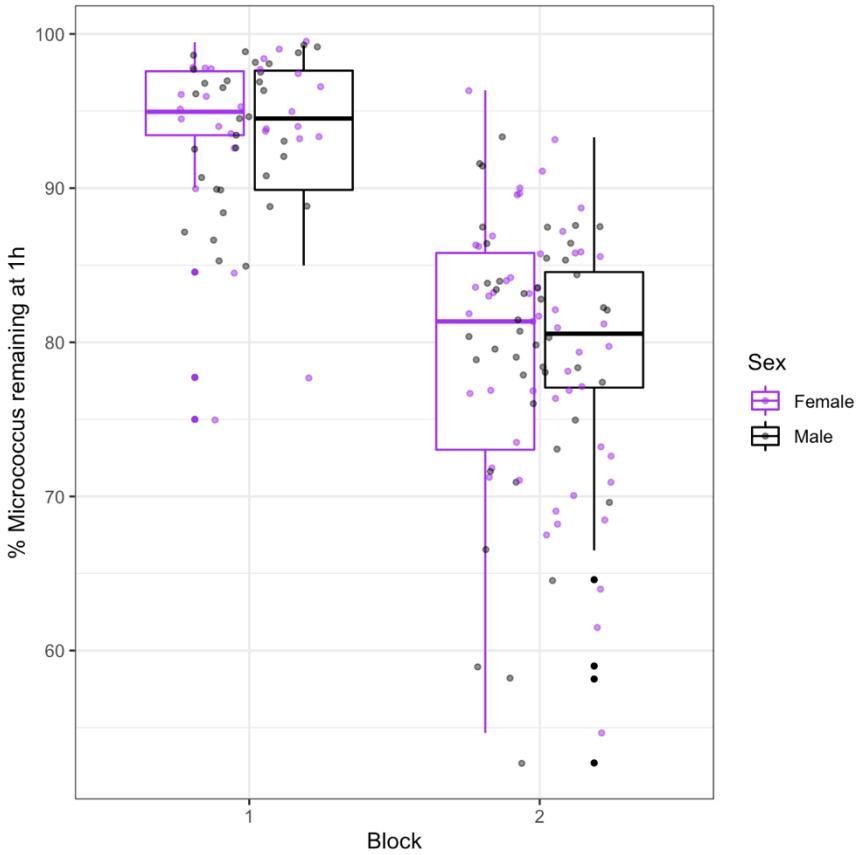


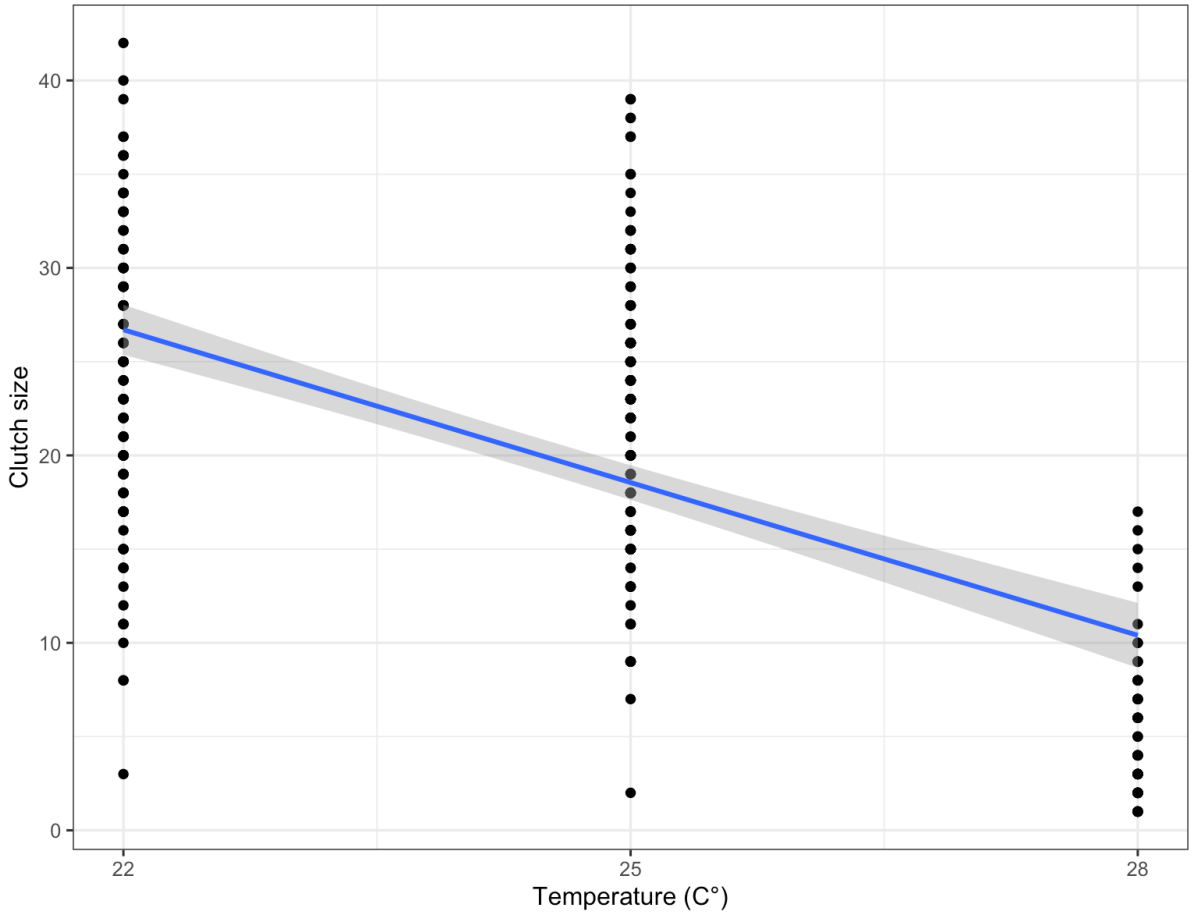
Figure S5 - The effect of experimental block on lytic activity of sexually mature beetles' anal exudate. Lytic activity is defined as the percentage of cell degradation of Micrococcus lysodeikticus *as recorded at 450 nm after 60 minutes. Higher remaining percentages of* M. lysodeikticus *remaining indicate lower exudate lytic activity. Each point represents one beetle (purple = females, black = males), thick*

969 *horizontal lines represent the median for each treatment, whiskers extend to the farthest data point*

970 *which is no more than 1.5 times the interquartile range from the box. N = 75 males and 75 females.*

971

972



973

974 *Figure S6 – The effect of incubator temperature during sexual maturation on clutch size for those pairs*

975 *that laid at least one egg. Each point represents a different clutch, the line represents the fit for the*

976 *linear model, and the shaded areas represent the 95 % confidence intervals. N = 304 clutches.*

977

978

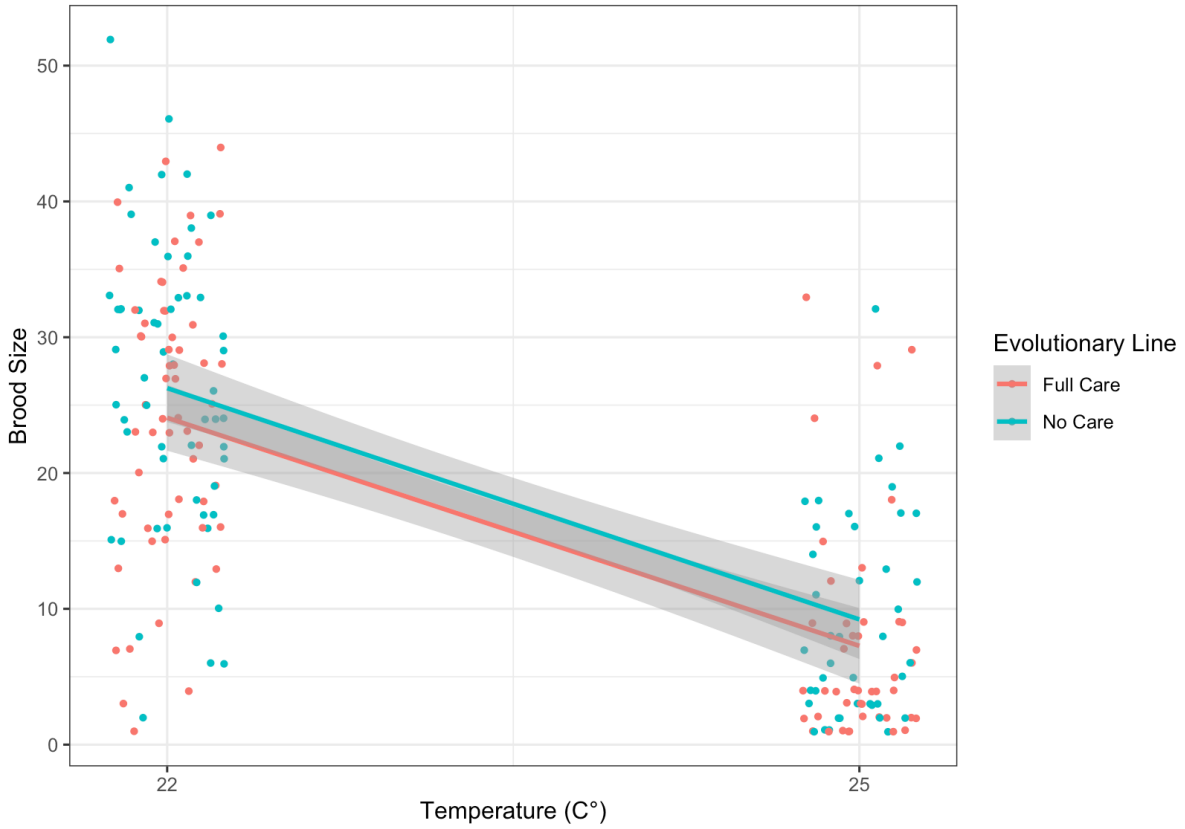


Figure S7 – The effect of temperature during sexual maturation and evolutionary population on the number of larvae hatched (brood size). No eggs successfully hatched from beetles kept at 28 °C, so this graph only shows data from 22 °C and 25 °C. Each datapoint represents one clutch, lines represent the reaction norms for evolutionary lines (red = Full Care, blue = No Care) and the shaded areas represent the 95 % confidence intervals. N = 383 broods.