Parental care maintains genetic variation by relaxing selection

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Abstract

Benevolent social behaviours, such as parental care, are predicted to relax selection against deleterious mutations, enabling them to persist. We tested this prediction experimentally using burying beetles *Nicrophorus vespilloides*, which make an edible nest for their larvae, whom they nourish and defend. For 20 generations, we allowed replicate experimental burying beetle populations to evolve either with post-hatching care ('Full Care' populations) or without it ('No Care' populations). Lineages were seeded from these experimental populations and then inbred to expose differences in their mutation load. Outbred lineages served as controls. Half the lineages received post-hatching care, half did not. We found that inbred lineages derived from the Full Care populations had lower breeding success and went extinct more quickly than lineages derived from the No Care populations – but only when offspring received no post-hatching care. We infer that Full Care lineages carried more recessive deleterious mutations. When parents provided care, the developmental environment was sufficiently benign that broods had higher survival, whether the population had a high mutation load or not. We suggest that the increased mutation load caused by parental care increases a population's dependence upon care. This could explain why care is seldom lost once it has evolved.

Introduction

Classical population genetics models imagine that populations attain an equilibrium level of genetic variation (known as mutation-selection balance [1-5]). New genetic mutations arise spontaneously, through diverse mechanisms, and increase genetic variation in the population [e.g. 5, 6]. However, since the majority of new mutations are mildly deleterious [e.g. 5, 6], they are quickly purged by natural selection. Mutation-selection balance is theoretically achieved when the rate of input of new genetic variants through spontaneous mutation is perfectly balanced by the rate of their elimination by selection [1-5].

The concept of mutation-selection balance has long been used as a theoretical reference point for understanding the effects of mutation rate on the health of human populations, partly because it is recognised that humans can modify their own environment and so change the forces of natural selection to which they are exposed [1-5]. Better quality housing, improved diets, and benevolent social activities, such as a welfare state or the universal provision of medical care, are suggested to have been particularly influential in preventing natural selection from purging deleterious mutations in human populations [1, 2, 4, 5]. Consistent with this suggestion, recent comparative genomic analyses have revealed a greater incidence of genetic pathologies in western industrialised populations than in traditional, pre-industrial human societies which are more exposed to natural selection [4, 5, 7-9]. Nevertheless, it is impossible to demonstrate that a more benign physical and social environment, in which selection is relaxed, has *caused* this difference.

Elaborate architecture, enhanced access to resources and benevolent social behaviours are relatively commonplace in other animals too, especially among the many bird, mammal and

insect species that cooperate with each other and live socially [10]. For these species, any *causal* effects of this social and physical environment on genetic variation can more easily be investigated. A complication in other animal societies, however, is that additional factors might perturb the mutation-selection balance. For example, animals that breed cooperatively also tend to produce fewer, larger offspring. This life history strategy is known to reduce genetic diversity [11] and could potentially oppose, or even conceal, any increases in genetic variation that are due to cooperation buffering the effects of natural selection. Cooperative animal societies are also commonly associated with a high incidence of reproductive skew. Since only a few dominant individuals are typically able to reproduce, the effective population size is greatly reduced [12]. This can lead to a reduction in the efficiency of natural selection and a greater influence of genetic drift [6], potentially confounding any increases in genetic variation that are due solely to relaxed selection. Similarly, animal societies typically comprise related individuals that derive kin-selected benefits from their cooperative social interactions. Theoretical analyses have shown that kin selection acts more weakly than direct selection [13]. Consequently, loci under kin selection are predicted to harbour more sequence variation than loci under direct selection [3, 13].

We tested the effect of kin-selected cooperative actions on the maintenance of genetic variation by focusing on parental care, a widespread form of cooperation [14]. Since care is commonly exhibited by pair-breeding individuals, this form of cooperation is unlikely to change effective population size – eliminating this potentially confounding effect. By building protective nests, defending their brood from attack and nourishing them, animal parents shield their young from environmental stressors [15] and weaken the correlation between the phenotypic variation seen by selection and the underlying genetic variation [3, 16]. In these ways, parents relax selection on the offspring phenotype [3, 15-18] theoretically allowing

88 mildly deleterious alleles to accumulate [3, 6, 15]. Previous experimental work with insects
89 has shown that parental care relaxes selection sufficiently that new mutants [15] and inbred
90 offspring [19] can survive - at least for one generation. However, it is not yet known whether
91 parental care also enables mildly deleterious mutations to persist over multiple generations.

92

93 We tested this prediction by evolving replicate laboratory populations of burying beetles
94 *Nicrophorus vespilloides* under sharply contrasting levels of parental care, for 20 generations.
95 Comparing populations within species also allowed us to eliminate the confounding effect of
96 propagule number on genetic diversity [11]. Burying beetles breed on the body of a small
97 dead vertebrate [20], which the parents jointly convert into a carrion nest by removing the fur
98 or feathers, rolling the flesh into a ball, covering it with anti-microbial anal exudates, and
99 burying it. This is pre-hatching parental care [21]. Parents also guard and feed larvae after
100 hatching, though larvae can survive in the lab with no post-hatching care at all [22]. In two of
101 our evolving populations, larvae were able to receive both pre-hatching and post-hatching
102 parental care (these were called the 'Full Care' lines) while in two other populations we
103 prevented parents from supplying any post-hatching care by removing them before the larvae
104 hatched, after the carrion nest was complete (these were called the 'No Care' lines). During
105 the first 20 or so generations of experimental evolution, No Care lines rapidly adapted to a life
106 without parental care [23], through divergent phenotypic change in both larval [e.g. 24]) and
107 parental [21] traits.

108

109 To determine whether parental care causes deleterious genetic variation to accumulate over
110 the generations, we inbred sub-populations, each derived from the replicate experimental
111 evolving populations, for 8 successive generations (we called this The Evolutionary History

Experiment). For these 8 generations, we measured the extent to which inbreeding reduced measures of reproductive success in comparison with control outbred populations. To determine whether parental care could temper the rate of extinction (as implied by [19]), in half of all our treatments parents were allowed to provide care after their offspring hatched, while in the remainder they were prevented from supplying post-hatching care. This generated 8 different treatments in total (see Supplementary Figure 1, for the design of the Evolutionary History Experiment).

We used the data from the Evolutionary History Experiment, to test three predictions: (1) that the No Care environment is harsher than the Full Care environment. (2) That the more benign conditions of the Full Care environment relax selection and promote the survival of more genetic variants. (3) That inbred populations from the Full Care lines should exhibit greater inbreeding depression than inbred populations from the No Care lines (having accumulated a greater number of deleterious recessive mutations under relaxed selection). The outbred populations acted as a control treatment for tests of all three predictions.

Methods

Nicrophorus vespilloides natural history.

The common burying beetle *N. vespilloides* breeds on a small dead vertebrate (like a songbird or mouse). The larvae hatch from eggs laid nearby in the soil and crawl to their carrion nest, which they can feed upon themselves [20]. Once at the carcass, larvae receive post-hatching biparental care. Parents supply fluids to their offspring through oral trophallaxis, and defend their brood and the carrion nest from attack by predators, microbes and rival beetles [20]. The duration and extent of post-hatching care are highly variable, however. For example, when

136 wild beetles are brought into the lab to breed, roughly 5% of larvae receive no post-hatching
137 care at all, yet larvae can still survive to become reproductively competent adults (e.g. [22,
138 25]. Within roughly a week of hatching, the larvae complete development and at this point
139 (which we refer to as 'dispersal), they start to crawl away from the scant remains of the
140 carcass to pupate in the soil. The parents, meanwhile, fly off in search of a new carcass.

141

142 **Experimental evolution**

143 The experimental lines used in this work have been described in detail elsewhere [e.g. 21, 24].
144 In brief, we established a large founding population of *N. vespilloides* by interbreeding wild-
145 caught individuals from four different woodlands. This was then divided into four
146 experimental lines. In two lines, larvae experienced 'Full Care' at each generation, with both
147 parents staying in the breeding box throughout the breeding bout and able to provide post-
148 hatching care as well as pre-hatching care. In the other two 'No Care' lines, parents engaged
149 in pre-hatching care but at each generation they were removed from the breeding box around
150 53 h after they were paired, so that they never interacted with their larvae. The work reported
151 here began when these lines had been exposed to 20 generations of experimental evolution
152 under these contrasting regimes of care.

153

154 **Evolutionary History Experiment**

155 **Preparatory common garden generation**

156 The experiment began by exposing individuals drawn from the four lines (Full Care replicated
157 twice and No Care replicated twice) to a common garden Full Care environment for one
158 generation (N = 60 pairs for each No Care line (to counter-balance the slightly lower breeding
159 success caused by the No Care environment) and N = 50 pairs for each Full Care line). In this

160 way, we minimised any potentially confounding transgenerational effects prior to starting the
161 Evolutionary History Experiment.

162 **Overview (see Supplementary Figure 1)**

163 Broods from the common garden generation were used to seed lineages in the experimental
164 treatments: broods derived from the Full Care populations (FC_{POP}) founded lineages that were
165 Inbred or Outbred, in either a Full Care (FC_{ENV}) or No Care (NC_{ENV}) environment, and the
166 same was true for broods derived from the No Care populations (NC_{POP}). Thus, for each
167 experimental line of origin, individuals in the different treatments came from a similar genetic
168 pool. From Generation 1 onwards, half of the beetles drawn from each line were exposed to
169 continuous inbreeding (full-sibling crosses) for up to 8 generations (by which point all the
170 inbred lineages had gone extinct) (N = c. 45 crosses per treatment at Generation 1). The
171 remaining beetles were outbred in identical conditions to provide a control baseline for
172 comparison with the inbred lineages (N = c. 35-40 crosses per treatment, per generation). Half
173 of all inbred lineages, and half of the outbreeding populations, were allowed to provide post-
174 hatching care for their young (Full Care environment), while the remaining beetles were only
175 allowed to provide pre-hatching care (No Care environment). The experiment therefore had a
176 2 x 2 x 2 design, with 8 treatments in all (Full Care versus No Care line of origin; Inbred
177 versus Outbred; Full Care environment versus No Care environment), with each treatment
178 replicated twice due to replicate Full Care and No Care populations (Supplementary Figure
179 1).

180 **Detailed methods**

181 Beetle maintenance was carried out following standard protocols [23]. Briefly, adult beetles
182 were kept individually in plastic boxes (12 x 8 x 6cm) filled with moist soil and fed twice a
183 week with raw beef mince. Adults were bred at 2-3 weeks post-eclosion in a breeding box (17

184 x 12 x 6cm) with soil and a mouse carcass (11-13 g for all treatments except for the
185 individuals derived from the Full Care lines, that were outbred under Full Care conditions (8-
186 14 g)). To ease the considerable burden of work, data for broods in this treatment were
187 collected from the ongoing experimental evolution lines in the laboratory. Carcass size was
188 included, where appropriate, as a factor in the statistical analyses (see below).

189 For the inbreeding treatments, we paired full siblings (one pair per family) whereas for the
190 outbreeding treatments we paired males and females at random and did not pair siblings or
191 cousins. Each pair was given a breeding box with a dead mouse sitting on soil, and the
192 breeding boxes were placed in a dark cupboard to simulate natural underground conditions.
193 For broods assigned to a No Care environment, parents were removed around 53 h after
194 pairing. Eight days after pairing (which is when the larvae have completed their development
195 and start to disperse away from the carcass) we scored two standard measures of reproductive
196 success in burying beetles [21]: brood success (fail = no larvae produced; success = some
197 larvae produced) and brood size at dispersal. Larvae were then placed into cells (2 x 2 x 2cm)
198 in an eclosion box (10 x 10 x 2cm), with one eclosion box per brood, which was filled with
199 soil until larvae had developed into sexually immature adults (about 18 days after dispersal).
200 At this point, adults were transferred to individual boxes until they reached sexual maturity
201 roughly 2 weeks later. Both the eclosion boxes and the individual boxes were kept on shelves
202 in the laboratory at 21°C on a 16L:8D hour light cycle.

203

204 **Statistical Analyses**

205 All statistical tests were conducted in R version 3.5.1 [26]. Data handling and visualisation
206 were carried out using the 'tidyverse' [27] and 'survminer' [28] R packages. All data and

207 code presented in the manuscript is available through: https://github.com/r-
208 mashoodh/nves_MutationLoad.

209

210 *Testing predictions (1) and (2)*

211 To test predictions (1) and (2) we focused on the data collected from Generation 1. Using a
212 binomial generalised linear model (GLM) in the base 'statistics' package in R, we tested the
213 effect of evolutionary history (*i.e.* derived from a No Care evolving population or from a Full
214 Care evolving population), current care environment (*i.e*. experienced No Care or Full Care
215 during Generation 1), and inbreeding *(i.e.* inbred or outbred) on brood success. We defined
216 brood success at dispersal in the following way: broods that produced at least one larva that
217 survived to breed were defined as successful (following [21, 23]) whereas those that did not
218 produce any surviving young were classified as failures.

219

220 We subsequently ran analyses separately for the inbreeding and outbreeding conditions to
221 examine any interactions between evolutionary history (*i.e*. derived from a No Care evolving
222 population (NC_{POP}) or from a Full Care evolving population (FC_{POP})) and the current
223 environment (*i.e.* experienced No Care (NC_{ENV}) or Full Care (FC_{ENV}) during Generation 1)
224 dropping non-significant interaction terms where appropriate. We included block and carcass
225 weight as covariates to ensure any effects we detected occurred over and above any variation
226 in these variables.

227

228 *Testing prediction (3)*

229 *Calculation of inbreeding depression.*

For direct comparison with previous work [19], we calculated the inbreeding depression rate: $\delta = (w_o - w_i)/w_o$, where w_o and w_i are respectively the number of surviving outbred and inbred offspring at dispersal. We combined data from both blocks.

Survival analysis across generations

To determine the effect of evolutionary history (*i.e*. derived from a No Care evolving population or from a Full Care evolving population), and current care environment (*i.e.* experienced No Care or Full Care during Generation 1) on the survival of the different lineages in the Evolutionary History Experiment (Supplementary Figure 1), we fit accelerated time hazard models with a log-logistic distribution using the 'survival' R package [27]. Again carcass weight and block were included as covariates. A lineage was considered to be extinct if it did not survive to reproduce in the subsequent generation. We additionally used the non-parametric Kruskal Wallis test to determine if median survival times of each inbred lineage differed, by comparing the effect of evolutionary history (*i.e*. derived from a No Care evolving population or from a Full Care evolving population) in separate analyses, one for each current care environment (No Care versus Full Care). Model diagnostics were checked visually.

Results

To test predictions (1) and (2) we initially focused on the data collected from the first generation of breeding in the Evolutionary History Experiment. In support of prediction (1), we found that exposure to a No Care environment reduced reproductive success, regardless of the evolutionary history of the lineage (Figure 1, Table 1). However, in support of prediction (2), we found that a supply of post-hatching care enabled more broods to survive, even if they

254 were inbred - and regardless of the evolutionary history of their lineage (Figure 1, Table 1),
255 replicating previous work [19].

256

257 To test prediction (3), we continued to examine inbred families in the first generation of
258 breeding in the Evolutionary History Experiment. In this generation, we found an interaction
259 between evolved history and the current environment in inbred but not outbred lineages
260 (Table 2). We split the dataset by the current level of care supplied, to be able to examine the
261 effect of evolutionary history in more detail. In support of prediction (3), we found inbred
262 families derived from the Full Care populations had lower brood survival than inbred families
263 drawn from the No Care populations (log(OR) =1.12 [0.49-1.80], z=3.42, p<0.001) – though
264 only when broods were raised in a No Care current environment. No equivalent differences
265 were observed in the Full Care current environment (log(OR)=0.20 [-1.4,1.9], z=0.25,
266 p=0.80). For the outbred families, the evolutionary history of the lineage had no effect on
267 breeding success, though broods were in general less successful when they received no post-
268 hatching care (Table 2).

269

270 To further test prediction (3), we expanded our analyses to consider all generations, beginning
271 by calculating the extent of inbreeding depression at each generation. Inbreeding depression
272 was greater in Generation 1 for families descended from the Full Care evolving populations
273 than the No Care evolving populations, in the No Care current environment (Figure 2A). We
274 found the same pattern in the Full Care current environment – though here the differences
275 between lineages were first seen at Generation 3 (Figure 2A).

276

Finally, we compared the survival of all lines across generations in the Evolutionary History Experiment, by fitting accelerated failure time hazard models (Figure 2B). A lineage was considered extinct if none of its members survived to reproduce in the subsequent generation. Whilst all inbred lineages in our experiments eventually went extinct, outbred lineages were still reproducing successfully at the point at which the experiment was terminated (Supplementary Figure 2, Supplementary Table 2).

For the inbred lineages, there was once again an interaction between the evolutionary history of a population and current care received (Supplementary Table 1). This resulted, in part, from a No Care current environment causing particularly rapid extinction (Figure 2B; Supplementary Table 2). When there was No Care, inbred lineages seeded from the Full Care evolving populations had significant lower median survival than inbred lineages seeded from the No Care evolving populations (Estimate=0.20 [0.05-0.36], $p<0.01$; Figure 2B). Lineages seeded from the Full Care evolving populations reached 50% extinction one generation sooner under a No Care environment than inbred lineages seeded from the No Care evolving populations (non-parametric Kruskal Wallis test: $H(1)=4.59$, $p=0.03$; Supplementary Table 2). In a Full Care environment, by contrast, we could detect no equivalent difference in lineage survival between the No Care and Full Care populations (Estimate=-0.01 [-0.13-0.10], $p=0.85$; Figure 2B; Supplementary Table 2).

Discussion

Burying beetles care for their offspring by making a nest for them to inhabit during development, providing them with plentiful carrion to feed upon and defending them from attack by rival microbes and animals [20]. Our experiments show that the supply of post-hatching care is sufficient to perturb the mutation-selection balance by relaxing selection - as predicted generally by evolutionary theory [1-5]. We cannot tell from our experiments

302 whether selection is relaxed because the primary beneficiaries of care are kin [3] or because
303 parental care more generally buffers against harsh environments and so weakens the effects of
304 natural selection [14], or both.
305
306 By supplying care, parents shield their young from relatively harsh environmental conditions:
307 larvae receiving parental care had higher survival than those that had no care. Indeed, we
308 found that when parents provided care, the developmental environment was sufficiently
309 benign, and the strength of selection then sufficiently weak, that diverse genetic variants were
310 able to survive - even those that were inbred, just as previous work has shown [19].
311 Consequently, after 20 generations of experimental evolution in these contrasting
312 environments, we found that the Full Care populations carried a greater mutation load than
313 the No Care populations (confirmed in a companion paper [29] which uses SNPs to quantify
314 the extent of genetic variation in the two types of experimental population). The difference
315 between the populations was especially pronounced during the first generation of inbreeding,
316 and most readily detectable when inbred individuals were prevented from supplying care.
317 This suggests that some of the additional mutations present in the Full Care populations were
318 recessive and / or only mildly deleterious [5]. Given the relatively short timeframe of this
319 experiment, we presume that these mutations were present in the founding populations of
320 wild-caught beetles but were removed from the No Care populations by selection acting more
321 strongly against them. In this sense, our findings are similar to previous work on *Tribolium*
322 which found that deleterious genetic variation was purged when populations were exposed
323 experimentally to more intense sexual selection [30].
324
325 Although it is now well-understood why individuals evolve cooperative behaviour, the
326 mechanisms that cause cooperation to persist and diversify remain relatively unclear [31].

Recent theoretical work suggests that positive feedback cycles could play a key role in entrenching cooperation, following its initial evolution [32]. Cooperative social interactions facilitate the transfer of beneficial microbes, for example, upon which social partners might then become dependent over evolutionary time, ensuring that cooperation must persist [e.g. 33-36]. Likewise, cooperative interactions can promote the division of labour between social partners, causing a degree of interdependence that ensures cooperation must continue [37]. Our results, together with those obtained by Pilakouta et al. [19], suggest a third mechanism through which cooperation can become entrenched, hinted at originally by Crow [2]. We have shown that parental care creates a problem (increased mutation load: our results) for which it is also the solution (enhanced survival of all genetic variants: [19], our results). By relaxing selection, parental care causes an increase mutation load which increases the population's dependence upon care. Care ensures that the diverse genetic variants, whose existence it has facilitated, are able survive until the end of development. This could explain why parental care has evolved more frequently than it has been evolutionarily lost [14]. As Crow [2] put it: 'there is no turning back…A return to the original conditions leads to the immediate full impact of all the mutants that have accumulated during the period of improved environment". In principle, this reasoning can be extended to any form of cooperation that relaxes selection. Indeed, Crow [2] made the argument originally in the context of environmental improvements in human societies and their effect on genetic variation.

Finally, we have focused on the immediate effects of parental care on genetic variation, but the longer-term consequences are still unclear and need not match the effects seen in the short-term. For example, although greater intensity of intrasexual selection is beneficial in the short term, because it purges deleterious mutations from the population [30], in the longer run more intense intrasexual selection can make lineages more prone to extinction [38]. This

352 might be due to a lack of beneficial genetic diversity. Likewise, although parental care
353 enables mildly deleterious mutations to persist in the short-term, perhaps in the longer-term it
354 builds up genetic diversity that could be beneficial and underpin rapid evolution, especially if
355 environmental conditions change suddenly, or if mutations promote novelty through
356 compensatory evolution [26]. In future work, it would be interesting to isolate the longer-term
357 effects of parental care on genetic diversity and the effects it might have on the evolutionary
358 resilience of wild populations in a changing world [39].

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Author contributions: Conceived the idea and designed the Evolutionary History Experiment: RMK, SP; Performed the Evolutionary History Experiment: SP; Collected data from Experimentally evolving populations: RM; Analysed the data: RM, HS; Wrote the manuscript: RMK, RM. All authors discussed the results and commented on the manuscript.

Competing interests: The authors declare no competing financial or nonfinancial interests.

Data and materials availability: Data and code needed to evaluate the conclusions in the paper are available from https://github.com/r-mashoodh/nves_MutationLoad.

484 **TABLES**

485 **Table 1.** Summary of binomial generalised linear model estimates for brood success in all
486 treatments in Generation 1 of the Evolutionary History Experiment, predicted by the type of
487 population in which they Evolved (*i.e.* whether families were derived from the No Care or
488 Full Care evolving populations), Current Environment (*i.e.* whether families experienced No
489 Care or Full Care in the current generation) and Breeding Condition (*i.e.* whether families
490 were inbred or outbred). Carcass weight and Block were included as a covariate for inbred
491 lineages (see Supplementary Figure 1 for experimental design).

Characteristic	log(HR)[1]	95% CI[1]	p-value
Evolved			
Full Care	—	—	
No Care	-0.05	-0.40, 0.30	0.8
CurrentEnv			
Full Care	—	—	
No Care	**-1.4**	**-1.7, -1.0**	**<0.001**
Breeding			
inbred	—	—	
outbred	**0.33**	**0.10, 0.56**	**0.006**
as.numeric(Block)	-0.02	-0.24, 0.20	0.9
Carcass.weight	0.12	-0.01, 0.25	0.10
Evolved * CurrentEnv			
No Care * No Care	**0.48**	**0.02, 0.93**	**0.042**

[1]HR = Hazard Ratio, CI = Confidence Interval

492

493 **Table 2** Summary of binomial generalised linear model estimates for brood success in
494 Generation 1 of the Evolutionary History Experiment, with Inbred and Outbred populations
495 analysed separately. For each analysis we tested whether brood success was predicted by the
496 type of population in which they Evolved (*i.e.* whether families were derived from the No
497 Care (NC_{POP}) or Full Care (FC_{POP}) evolving populations) and Current Environment (*i.e.*
498 whether families experienced No Care (NC_{ENV}) or Full Care (FC_{ENV}) in the current generation).
499 Carcass weight and Block were included as covariates (see Supplementary Figure 1 for
500 experimental design). Separate models were run for Inbred and Outbred families.

501

	Inbred			**Outbred**		
Characteristic	log(HR)[1]	95% CI[1]	p-value	log(HR)[1]	95% CI[1]	p-value
Evolved						
Full Care	—	—		—	—	
No Care	0.08	-0.38, 0.54	0.7	-0.13	-0.71, 0.45	0.7
CurrentEnv						
Full Care	—	—		—	—	
No Care	**-1.7**	**-2.2, -1.3**	**<0.001**	**-0.92**	**-1.4, -0.43**	**<0.001**
as.numeric(Block)	-0.09	-0.40, 0.22	0.6	0.11	-0.21, 0.45	0.5
Carcass.weight	0.20	-0.05, 0.45	0.13	0.05	-0.13, 0.22	0.7
Evolved * CurrentEnv						
No Care * No Care	**0.64**	**0.02, 1.3**	**0.043**	0.25	-0.46, 0.97	0.5

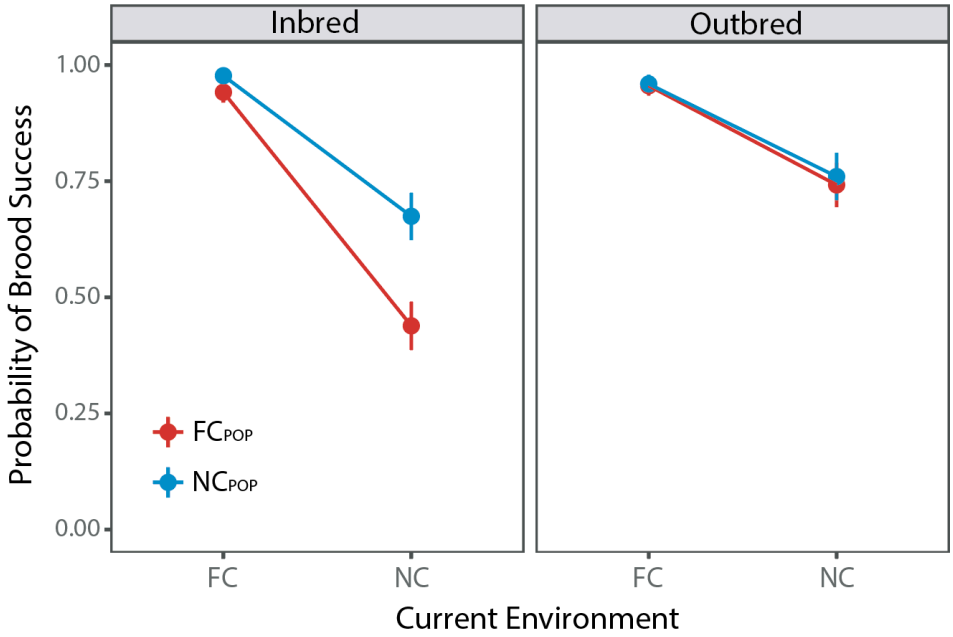
[1]HR = Hazard Ratio, CI = Confidence Interval

502

503

504 **FIGURES**

505 **Figure 1**



506

507 **Figure 1. Breeding success of evolved populations in Generation 1 of the Evolutionary**

508 **History Experiment.** Predicted means ± S.E brood survival probabilities in populations that

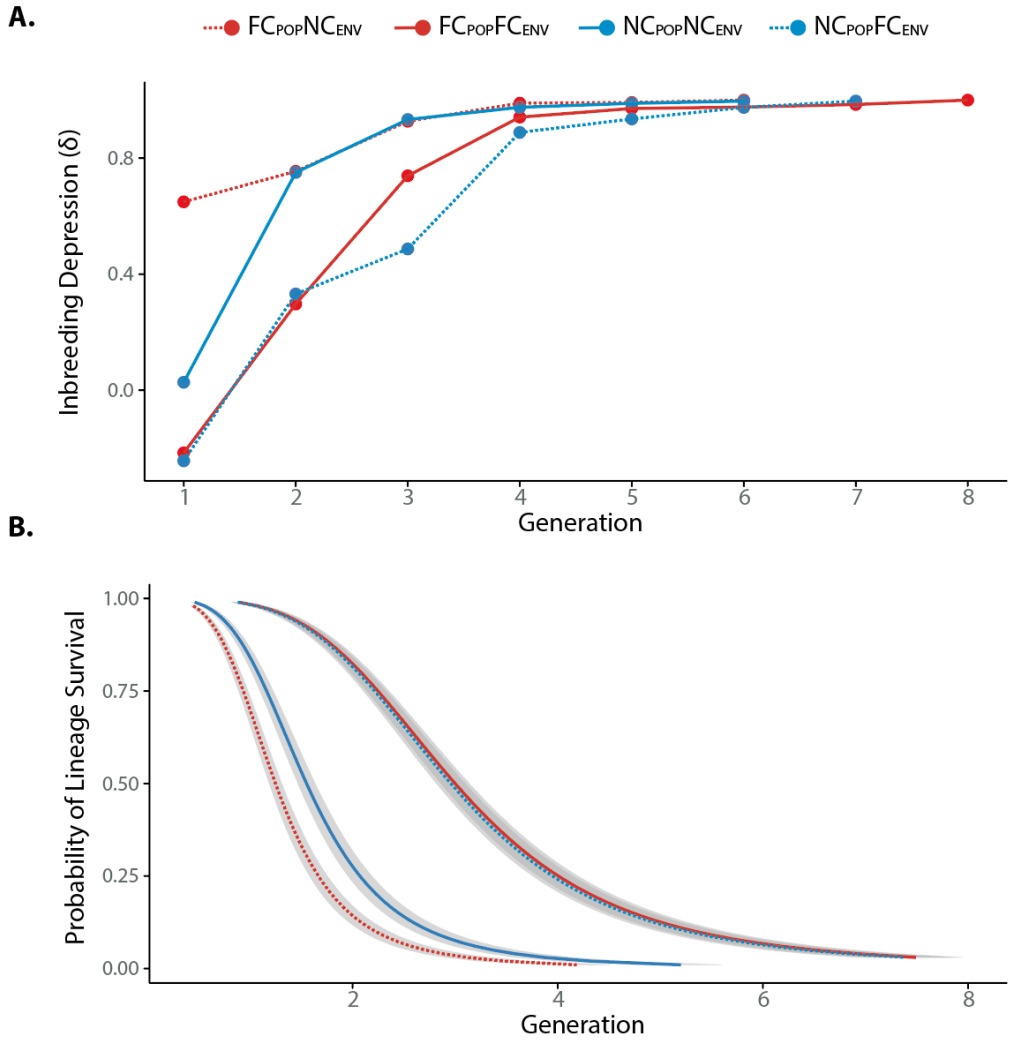
509 have evolved under Full Care (red lines and datapoints, FC_{POP}) or No Care (blue lines and

510 datapoints NC_{POP}) are shown, based on the current care environment (Full Care *vs* No Care)

511 under inbred (left) and outbred (right) conditions.

512

513 **Figure 2**



514

515 **Figure 2. Survival of inbred populations across generations.**

516 **A.** Inbreeding depression values for population lineages from the Evolutionary History

517 Experiment, shown in their native ($FC_{POP}FC_{ENV}$ and $NC_{POP}NC_{ENV}$) and reciprocal current

518 environments ($FC_{POP}NC_{ENV}$ and $NC_{POP}FC_{ENV}$). **B.** Survival curves for inbred population

519 lineages from the Evolutionary History Experiment and associated 95% confidence intervals

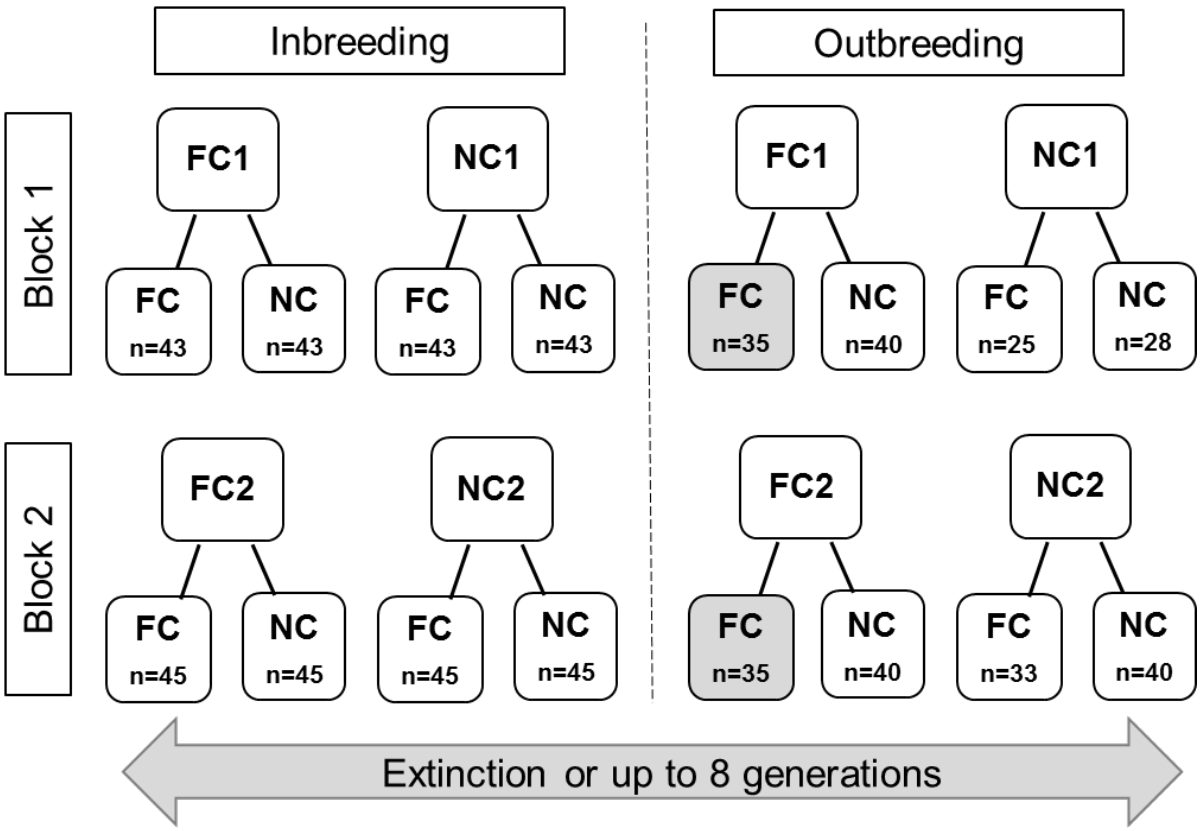
520 for the inbred lines, also shown in their native ($FC_{POP}FC_{ENV}$ and $NC_{POP}NC_{ENV}$) and reciprocal

521 current environments ($FC_{POP}NC_{ENV}$ and $NC_{POP}FC_{ENV}$). (FC = Full Care, NC = No Care).

522

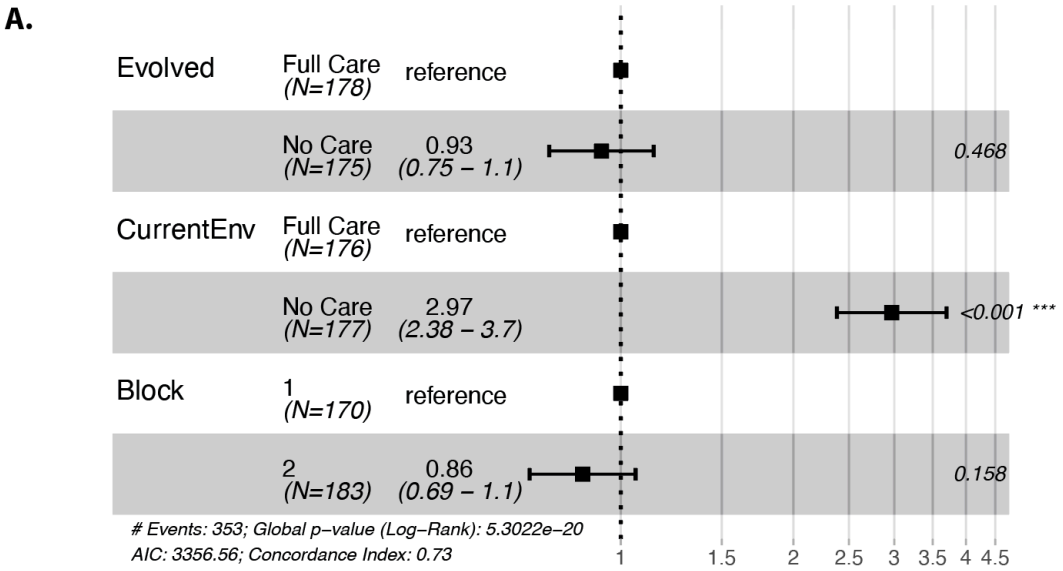
SUPPLEMENTARY FIGURES AND TABLES

Supplementary Figure 1. Overview of the Evolutionary History Experiment. Beetles that had evolved in the Full Care (FC) or No Care (NC) lines for 20 generations were used to seed new experimental populations in the lab. Before inbreeding began, all beetles from these lines experienced one generation of a FC common garden environment to minimise potentially confounding transgenerational effects. Sequential inbreeding or outbreeding was then applied for up to eight generations under both FC and NC environments. n: number of families per population in generation one of inbreeding (*i.e.* the generation immediately after the common garden FC generation). The design includes two replicate populations organised into Blocks (Block 1 and Block 2) whose breeding was staggered by one week (to ease the workload of maintaining them). Grey boxes: data for these two populations were collected from the evolving populations.



537 **Supplementary Figure 2. Survival analysis in inbred lineages.** Forest plots of Cox
538 proportional hazard survival model estimates (with 95% confidence intervals) for CurrentEnv
539 (*i.e.* whether families experienced No Care or Full Care in the current generation) and
540 Evolved history (*i.e.* whether families were derived from the No Care or Full Care evolving
541 populations). Block was included as a covariate for inbred lineages (see Supplementary
542 Figure 1 for experimental design).

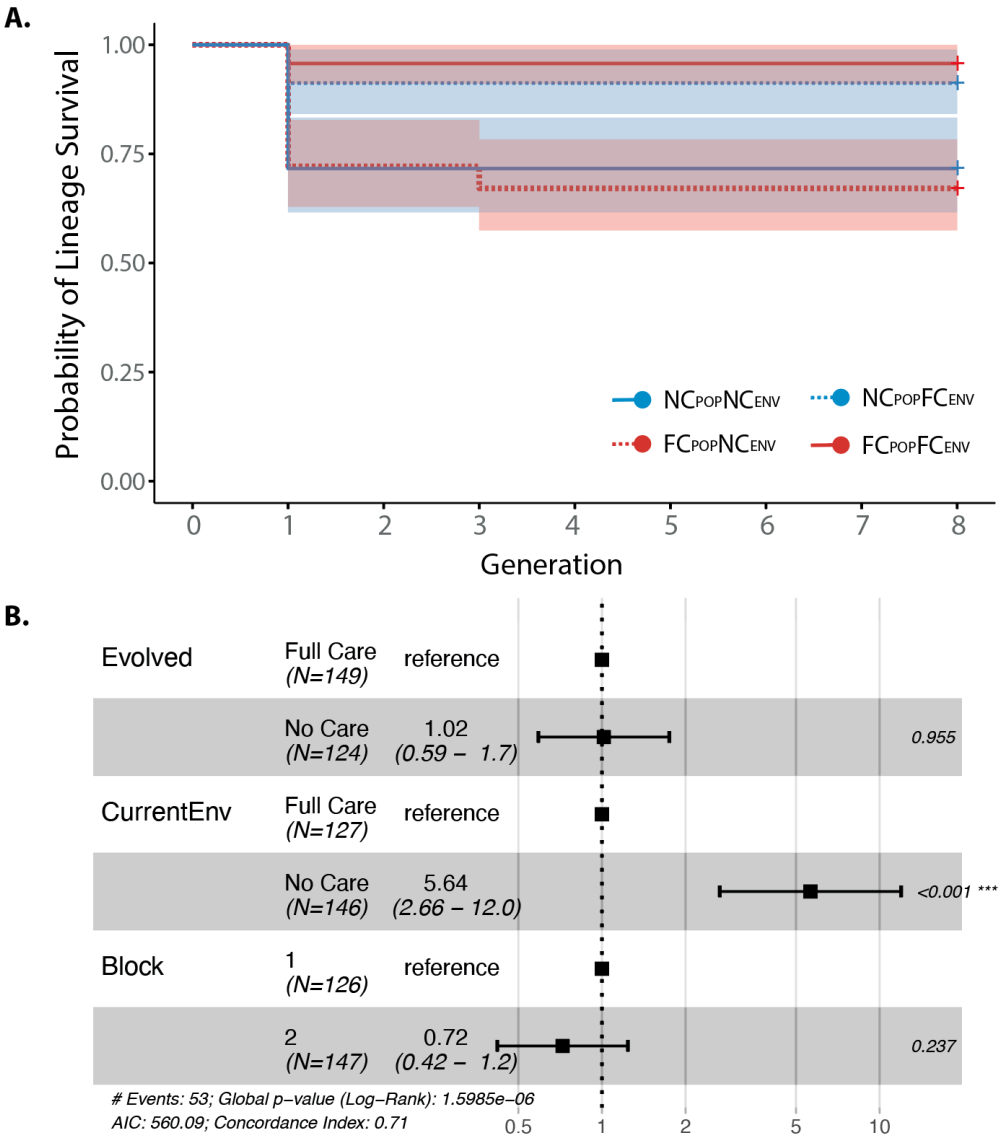
543



544

545

Supplementary Figure 3. Survival of outbred lineages across generations. (A) Survival curves for outbred population lineages from the Evolutionary History Experiment shown in their native ($FC_{POP}FC_{ENV}$ and $NC_{POP}NC_{ENV}$) and reciprocal current environments ($FC_{POP}NC_{ENV}$ and $NC_{POP}FC_{ENV}$) and associated 95% confidence intervals (FC: Full Care; NC: No Care). (B) Forest plots of Cox proportional hazard survival model estimates (with 95% confidence intervals) for CurrentEnv (*i.e.* whether families experienced No Care or Full Care in the current generation) and Evolved (*i.e.* whether families were derived from the No Care or Full Care evolving populations). Block was included as a covariate for inbred lineages (see Supplementary Figure 1 for experimental design).



558 **Supplementary Table 1.** Summary of binomial generalised linear model estimates for brood
559 success in Generation 1 of the Evolutionary History Experiment, predicted by Evolution
560 History (*i.e.* whether families were derived from the No Care or Full Care evolving
561 populations), Current Environment (*i.e.* whether families experienced No Care or Full Care in
562 the current generation) and Breeding Condition (*i.e.* whether families were inbred or outbred).
563 Carcass weight and Block were included as a covariate for inbred lineages (see
564 Supplementary Figure 1 for experimental design).

565

Factor	log(OR)[1]	95% CI[1]	p-value
Evolution History			
Full Care	—	—	
No Care	-0.06	-1.2, 1.1	>0.9
Current Environment			
Full Care	—	—	
No Care	**-3.0**	**-3.9, -2.2**	**<0.001**
Breeding Condition			
Inbred	—	—	
Outbred	**0.82**	**0.37, 1.3**	**<0.001**
Block	-0.18	-0.63, 0.26	0.4
Carcass.weight	0.30	-0.02, 0.63	0.068
Evolved * CurrentEnv			
N * N	0.80	-0.46, 2.0	0.2

[1]OR = Adjusted Odds Ratio, CI = Confidence Interval

566

567

568 **Supplementary Table 2** Summary of binomial generalised linear model estimates for brood
569 success in Generation 1 of the Evolutionary History Experiment, predicted by Evolutionary
570 History (*i.e.* whether families were derived from the No Care or Full Care evolving
571 populations) and Current Environment (*i.e.* whether families experienced No Care or Full
572 Care in the current generation). Carcass weight and Block were included as covariates (see
573 Supplementary Figure 1 for experimental design). Separate models were run for Inbred and
574 Outbred families.

	Inbred			**Outbred**		
Characteristic	log(OR)[1]	95% CI[1]	p-value	log(OR)[1]	95% CI[1]	p-value
Evolutionary History						
Full Care	—	—		—	—	
No Care	**1.0**	**0.39, 1.6**	**0.001**	0.10	-0.61, 0.81	0.8
Current Environment						
Full Care	—	—		—	—	
No Care	**-3.0**	**-3.9, -2.3**	**<0.001**	**-2.0**	**-3.1, -1.1**	**<0.001**
Block	-0.48	-1.1, 0.10	0.11	0.29	-0.41, 1.0	0.4
Carcass.weight	0.28	-0.18, 0.74	0.2	0.13	-0.32, 0.59	0.6

[1]OR = "Adjusted" Odds Ratio, CI = Confidence Interval

575

576

577 **Supplementary Table 3** Median survival times of each lineage (measured in generations) by
578 Breeding Condition (Inbred versus Outbred), Evolutionary History (*i.e*. whether families were
579 derived from the No Care or Full Care evolving populations) and Current Environment (*i.e.*
580 whether families experienced No Care or Full Care in the current generation) with 95%
581 confidence intervals (CI). (NA = populations did not go extinct).

582

Breeding Condition	Evolutionary History	Current Environment	median	lower CI	upper CI
Inbred	Full Care	Full Care	3	3	3
Inbred	Full Care	No Care	1	1	1
Inbred	No Care	Full Care	3	3	3
Inbred	No Care	No Care	2	1	2
Outbred	Full Care	Full Care	NA	NA	NA
Outbred	Full Care	No Care	NA	NA	NA
Outbred	No Care	Full Care	NA	NA	NA
Outbred	No Care	No Care	NA	NA	NA

583

584

585