Parental care allows genetic variation to accumulate by relaxing selection

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Benign social environments are thought to relax selection against deleterious mutations, enabling them to accumulate in the genome. However, it has not proved straightforward to demonstrate causality in this relationship. We allowed burying beetle populations to evolve with and without the benign effects of parental care for over 20 generations, before inbreeding lineages that were seeded from these experimental populations to expose any differences in their mutation load. We found that inbred lineages with a history of parental care went extinct most quickly, indicating that they harboured more recessive deleterious alleles.

Classical population genetics models imagine that populations attain an equilibrium level of genetic variation, at which the rate of input of new genetic variants is balanced by the rate of their elimination by natural selection (known as mutation-selection balance[1-4]). The concept of mutation-selection balance has long been used to understand the effects of genetic 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[2-4]. Benevolent social activities, such as the widely available provision of medical care, are suggested to have been particularly influential in preventing natural selection from purging deleterious mutations[2-4]. Consistent with this suggestion, recent comparative genomic analyses have revealed a greater incidence of genetic pathologies in western industrialised populations than in more traditional human societies[3-7] - though it is impossible to demonstrate that benevolent social behaviours have caused this difference.

Benevolent social behaviours are relatively commonplace in other animals too, where they are more formally defined as acts of cooperation[8]. Here the effect on mutation-selection balance has received relatively little empirical attention[9], though similar effects are expected in theory[1]. Parental care is a widespread example of cooperation[10]. By building protective

46 nests, defending their brood from attack and nourishing them, animal parents shield their
47 young from environmental stressors[11] and weaken the correlation between the phenotypic
48 variation seen by selection and the underlying genetic variation[1,12]. In these ways, parents
49 relax selection on the offspring phenotype[1, 11-14], theoretically allowing mildly deleterious
50 alleles to accumulate[1,11]. Previous experimental work with insects has shown that parental
51 care enables new mutants[11] and inbred offspring[8] to survive for one generation. However, it is
52 not yet known whether parental care also enables mildly deleterious mutations to accumulate
53 and persist over multiple generations.

54 We tested this suggestion by evolving replicate laboratory populations of burying
55 beetles *Nicrophorus vespilloides* under sharply contrasting levels of parental care, for more
56 than 20 generations. Burying beetles breed on the body of a small dead vertebrate[15], which the
57 parents jointly convert into a carrion nest by removing the fur or feathers, rolling the flesh
58 into a ball, covering it with anti-microbial anal exudates, and burying it. This is pre-hatching
59 parental care[16]. Parents also guard and feed larvae after hatching, though larvae can survive in
60 the lab with no post-hatching care at all[17]. In two of our evolving populations, larvae were
61 able to receive both pre-hatching and post-hatching parental care (these were called the 'Full
62 Care' lines) while in two other populations we prevented parents from supplying any post-
63 hatching care by removing them before the larvae hatched, after the carrion nest was complete
64 (these were called the 'No Care' lines). During the first 20 or so generations of experimental
65 evolution, No Care lines rapidly adapted to a life without parental care[18], through divergent
66 phenotypic change in both larval[19] and parental traits[16].

67 To determine whether parental care causes deleterious genetic variation to accumulate
68 over the generations, we inbred sub-populations, each derived from the replicate experimental
69 evolving populations, for 8 successive generations (we called this The Evolutionary History
70 Experiment). For these 8 generations, we measured the extent to which inbreeding reduced

71 measures of reproductive success in comparison with control outbred populations. To
72 determine whether parental care could temper the rate of extinction (as implied by [9]), in half
73 of all our treatments parents were allowed to provide care after their offspring hatched, while
74 in the remainder they were prevented from supplying post-hatching care. This generated 8
75 different treatments in total (see Figure 1 in Supplementary Information, for the design of the
76 Evolutionary History Experiment).

77 We used the data from the Evolutionary History Experiment, to test three predictions:
78 (1) that the harsh conditions of the No Care environment eliminates any genetic variants that
79 are not adapted to survive under these conditions. (2) That the more benign conditions of the
80 Full Care environment relaxes selection and promotes the survival of more genetic variants.
81 (3) That inbred populations from the Full Care lines should exhibit greater inbreeding
82 depression than inbred populations from the No Care lines (due to a greater accumulation of
83 deleterious recessive mutations under relaxed selection). The outbred populations acted as a
84 control treatment for tests of all three predictions.

85 To test predictions (1) and (2) we initially focused on the data collected from the first
86 generation of breeding in the Evolutionary History Experiment. The results supported both
87 predictions in showing that the No Care is harsher than the Full Care environment, and that a
88 Full Care environment can relax selection against genetic variants that would otherwise be
89 eliminated in the harsher No Care environment. We found that inbreeding reduced
90 reproductive success, regardless of the evolutionary history of the lineage (Figure 1,
91 Supplementary Table 1). However, a supply of post-hatching care enabled more broods to
92 survive, even if they were inbred - replicating the findings of [9]. In addition, we found that
93 families that were allowed to provide care for their offspring were more likely to produce
94 surviving offspring, whether or not the parents were inbred, and regardless of the evolutionary
95 history of their lineage (Figure 1, Supplementary Table 1).

96 To test prediction (3), we continued to examine inbred families in the first generation
97 of breeding in the Evolutionary History Experiment. We found that the chance of brood
98 survival depended on the levels of current care supplied as well as on the evolutionary history
99 of the lineage (Supplementary Table 2). To examine the effect of evolutionary history in more
100 detail, we split the dataset by the current level of care supplied. In support of prediction (3),
101 inbred families derived from the Full Care populations had lower brood survival than families
102 drawn from the No Care populations (log(OR) =1.12 [0.49-1.80], z=3.42, p<0.001) – in a No
103 Care current environment. No equivalent differences were observed in the Full Care current
104 environment (log(OR)=0.20 [-1.4,1.9], z=0.25, p=0.80). For the outbred families, the
105 evolutionary history of the lineage had no effect on breeding success, though broods were in
106 general less successful when they received no post-hatching care (Supplementary Table 2).
107 To further test prediction (3), we calculated the extent of inbreeding depression at each
108 generation. Inbreeding depression was greater in Generation 1 for families descended from
109 the Full Care evolving populations than the No Care evolving populations, in the No Care
110 current environment. We found the same pattern in the Full Care current environment –
111 though here the differences between lineages were seen at Generation 3 (Figure 2A).

112 Finally, we compared the survival of all lines in the Evolutionary History Experiment,
113 by fitting multivariate Cox proportional hazard models (Figure 2B). A lineage was considered
114 extinct if none of its members survived to reproduce in the subsequent generation. Whilst all
115 inbred lineages in our experiments eventually went extinct, outbred lineages were still
116 reproducing successfully at the point at which the experiment was terminated (Supplementary
117 Figure 3A, B). For the inbred lineages a No Care current environment resulted in particularly
118 rapid extinction (Figure 2B). Of these, lineages seeded from the Full Care evolving
119 populations had significant lower median survival than inbred lineages seeded from the No
120 Care evolving populations (non-parametric Kruskal Wallis test: $H(1)$=4.59, p=0.03). The

121 lineages seeded from the Full Care evolving populations reached 50% extinction one
122 generation sooner than those seeded from the No Care evolving populations (Supplementary
123 Table 3). We could detect no equivalent difference in lineage survival between the evolved
124 populations in the Full Care current environment ($H(1)=0.06$, $p=0.81$).

125 Together, these analyses support all three of our predictions, and demonstrate that the
126 benign social environment created by parental care can weaken selection sufficiently to
127 enable the accumulation of genetic mutations within populations. In the short-term, such
128 genetic mutants are overwhelmingly likely to be recessive and mildly deleterious [1-7]. It would
129 be interesting to determine in future work whether this sets in motion a positive evolutionary
130 feedback loop between the extent of cooperation and mutation load, due to a greater reliance
131 on cooperative behaviour to compensate for any genetic deficiencies. In the longer-term
132 though, benevolent social interactions could build up genetic diversity that might turn out to
133 be beneficial and cause rapid evolution, especially if environmental conditions change
134 suddenly, or if mutations promote novelty through compensatory evolution[20].
135

136 **METHODS**

137 ***Nicrophorus vespilloides* natural history.** The common burying beetle breeds on a small
138 dead vertebrate (like a songbird or mouse). The larvae hatch from eggs laid nearby in the soil
139 and crawl to their carrion nest, which they can feed upon themselves[15]. Once at the carcass,
140 larvae receive post-hatching biparental care. Parents supply fluids to their offspring through
141 oral trophallaxis, and defend their brood and the carrion nest from attack by predators,
142 microbes and rival beetles[15]. The duration and extent of post-hatching care are highly
143 variable, however. For example, when wild beetles are brought into the lab to breed, roughly
144 5% of larvae receive no post-hatching care at all, yet larvae can still survive to become
145 reproductively competent adults (see[19] and references therein). Within roughly a week of

146 hatching, the larvae complete development and at this point (which we refer to as 'dispersal),
147 they start to crawl away from the scant remains of the carcass to pupate in the soil. The
148 parents, meanwhile, fly off in search of a new carcass.

149 **Experimental evolution.** The experimental lines used in this work have been described in
150 detail elsewhere[16, 19]. In brief, we established a large founding population of *N. vespilloides* by
151 interbreeding wild-caught individuals from four different woodlands. This was then divided
152 into four experimental lines. In two lines, larvae experienced 'Full Care' at each generation,
153 with both parents staying in the breeding box throughout the breeding bout and able to
154 provide post-hatching care as well as pre-hatching care. In the other two 'No Care' lines,
155 parents engaged in pre-hatching care but at each generation they were then removed from the
156 breeding box, around 53 hours after they were paired, so that they never interacted with their
157 larvae. The work reported here began when these lines had been exposed to 20 generations of
158 experimental evolution under these contrasting regimes of care.

159

160 **Evolutionary History Experiment (see Supplementary Figure 1 for overview of design).**

161 **Preparatory common garden generation** The experiment began by exposing individuals
162 drawn from the four lines (Full Care replicated twice and No Care replicated twice) to a
163 common garden Full Care environment for one generation (N = 60 pairs for each No Care line
164 (to counter-balance the slightly lower breeding success caused by the No Care environment)
165 and N = 50 pairs for each Full Care line). In this way, we minimised any potentially
166 confounding transgenerational effects prior to starting the Evolutionary History Experiment.

167 **Overview.** Broods from the common garden generation seeded lineages in the experimental
168 treatments: broods derived from the Full Care lines founded lineages that were Inbred or
169 Outbred, in either a Full Care or No Care environment, and the same was true for broods

170 derived from the No Care lines. Thus, for each line of origin, individuals in the different
171 treatments came from a similar genetic pool. From Generation 1 onwards, half of the beetles
172 drawn from each line were exposed to continuous inbreeding (full-sibling crosses) for up to 8
173 generations (by which point all the inbred lineages had gone extinct) (N = c. 45 crosses per
174 treatment at Generation 1. The remaining beetles were outbred in identical conditions to
175 provide a control baseline for comparison with the inbred lineages (N = c. 35-40 crosses per
176 treatment, per generation). Half of all inbred lineages, and half of the outbreeding
177 populations, were allowed to provide post-hatching care for their young (Full Care
178 environment), while the remaining beetles were only allowed to provide pre-hatching care
179 (No Care environment). The experiment therefore had a 2 x 2 x 2 design, with 8 treatments in
180 all (Full Care versus No Care line of origin; Inbred versus Outbred; Full Care environment
181 versus No Care environment), with each treatment replicated twice due to replicate Full Care
182 and No Care lines (Supplementary Figure 1).

183 **Detailed methods:** Beetle maintenance was carried out following standard protocols [18].
184 Briefly, adult beetles were kept individually in plastic boxes (12 x 8 x 6cm) filled with moist
185 soil and fed twice a week with raw beef mince. Adults were bred at 2-3 weeks post-eclosion
186 in a breeding box (17 x 12 x 6cm) with soil and a mouse carcass (11-13 g for all treatments
187 except for the individuals derived from the Full Care lines, that were outbred under Full Care
188 conditions (8-14 g)). To ease the considerable burden of work, data for broods in this
189 treatment were collected from the ongoing experimental evolution lines in the laboratory, and
190 so received mice in the size range assigned to that treatment for breeding. Carcass size was
191 included, where appropriate, as a factor in the statistical analyses (see below).

192 For the inbreeding treatments, we paired full siblings (one pair per family) whereas for the
193 outbreeding treatments we paired males and females at random and did not pair siblings or
194 cousins. Each pair was given a breeding box with a dead mouse sitting on soil, and the

195 breeding boxes were placed in a dark cupboard to simulate natural underground conditions.
196 For broods assigned to a No Care environment, parents were removed around 53 hours after
197 pairing. Eight days after pairing (which is when the larvae have completed their development
198 and start to disperse away from the carcass) we scored two standardly used measures of
199 reproductive success in burying beetles[16]: brood success (fail = no larvae produced; success =
200 some larvae produced) and brood size at dispersal. Larvae were then placed into cells (2 x 2 x
201 2cm) in an eclosion box (10 x 10 x 2cm), with one eclosion box per brood, which was filled
202 with soil until larvae had developed into sexually immature adults (about 18 days after
203 dispersal). At this point, adults were transferred to individual boxes until they reached sexual
204 maturity roughly 2 weeks later. Both the eclosion boxes and the individual boxes were kept
205 on shelves in the laboratory at 21°C on a 16L:8D hour light cycle.

206

207 **Statistical Analyses**

208 All statistical tests were conducted in R version 3.5.1[21]. Data handling and visualisation were
209 carried out using the 'tidyverse'[24] and 'survminer'[23] R packages. All data and code presented
210 in the manuscript is available through: https://github.com/r-mashoodh/nves_MutationLoad.

211

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

213 To test predictions (1) and (2) we focused on the data collected from Generation 1. Using a
214 binomial generalised linear model (GLM) in the base 'statistics' package in R, we tested the
215 effect of evolutionary history (*i.e.* derived from a No Care evolving population or from a Full
216 Care evolving population), current care environment (*i.e.* experienced No Care or Full Care
217 during Generation 1), and inbreeding *(i.e.* inbred or outbred) on brood success. We defined
218 brood success at dispersal in the following way: broods that produced at least one surviving

219 larva were defined as successful (following [16,18]) whereas those that did not produce any
220 surviving young were classified as failures.

221

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

228 *Testing prediction (3)*

229 *Calculation of inbreeding depression.*

230 For direct comparison with [9], we calculated the inbreeding depression rate: $\delta = (w_o -$
231 $w_i)/w_o$, where w_o and w_i are respectively the number of surviving outbred and inbred
232 offspring at dispersal. We combined data from both blocks to derive the measures shown in
233 Figure 2A.

234

235 *Survival analysis across generations*

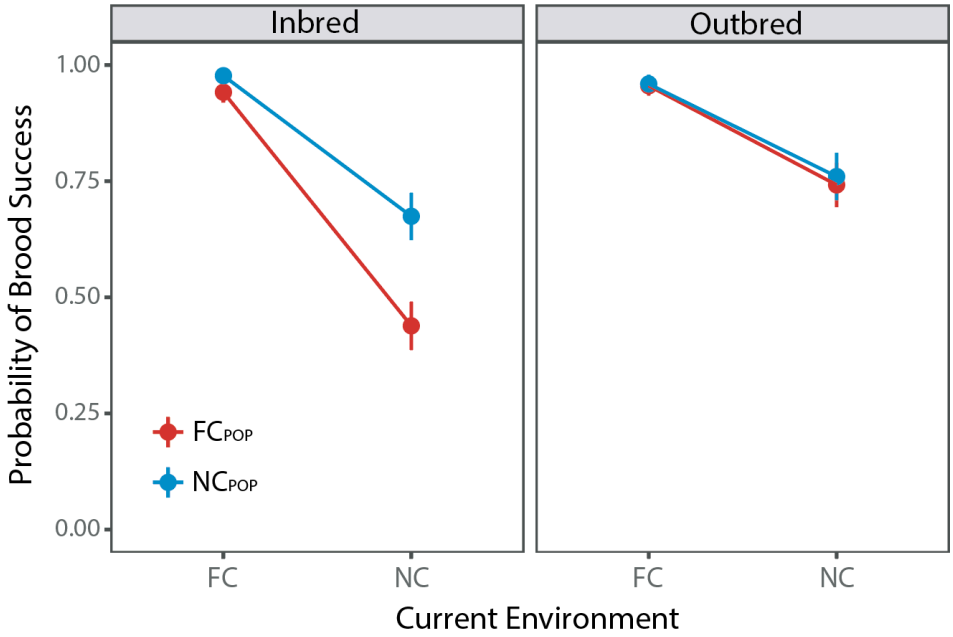
236 To determine the effect of evolutionary history (*i.e.* derived from a No Care evolving
237 population versus a Full Care evolving population), current care environment (*i.e.*
238 experienced No Care or Full Care) on the survival of the different lineages in the
239 Evolutionary History Experiment (Supplementary Figure 1), we fit multivariate Cox
240 proportional hazard models using the 'survival' R package [24], again adding carcass weight
241 and block as covariates. A lineage was considered to be extinct if it did not survive to
242 reproduce in the subsequent generation. The outbred lineages did not go extinct and so were

243 analysed separately from the inbred lineages. We additionally used the non-parametric
244 Kruskal Wallis test to determine if median survival times of each inbred lineage differed, by
245 comparing the effect of evolutionary history (*i.e.* derived from a No Care evolving population
246 or from a Full Care evolving population) in separate analyses, one for each current care
247 environment (No Care versus Full Care). Model diagnostics were checked visually and using
248 the cox.zph function.

249

250 **Figures**

251 **Figure 1**



252

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

254 **History Experiment.** Predicted means ± S.E survival probabilities of populations that have

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

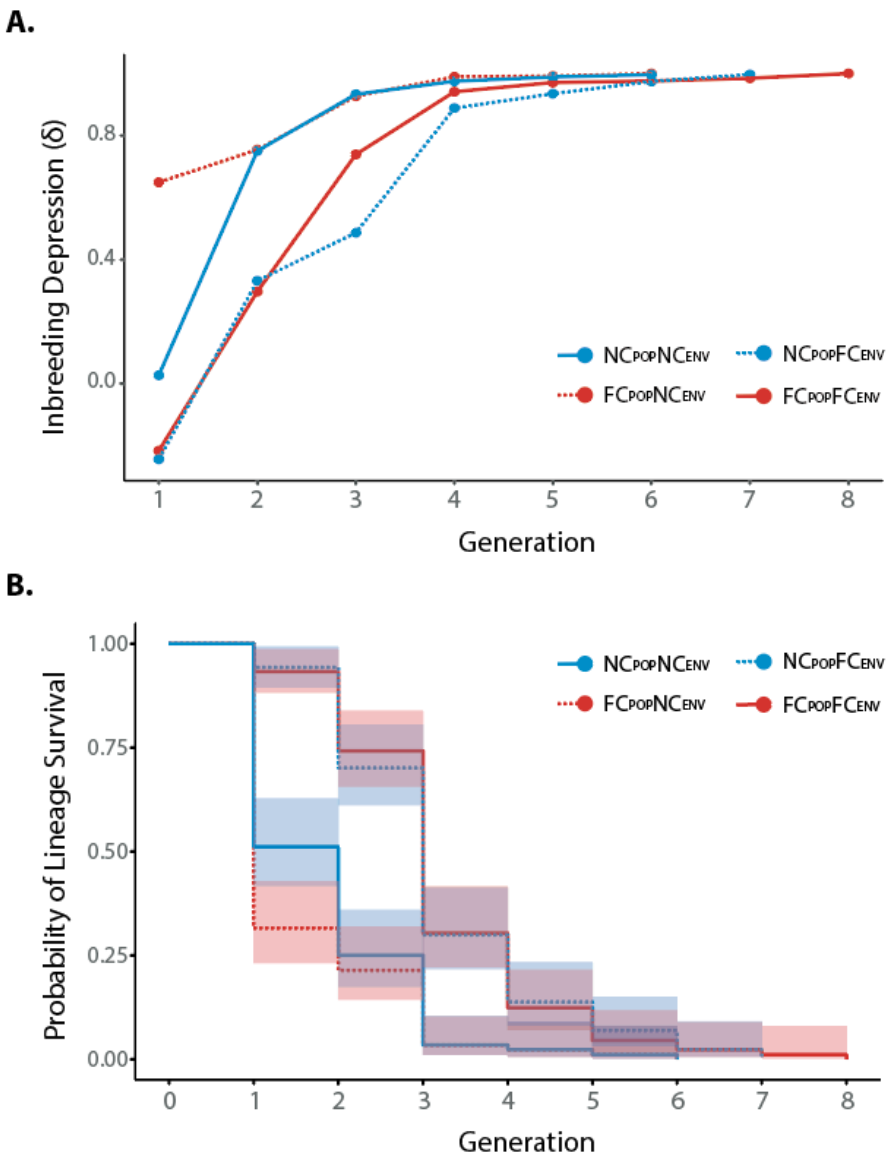
256 datapoints NC_{POP}) are shown, based on the current care environment (Full Care (FC) *vs* No

257 Care (NC)) under inbred (left) and outbred (right) conditions.

258

259 **Figure 2**

260



261

262 **Figure 2. Survival of inbred populations across generations.** A) inbreeding depression

263 values for population lineages from the Evolutionary History Experiment, shown in their

264 native ($FC_{POP}FC_{ENV}$ and $NC_{POP}NC_{ENV}$) and reciprocal current environments ($FC_{POP}NC_{ENV}$

265 and $NC_{POP}FC_{ENV}$). (B) Survival curves for inbred population lineages from the Evolutionary

266 History Experiment and associated 95% confidence intervals for the inbred lines.

267

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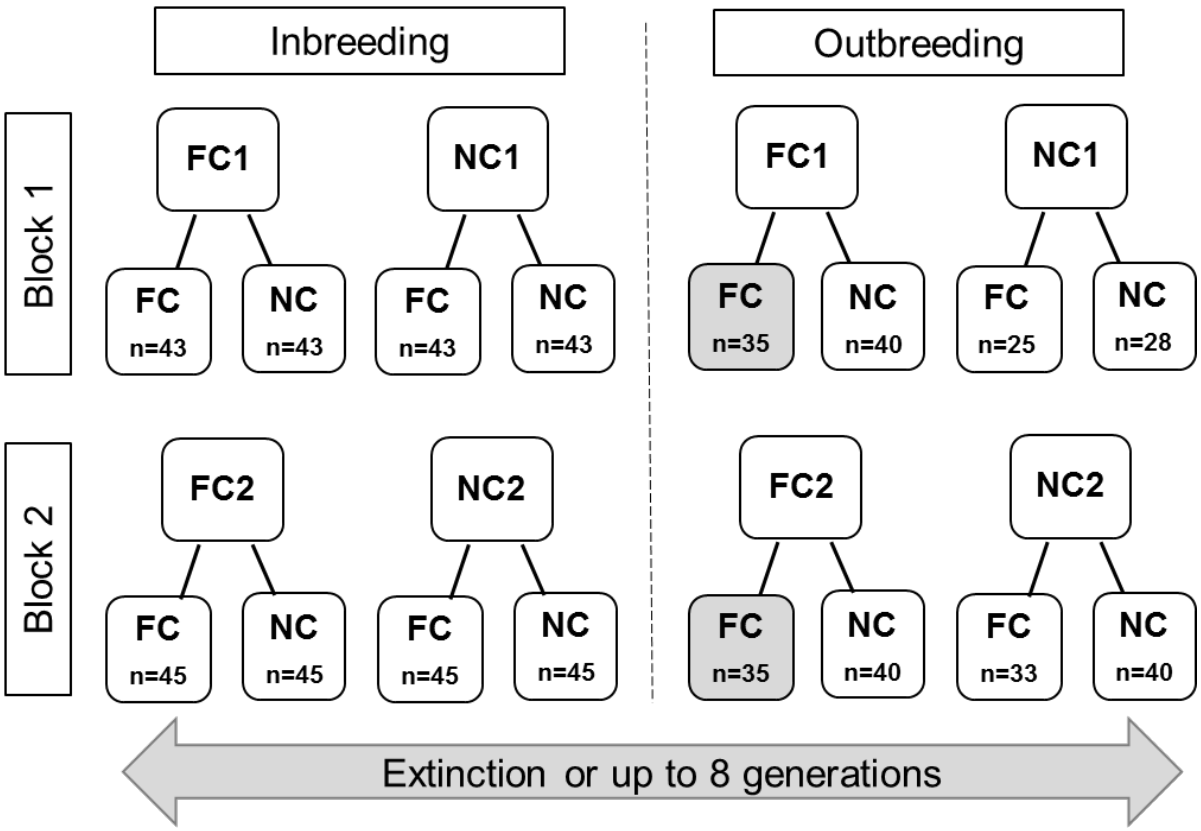
Authors contribution: Conceived the idea and designed the Evolutionary History Experiment: RMK, SP; Performed the Evolutionary History Experiment: SP; Collected data from Experimentally evolving populations: RM; Analysed the data: RM, HS; Wrote the manuscript: RMK, RM, SP. All authors discussed the results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

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.



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

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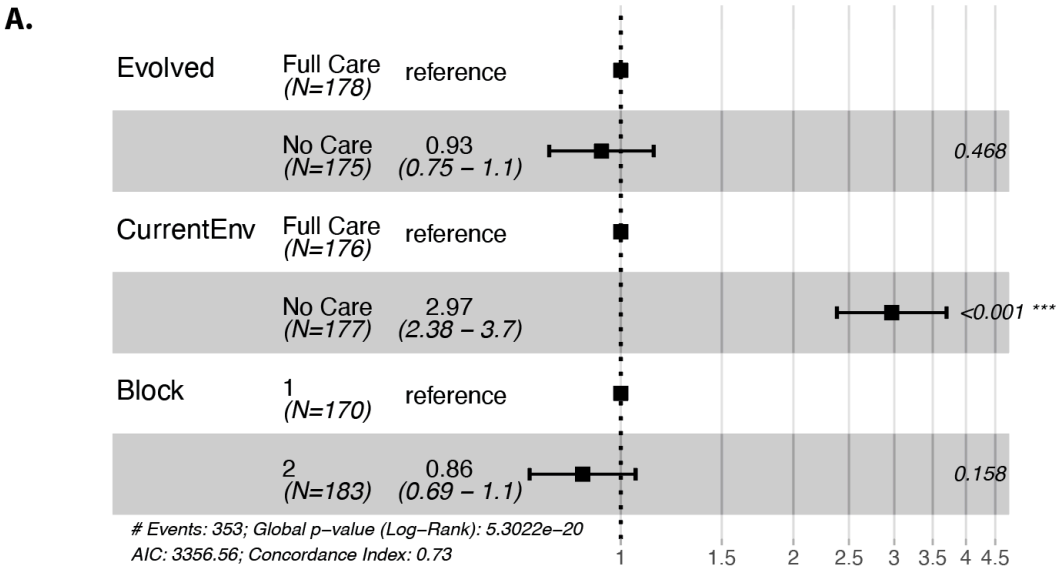

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372

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

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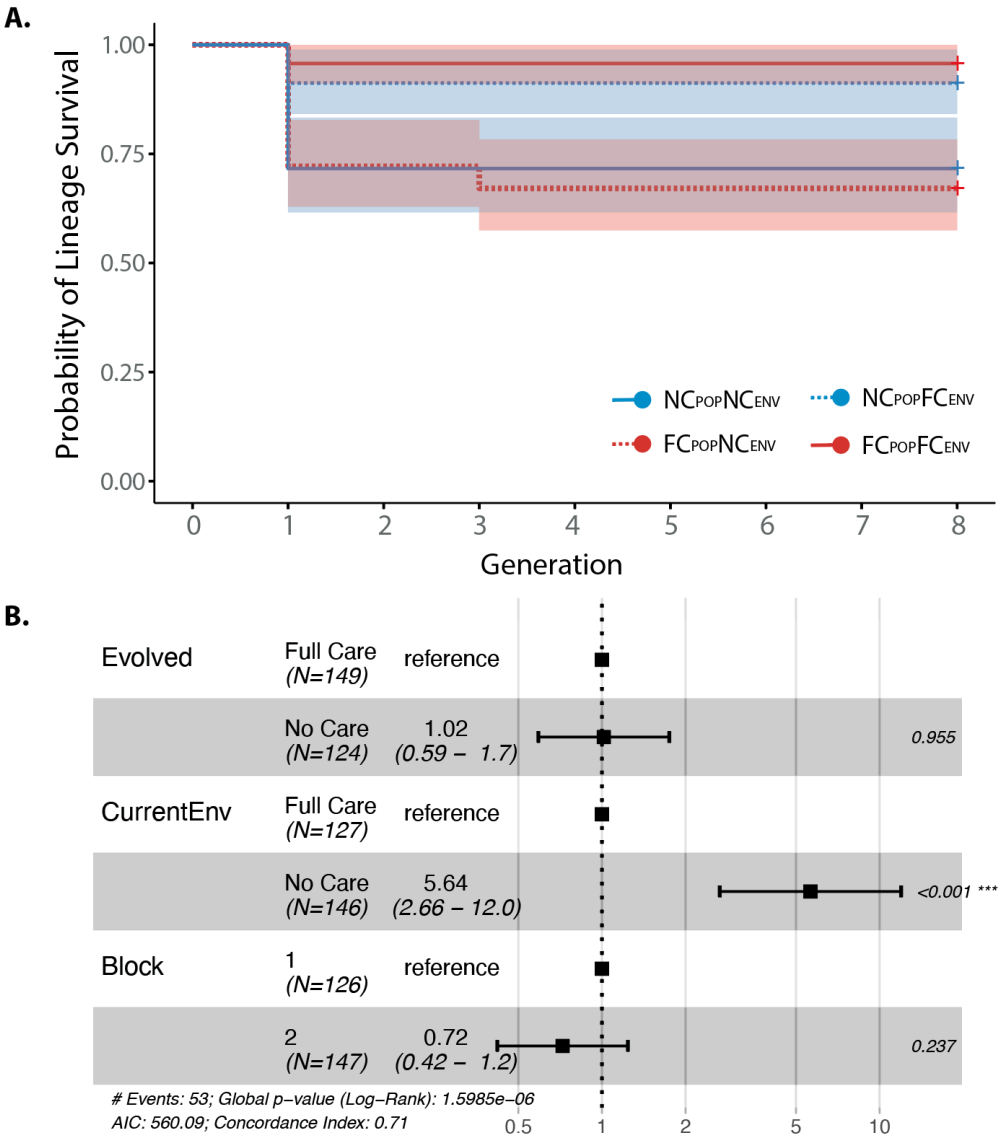

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385 **Supplementary Table 1.** Summary of binomial generalised linear model estimates for brood
386 success in Generation 1 of the Evolutionary History Experiment, predicted by Evolution
387 History (*i.e.* whether families were derived from the No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

392

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

393

394

395 **Supplementary Table 2** Summary of binomial generalised linear model estimates for brood
396 success in Generation 1 of the Evolutionary History Experiment, predicted by Evolutionary
397 History (*i.e.* whether families were derived from the No Care or Full Care evolving
398 populations) and Current Environment (*i.e.* whether families experienced No Care or Full
399 Care in the current generation). Carcass weight and Block were included as covariates (see
400 Supplementary Figure 1 for experimental design). Separate models were run for Inbred and
401 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

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

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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

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