

1 Title:

2 Cooperative interactions within the family enhance  
3 the capacity for evolutionary change of body size

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25 Abstract:

26 Classical models of evolution seldom predict the rate at which populations evolve in  
27 the wild. One explanation is that the social environment affects how traits change in  
28 response to natural selection. Here, we determine how social interactions between  
29 parents and offspring, and among larvae, influence the response to experimental  
30 selection on adult size. Our experiments focus on burying beetles (*Nicrophorus*  
31 *vespilloides*), whose larvae develop within a carrion nest. Some broods exclusively  
32 self-feed on the carrion while others are also fed by their parents. We found  
33 populations responded to selection for larger adults but only when parents cared for  
34 their offspring. We also found populations responded to selection for smaller adults  
35 too, but only by removing parents and causing larval interactions to exert more  
36 influence on eventual adult size. Comparative analyses revealed a similar pattern:  
37 evolutionary increases in species size within the genus *Nicrophorus* are associated  
38 with the obligate provision of care. Synthesising our results with previous studies, we  
39 suggest that cooperative social environments enhance the response to selection  
40 whereas excessive conflict can prevent further directional selection.

41

42 Introduction:

43 Predicting the rate at which populations can evolve and adapt in a rapidly changing  
44 world is a major challenge for evolutionary biology<sup>1</sup>. A key problem is to explain how  
45 rapidly traits change in response to selection. The breeder's equation summarizes  
46 classical genetic models of evolution by suggesting that the magnitude of  
47 evolutionary change in any given trait depends simply on the extent to which that trait  
48 contributes to fitness (the strength of selection), and the degree to which it is  
49 transmitted to the next generation by genetic variation (the trait's heritability)<sup>2</sup>. Yet

50 these two parameters are seldom sufficient to predict how evolution will proceed in  
51 the wild<sup>3,4</sup>. One suggestion is that this is because the social environment has an  
52 additional causal influence on the response to selection<sup>5-9</sup>. An individual's social  
53 environment derives from its behavioural interactions with conspecifics. Variation in  
54 the social environment can contribute to variation in an individual's phenotype, much  
55 as the abiotic environment does<sup>10,11</sup>. An important difference, though, is that there is  
56 genetic variation in the social environment. This means that the social environment  
57 can be inherited and can therefore change the response to selection of the traits that it  
58 induces<sup>6-9</sup>.

59

60 Specifically, mathematical analyses show that when the effect of the social  
61 environment on trait expression (typically denoted  $\psi$ ) is large and positive, it  
62 increases a trait's response to selection and accelerates evolutionary change. But if the  
63 effect of the social environment is negative, it prevents any response in the trait to  
64 selection and impedes evolutionary change<sup>6-9,12-16</sup>. Previous experiments with  
65 domesticated species have supported that latter prediction by showing that  
66 competitive interactions can prevent selection for traits of greater economic value to  
67 farmers, such as increased body size<sup>13-17</sup>. However, it is unclear whether the social  
68 environment can ever causally accelerate trait evolution in animal populations.  
69 Nevertheless, theoretical work<sup>6-9</sup> and correlational analyses of the outcome of natural  
70 selection using large pedigreed datasets collected from wild animals, both suggest it is  
71 likely<sup>18</sup>.

72

73 Results and Discussion:

74 We tested whether the social environment within the family can promote the

75 evolution of burying beetle size (*Nicrophorus vespilloides*) using experiments on  
76 wild-caught individuals. This species exhibits facultative biparental care, which  
77 makes it ideal for experimental manipulations of the social environment (e.g. ref. 19).  
78 Both parents work together to prepare the carrion nest by removing the fur or feathers  
79 from the dead body, rolling the flesh into a ball and burying it underground. Larvae  
80 hatch from eggs laid in the soil nearby and crawl to the carcass nest, where they take  
81 up residence. There they feed on the flesh themselves, but are also tended by their  
82 parents who guard them and transfer resources through regurgitation<sup>20</sup>. However, if  
83 parents are removed after nest preparation is complete, but before the larvae hatch,  
84 then larvae can complete development without any post-hatching parental care at  
85 all<sup>19,21</sup>. After roughly five days, larvae disperse away from the carcass to pupate in the  
86 soil.

87

88 We focused on the evolution of adult size for three reasons. First, size is strongly  
89 associated with fitness in this species<sup>20</sup>. Competition for the carrion breeding  
90 resource can be intense, and larger beetles are more likely to win fights for ownership  
91 of carcass (e.g. ref. 22). Second, adult size is known from previous work to vary with  
92 aspects of the family social environment that larvae experience during development,  
93 including social interactions with siblings<sup>23</sup> and parents<sup>21</sup>. Third, we found that the  
94 heritability of adult size is very low. We used techniques from classical quantitative  
95 genetics to estimate the heritability of adult size, in environments where parents  
96 provided post-hatching care for offspring (hereafter Full Care), and in environments  
97 where they provided no post-hatching care, because parents were experimentally  
98 removed (hereafter No Care). In both environments, the heritability of adult body size  
99 did not differ from zero (estimate  $\pm$  s.e., Full Care:  $h^2 = 0.08 \pm 0.12$ ; No Care:  $h^2 =$

100  $0.05 \pm 0.30$ , see Supplementary Materials). Our estimates are similar to estimates of  
101 the heritability of adult size in the congeneric *N. pustulatus*<sup>24</sup>. This result gave us the  
102 opportunity to distinguish the effect of the social environment from effects due to the  
103 heritability of body size on the way in which body size responds to selection. This is  
104 because body size should exhibit negligible change as a function of its heritability.

105

106 To test whether the social environment causally influences the response to selection,  
107 we carried out an artificial selection experiment on eight laboratory populations (see  
108 Methods). Importantly, we varied the social environment among the populations so  
109 that we could analyse its causal influence on the response to selection: half the  
110 populations experienced Full Care during development (N = 4 populations), the other  
111 half had No Care (N = 4 populations). We then exposed half of the populations within  
112 each Care environment to selection for increased adult body size (Large), while the  
113 remaining populations experienced selection for decreased adult body size (Small, see  
114 Methods). Thus we had four types of experimental populations, each replicated twice:  
115 Full Care Large, Full Care Small, No Care Large, and No Care Small. We selected on  
116 body size for seven generations, generating over 25,000 beetles.

117

118 For each experimental treatment, we measured the cumulative selection differential  
119 and response to selection, and used these measures to estimate the realised heritability  
120 of adult body size (see Methods). This gave us a measure of the extent to which body  
121 size could be changed by artificial selection. The breeder's equation predicts that the  
122 realised heritability of body size should not differ among the treatments. However, we  
123 found instead that the realised heritability of adult body size varied among the four  
124 types of experimental treatments (ANCOVA, care  $\times$  selection  $\times$  cumulative selection

125 differential:  $F_{3,44} = 6.87$ ,  $P < 0.001$ ,  $N = 48$ , Fig. 1). Furthermore, the realised  
126 heritability of body size was relatively high, and significantly different from zero, for  
127 the Full Care Large treatment ( $0.09 \pm 0.02$ ), where mean body size increased across  
128 the generations, and for the No Care Small treatment ( $0.11 \pm 0.03$ ), where mean body  
129 size correspondingly decreased. For these two treatments we therefore conclude that  
130 the social environment during development enhanced the capacity for evolutionary  
131 change in adult body size, and to a similar degree whether selection was for increased  
132 or decreased body size.

133

134 By contrast, in the Full Care Small and the No Care Large treatments, the realised  
135 heritability of adult body size was not significantly different from zero (Full Care  
136 Small:  $-0.01 \pm 0.02$ ; No Care Large:  $0.01 \pm 0.03$ ). Mean adult body size did not  
137 change over the course of the selection experiment for individuals from either of these  
138 treatments (Fig. 1).

139

140 The next step was to determine how the two contrasting social environments in our  
141 selection experiment could influence evolutionary change in adult size. Previous work  
142 has shown that the mass a larva attains by the time it disperses away from the carcass  
143 strongly influences the size of the adult that then emerges<sup>25</sup>. Furthermore, larval mass  
144 at dispersal depends on the number of larvae competing during development for the  
145 finite resources on a carcass<sup>23</sup>. Building on these results, we identified three social  
146 factors that influence larval mass at dispersal. The first is clutch size, because it  
147 influences the number of larvae competing for carrion. However, it is not the sole  
148 determinant of brood size on a carcass. Larger females lay a larger clutch<sup>26</sup> but have  
149 fewer surviving larvae that disperse from the carcass (see Methods, Supplementary

150 Fig. 1), presumably due to a greater incidence of filial cannibalism<sup>27</sup>. Brood size at  
151 dispersal is therefore different from clutch size, and is the second factor influencing  
152 larval mass at dispersal. The third factor is the presence or absence of parents after  
153 hatching. This factor is important because it influences the relationship between brood  
154 size and larval size at dispersal, especially for broods of 10 or fewer larvae. When  
155 parents are present, and there are only a few larvae on the carcass, each consumes  
156 more carrion and is larger at dispersal<sup>23</sup>. However, when parents are absent, each  
157 larva typically attains only a low mass by the time it disperses to pupate, because  
158 larvae seemingly help each other to colonize and consume the carcass<sup>23</sup>. Thus larvae  
159 in small broods cannot attain a large mass at dispersal when parents are absent, but  
160 they can when parents are present<sup>23</sup>.

161

162 We suggest that selection on these three elements of the social environment combined  
163 to cause correlated change in body size in the Full Care Large lines and the No Care  
164 Small lines (see Supplementary Materials). In the Full Care Large treatment (Fig. 2a),  
165 we selected for larger adults. They produced larger clutches (Supplementary Fig. 2),  
166 but produced fewer (Supplementary Fig. 3) and therefore larger dispersing larvae  
167 (presumably due to greater levels of filial cannibalism). They matured into larger  
168 adults themselves. Likewise, in the No Care Small treatment (Fig. 2b) we selected for  
169 smaller adults and they laid a smaller clutch (Supplementary Fig. 2). Since these  
170 broods developed without parents, the resulting smaller broods yielded smaller larvae  
171 (Supplementary Fig. 3), which matured into smaller adults. In each treatment, we  
172 effectively selected a social environment on the carcass that induced the production of  
173 more individuals with either a larger (Full Care Large) or smaller (No Care Small)  
174 body size. Furthermore, these selected individuals then produced a similar social

175 environment for their offspring. This explains why these lines responded to selection  
176 on body size, despite the very low heritability of body size.

177

178 We observed very little change in body size in the other experimental populations (No  
179 Care Large, Full Care Small). This was predicted by the classical estimates of  
180 heritability, but it might also be attributed to effects of the social environment, which  
181 could have cancelled out the effects of selection at each generation (see ref. 28). For  
182 example, in the No Care Large treatment (Fig. 2c), selecting for larger adults yielded  
183 smaller individuals in the next generation. The larger adults laid a larger clutch  
184 (Supplementary Fig. 2), but with no parents present after hatching to cannibalize  
185 offspring, these larger clutches yielded relatively large broods (Supplementary Fig. 3)  
186 of smaller larvae, which matured into smaller adults. Similarly, in the Full Care Small  
187 treatment (Fig. 2d) selection for smaller adults yielded larger adults in the following  
188 generation. The smaller adults laid a smaller clutch (Supplementary Fig. 2), which in  
189 turn yielded a smaller brood (Supplementary Fig. 3) of relatively large larvae that  
190 matured into large adults.

191

192 We explicitly tested the conclusions set out in Fig. 2, by comparing the slope of the  
193 regression between dam size and progeny size (see Supplementary Materials). Fig. 2a  
194 and 2b predict that in the Full Care Large and No Care Small treatment, this  
195 correlation should be positive, whereas Fig. 2c and Fig. 2d predict it should be  
196 negative in the No Care Large and Full Care Small treatment. We found that the  
197 slopes of these correlations differed significantly among treatments (linear mixed  
198 model, care  $\times$  selection  $\times$  dam pronotum:  $\chi^2_1 = 4.13$ ,  $P = 0.042$ ,  $N = 15,484$ ). The  
199 slopes were positive in the Full Care Large ( $0.13 \pm 0.09$ ) and No Care Small



200 treatments ( $0.09 \pm 0.09$ ). However, although they were negative in the Full Care  
201 Small treatment ( $-0.06 \pm 0.06$ ), as we predicted, they were positive in the No Care  
202 Large treatment ( $0.12 \pm 0.1$ ), which we did not predict.  
203  
204 Our experiments thus find no clear evidence to support the suggestion that the social  
205 environment within the family alone prevented evolutionary change in the Full Care  
206 Small and No Care Large treatments. They do, however, show that social interactions  
207 within the family enhanced the response to selection in the Full Care Large and No  
208 Care Small treatment. Specifically, our experiments indicate that parental care is  
209 required to promote a rapid evolutionary increase in body size in *N. vespilloides*.  
210  
211 We tested the merits of this conclusion in a final comparative analysis, in an attempt  
212 to link our experimental results to the processes that might have underpinned the  
213 diversification of the *Nicrophorus* genus (see Methods). Different species of burying  
214 beetle are remarkably alike in their ecology and appearance<sup>29</sup>. They differ principally  
215 in their relative size and in the extent to which parental care is essential for larval  
216 growth and survival<sup>30</sup>. Observations of natural burying beetle populations show that  
217 adult size is correlated with variation in the size of carrion used by different species  
218 for reproduction<sup>20</sup>. Variation in adult body size is correlated with the partitioning of  
219 the carrion niche by sympatric species, and enables larger species to favor larger  
220 carrion and smaller species to breed on smaller carcasses<sup>20</sup>. We mapped the changes  
221 in adult body size across the *Nicrophorus* genus by measuring museum specimens of  
222 49 of the 68 extant species<sup>29</sup> and placing them on a recent molecular phylogeny of the  
223 genus (Fig. 3)<sup>30</sup>. We found that there is considerable variation in body size across the  
224 phylogeny, with multiple shifts to both larger and smaller species relative to the

225 ancestral phenotype (Fig. 3). Consistent with our experimental results, we also found  
226 that the evolution of very large burying beetles is associated with obligate provision  
227 of parental care (PGLS: estimate =  $1.57 \pm 0.66$ ,  $t_{12} = 2.40$ ,  $P = 0.035$ ,  $N = 14$ ).  
228

229 Setting our results alongside previous work on other species suggests that in general,  
230 the way in which the social environment influences a trait's response to selection  
231 depends on whether it is associated with social interactions that are cooperative or that  
232 promote excessive conflict (see ref. 10 for formal definitions of these terms). For  
233 example, previous studies have shown that selection for increased size or productivity  
234 in pigs and poultry also selects for increased aggression. Increased aggression reduces  
235 fitness so much that any effects of selection on size cannot be transmitted to the next  
236 generation and this prevents evolutionary change<sup>13,17</sup>. This suggests that traits  
237 associated with social environments which induce high levels of conflict could have  
238 limited capacity for further directional evolutionary change. Previous work has also  
239 demonstrated that, under these conditions, the only way in which increased  
240 productivity or size can be artificially selected is by imposing multilevel, group or kin  
241 selection<sup>12,13</sup>. That is, a response to selection can be restored only when an explicitly  
242 cooperative social environment is artificially created at the same time<sup>32</sup>.  
243

244 Our experiment provides more direct evidence that cooperative interactions enhance  
245 the response to selection, and can do so even when selection acts on individuals. In  
246 the Full Care Large treatment, selection for increased body size was possible because  
247 parents helped small broods of larvae to attain a large size at dispersal<sup>23</sup>. In the No  
248 Care Small treatment (Fig. 2), selection for smaller individuals decreased brood size,  
249 and smaller broods resulted in smaller larvae. This result can be explained by our

250 previous finding that larval cooperation is key to larval success when parents are  
251 removed<sup>23</sup>. Presumably, with fewer siblings to help penetrate and feed upon the  
252 carcass, individual larvae in small broods were able to attain only a low mass by the  
253 time they dispersed from the carcass. Reducing the number of cooperating larvae thus  
254 reduced larval mass. In these two different ways, cooperative interactions reinforced  
255 the response to selection in our experiment by magnifying changes in body size across  
256 the generations, causing increases and decreases in body size of a similar magnitude.  
257 Cooperative interactions within the family therefore enhanced the capacity for  
258 evolutionary change.

259

260 Our general conclusion is that the response to selection is likely to be reduced when  
261 trait expression is associated with excessive conflict, but enhanced for traits whose  
262 expression is associated with more cooperative social environments. Proper  
263 characterisation of the social environment in which traits are expressed is therefore  
264 important not only for understanding a trait's current adaptive value<sup>10</sup> but also for  
265 predicting its future capacity to evolve and adapt.

266

267 **Methods**

268 The burying beetle genus *Nicrophorus* is distributed primarily throughout the  
269 temperate regions of the Northern Hemisphere<sup>29</sup>. So far as is known, the natural  
270 history and reproductive biology of all *Nicrophorus* species are broadly similar<sup>20,29,31</sup>  
271 and centre on the use of small carrion as a breeding resource<sup>20</sup>. Although the two  
272 other extant genera in the Nicrophorinae also use carrion for reproduction, they lack  
273 the elaborate parental care exhibited by *Nicrophorus* species and the associated social  
274 interactions that it generates<sup>31,33</sup>. These genera are also less speciose than  
275 *Nicrophorus*: there are 68 known species in *Nicrophorus*, one in *Eonecrophorus* and  
276 three in *Ptomascopus*<sup>29</sup>. This suggests there is a correlation between the social  
277 environment during development and the capacity for diversification in each of these  
278 lineages.

279

280 **Estimating the heritability of body size in *N. vespilloides***

281 *Cultivating N. vespilloides in the lab*

282 All the individuals used in this experiment belonged to a captive colony (kept at a  
283 constant temperature: 21°C, with a 16h:8h light:dark cycle) established at the  
284 University of Cambridge in 2013 from wild-caught adults collected under licence  
285 from local field sites at Byron's Pool and Wicken Fen in Cambridgeshire, U. K.  
286 Adults were housed individually in plastic boxes (12 × 8 × 2cm) filled with moist soil  
287 (Miracle Grow) and fed twice a week with approximately 0.3g of minced beef. For  
288 breeding, pairs of unrelated individuals were placed into larger plastic boxes (17 × 12  
289 × 6cm) half-filled with moist soil, provided with a 8–13g freshly thawed mouse  
290 carcass and kept in the dark to simulate natural underground conditions. The larvae  
291 disperse from the carcass to pupate roughly eight days after pairing. Dispersing larvae

292 were transferred into population boxes ( $10 \times 10 \times 2\text{cm}$ ), each subdivided into equal  
293 cells of  $2 \times 2 \times 2\text{cm}$  and filled with soil. Once pupation was complete (approximately  
294 3 weeks after dispersal), each sexually immature adult was moved to its own  
295 individual, uniquely labeled box. Sexual maturity is reached approximately two  
296 weeks after eclosion, and beetles were paired for reproduction at this time. No  
297 siblings or cousins were paired for breeding.

298

### 299 *Methods*

300 We performed a full-sib/half-sib quantitative genetics experiment to estimate the  
301 heritability of body size in *N. vespilloides*. We used two populations of beetles for this  
302 experiment, both maintained under the same conditions as stock populations (Full  
303 Care) for 11 generations without any selection for body size. Four females were  
304 mated to a single male and then each female was given a recently defrosted mouse  
305 (10–12g) to breed upon. Once the carcass had been prepared and all eggs laid,  
306 approximately 53h after providing the mouse<sup>34</sup>, the female and carcass were removed.  
307 The female was placed in a new breeding box and provided with a fully prepared  
308 carcass from a donor female. At that time we also prepared an equal number of  
309 breeding boxes with just a donor-prepared carcass and no female. The breeding box  
310 where the female laid her eggs was checked three times each day for larval hatching.  
311 Once larvae started hatching, the larvae were transferred to either the carcass with  
312 their mother (Full Care) or to the other carcass without an adult (No Care). Larvae  
313 were added until a maximum of 12 larvae were present on each carcass, resulting in  
314 mean ( $\pm$  s.e.) brood sizes of  $7.85 \pm 0.25$  in the Full Care, and  $8.21 \pm 0.24$  in the No  
315 care environments.

316

317 We checked breeding boxes three times daily, and determined that the larvae were  
318 ready to disperse when two or more larvae were seen crawling away from the remains  
319 of the carcass<sup>24</sup>. At this point the contents of the breeding box were removed and the  
320 larvae were counted and weighed individually. The larvae were then placed into  
321 individual cells within an eclosion box in the order in which they were weighed so we  
322 could relate larval mass to adult size. After eclosion, we anaesthetized the adults with  
323 CO<sub>2</sub>. Once anaesthetized, each individual was placed flat under a Canon DSLR  
324 camera and photographed. The camera was attached to a stand to ensue consistency in  
325 the images obtained and connected to a computer for automatic image labeling. All  
326 photographs contained a scale against which the pronotum width of each individual  
327 was measured using a custom MatLab script. No statistical methods were used to  
328 predetermine sample size.

329

330 We analyzed data for each care regime separately, using the package ASreml-R 3.0<sup>35</sup>  
331 in R version 3.3.0<sup>36</sup>. Models included a fixed effect of the number of larvae surviving  
332 per brood (mean-centered), a random effect of brood ID to estimate variance due to  
333 permanent environmental (including maternal) effects, and a random effect of the  
334 pedigree term to estimate the additive genetic variance. (We were unable to partition  
335 variance due to maternal effects from that of the permanent environment because no  
336 females had multiple broods within a single environment). We then tested the  
337 significance of the additive genetic variance in adult size by comparing models with  
338 and without the pedigree term using a likelihood ratio test. We estimated  $\chi^2_{\text{nDF}}$  as  
339 twice the difference in model log likelihoods; given that we were testing the effect of  
340 a single variance component (nDF = 1), we assumed that the test statistic was  
341 asymptotically distributed as an equal mix of  $\chi^2_0$  and  $\chi^2_1$  (ref. 37). The heritability of

342 adult size was calculated as  $V_A / V_P$  where  $V_P$  is the sum of the variance components  
343 (additive genetic, permanent environment, and residual) from the model, having  
344 conditioned on the fixed effects. We used Wald  $F$ -tests to estimate the significance of  
345 fixed effects.

346

### 347 *Results*

348 The experiment yielded 186 maternal full-sib families and 56 paternal half-sib  
349 families in the Full Care environment, and 84 maternal full-sib families and 22  
350 paternal half-sib families in the No Care environment. Mean ( $\pm$  s.e.) brood size in the  
351 Full Care was  $7.69 \pm 0.24$  and  $5.31 \pm 0.30$  in the No Care.

352

353 We found no evidence for significant additive genetic variance in adult size in either  
354 the Full Care ( $V_A = 0.01 \pm 0.02$ ,  $\chi^2_{0,1} = 0.46$ ,  $P = 0.25$ ) or No Care ( $V_A = 0.01 \pm 0.05$ ,  
355  $\chi^2_{0,1} = 0.03$ ,  $P = 0.43$ , Supplementary Table 1) environments. The heritability  
356 estimates of adult size were correspondingly close to zero, with large standard errors  
357 ( $h^2_{\text{Full}} = 0.08 \pm 0.12$ ;  $h^2_{\text{No}} = 0.05 \pm 0.30$ ). Permanent environment effects (ie effects of  
358 the Care treatment and brood size) explained a significant amount of the total  
359 phenotypic variation in adult size (conditional on fixed effects) in both Full Care ( $V_{\text{PE}}$   
360  $= 0.05 \pm 0.01$ ,  $\chi^2_{0,1} = 16.22$ ,  $P < 0.001$ ; proportion of total phenotypic variance  
361 conditional on fixed effects  $= 0.263 \pm 0.065$ ) and No Care ( $V_{\text{PE}} = 0.05 \pm 0.03$ ,  $\chi^2_{0,1} =$   
362  $6.05$ ,  $P = 0.007$ ; proportion  $= 0.361 \pm 0.157$ ) environments. For completeness, we ran  
363 the same models without any fixed effects (see ref. 38), but this had no meaningful  
364 effect on our results.

365

### 366 **Selection experiment**

367 One way to analyse the effect of the social environment on the response to selection is  
368 to use an artificial selection experiment. We manipulated the social environment,  
369 imposed selection and measured the response. This enabled us to attribute changes in  
370 the response to selection to our manipulations of the social environment, without  
371 making any *a priori* assumptions about which particular aspects of the social  
372 environment were important in influencing trait expression.

373

374 All the individuals used in the selection experiment belonged to a captive colony  
375 established at Cambridge University in 2013 from wild caught adults collected under  
376 licence from local field sites at Byron's Pool and Wicken Fen in Cambridgeshire,  
377 U.K. Full details of the protocols used are given in (ref. 19).

378

### 379 *Methods*

380 From the genetically diverse founding population, we started eight populations  
381 consisting of four treatments with two replicates per treatment, randomly allocating  
382 individuals to treatments. We had two treatments, Provision of Care and Selection for  
383 Size, resulting in a  $2 \times 2$  factorial experiment. Provision of Care was manipulated by  
384 either leaving or removing both parents 53 hours after pairing, after carcass  
385 preparation and egg laying were complete<sup>34</sup>, resulting in a Full Care treatment, and a  
386 No Care treatment, respectively. We then imposed two selection regimes on the Full  
387 Care and No Care populations: Large and Small. We selected the largest third of the  
388 population with the Large regime, and the smallest third of the population under the  
389 Small regime. Selection was imposed at the population level and not at the family  
390 level. Once the population had been selected, individuals were paired haphazardly,  
391 although we ensured cousins and siblings did not breed. All beetles were maintained



392 under the conditions described above. Each population was maintained with at least  
393 25 families per generation, by breeding 40 pairs of beetles for the Full Care  
394 populations and 60 pairs for the No Care populations. When it became impossible to  
395 sustain populations of this size, the experiment ceased. (We bred extra pairs in the No  
396 Care population to ensure there were enough successful families: failure rates are high  
397 when initially removing parental care).

398

399 At eclosion members of the same sex from each family were temporarily housed in a  
400 box together and anaesthetised with CO<sub>2</sub>. Once anaesthetized, each individual was  
401 photographed and the body size measured in the same method as described above.  
402 Each individual was given a unique ID that we used to identify individuals that were  
403 retained to breed in the next generation.

404

405 To estimate the potential for evolutionary change in body size in each population, we  
406 calculated the realised heritability of body size, as the slope of the regression of the  
407 cumulative response to selection against the cumulative strength of selection<sup>39</sup>. Post-  
408 hoc pairwise comparisons were adjusted for multiple testing<sup>40</sup>. No statistical methods  
409 were used to predetermine sample size.

410

#### 411 *Results*

412 The realised heritability did not differ significantly between replicate populations for  
413 each treatment ( $F_{40} = 2.08$ ,  $P = 0.10$ ). Replicates were therefore pooled for all  
414 subsequent analyses. After running the global model, we used pairwise comparisons  
415 to compare measures of realised heritability across the different treatments. The Full  
416 Care Large and Full Care Small treatments significantly differed from one another in

417 realised heritability ( $F_{22} = 9.90$ ,  $P_{\text{adj}} = 0.015$ ), as did the Full Care Large and No Care  
418 Small ( $F_{22} = 26.44$ ,  $P_{\text{adj}} = 0.006$ ). There was marginal support for a difference in  
419 realised heritability between Full Care Large and No Care Large ( $F_{22} = 3.95$ ,  $P_{\text{adj}} =$   
420  $0.072$ ). Realised heritability in the No Care Small treatment differed significantly  
421 from that in the Full Care Small ( $F_{22} = 5.92$ ,  $P_{\text{adj}} = 0.03$ ) and the No Care Large  
422 populations ( $F_{22} = 6.36$ ,  $P_{\text{adj}} = 0.03$ ). The Full Care Small and No Care Large did not  
423 differ from one another in their realised heritability ( $F_{22} = 0.30$ ,  $P_{\text{adj}} = 0.59$ ). Realised  
424 heritability estimates for each population are in Supplementary Table 2.

425

#### 426 **The effects of the social environment on adult size**

427 The social environment that larvae experience during development influences the size  
428 the larvae attain by the time they disperse from the carcass and this, in turn, is  
429 strongly correlated with adult size<sup>25</sup>. Three factors contribute to this social  
430 environment (see main text): clutch size, brood size at dispersal and the presence (or  
431 absence) of parents during larval development<sup>23</sup>. To understand how these different  
432 elements of the social environment might have caused the outcome of the selection  
433 experiment, we began by investigating how clutch size and brood size are related to  
434 adult size.

435

#### 436 *a) Relationship between female size and clutch size, or brood size at dispersal*

437 To assess the effect of female size on clutch size we analysed data from<sup>26</sup> where we  
438 manipulated female size experimentally and destructively counted the total clutch size  
439 for a breeding attempt after 53 hours when egg laying has ceased<sup>34</sup>. Brood size data  
440 were taken from a stock population maintained in the laboratory under the same  
441 conditions as the Full Care populations, and assayed when the selected populations

442 were in generation five. Brood size was measured at the point of larval dispersal away  
443 from the carcass. Both clutch size and brood size were analysed with a Poisson  
444 distribution and a log link function with female size and carcass mass fitted as  
445 covariates.

446

447 We found that clutch size increased with female size even when accounting for  
448 carcass mass ( $t = 3.63$ ,  $P = 0.001$ ), whereas brood size at dispersal decreased with  
449 female size ( $t = -2.06$ ,  $P = 0.04$ , Supplementary Fig. 1).

450

451 The next step was to relate these effects of the social environment to the results of our  
452 selection experiment. If the outcome of the selection experiment is attributable to  
453 different elements of the social environment, then we predict we should see  
454 divergence in clutch size, and brood size at dispersal among the different  
455 experimental treatments.

456

#### 457 *b) Measurement of clutch size in the experimentally selected populations*

458 Based on the results in Supplementary Fig. 1, we predict that clutch size should be  
459 greater in populations where adults are selected to be larger (i.e. Full Care Large and  
460 No Care Large) than in populations where adults are selected to be smaller (i.e. Full  
461 Care Small and No Care Small). To test this prediction, we estimated clutch size in all  
462 eight populations at generation five by counting the number of eggs visible on the  
463 bottom of the breeding box. We know from previous work that this measure is  
464 strongly correlated with total clutch size<sup>26</sup>. We analysed estimated clutch size using a  
465 generalised linear model with a Poisson error structure, and log link function. We  
466 included carcass size as a covariate.

467

468 As predicted, we found that clutch size in generation five of the selection experiment  
469 was greater in the Large selected lines than in the Small selected lines ( $z = -7.53$ ,  $P <$   
470  $0.001$ ), independent of the parental care treatment ( $z = 1.32$ ,  $P = 0.19$ , Supplementary  
471 Fig. 2). There was no interaction between selection regime and parental care on clutch  
472 size ( $z = -0.38$ ,  $P = 0.70$ ).

473

474 *c) Measurement of brood size in the experimentally selected populations*

475 We predicted that brood size at larval dispersal should also differ among the  
476 experimental populations. Specifically, based on the results in Supplementary Fig. 1,  
477 we predicted that members of the Full Care Large populations should have a smaller  
478 brood size than members of the Full Care Small populations. In addition, since there  
479 is no possibility of filial cannibalism in the No Care populations, we predicted that in  
480 these populations brood size should vary in the same way as clutch size, and therefore  
481 should be greater in the No Care Large populations than in the No Care Small  
482 populations. We measured brood size at larval dispersal in Generation 7 of the  
483 selection experiment and pooled both replicates. We analysed estimated brood size  
484 using a generalised linear model with a Poisson error structure, and log link function,  
485 and tested our prediction by searching for a significant interaction between parental  
486 care (Full Care, No Care) and selection regime (Large, Small) on brood size at  
487 dispersal. We included carcass size as a covariate.

488

489 As predicted, we found a significant interaction between parental care and selection  
490 regime on brood size at larval dispersal in generation seven ( $z = -4.89$ ,  $P < 0.001$ ).

491 Full Care Large populations had fewer offspring at dispersal than the Full Care Small

492 populations, whereas No Care Large populations had more offspring at dispersal than  
493 No Care Small populations (Supplementary Fig. 3).

494

495 *d) Testing predictions from Figure 3*

496 From Fig. 3, we predicted that the slopes of offspring size regressed against dam size  
497 would differ among the experimental treatments. Specifically, we predicted that the  
498 slope would be positive for the Full Care Large and No Care Small lines, because  
499 these were the lines in which we observed phenotypic change. And we predicted that  
500 the slope would be negative in the No Care Large and Full Care Small lines. We took  
501 all the data from all the lines and combined both replicates per treatment for the seven  
502 generations of the experiment.

503

504 We used R<sup>36</sup> and the package lme4<sup>41</sup> to run a linear mixed model, where we ran a  
505 model coding the three-way interaction of Care treatment (Full Care or No Care),  
506 selection regime (Large or Small) and dam pronotum width. Also included in the  
507 model was carcass size and generation. Dam ID was fit as a random term.

508 Significance was determined by removing the three-way interaction from the model  
509 and comparing the output with the full model. The slopes for each experimental  
510 treatment were obtained in the same way, but with the appropriate subset of the data  
511 for each experimental treatment.

512

513 **Phylogenetic analysis of body size**

514 We collected data on *Nicrophorus* body size using the beetle collections at the Natural  
515 History Museum in London. We took standardized photographs of representatives  
516 from all the *Nicrophorus* species included in a recently published molecular

517 phylogeny<sup>31</sup>, with a constant distance between subject and camera, and including a  
518 scale-bar in each picture. There was no sexual size dimorphism in our dataset ( $t = -$   
519  $1.453$ ,  $P = 0.15$ ). Therefore body size data from both sexes were pooled for each  
520 species. We used the standard practice of quantifying body size by measuring  
521 pronotum width, and used a MatLab script to calibrate photographic measurements of  
522 pronotum width with the scale bar in each image, using the same method for both  
523 experiments detailed above. The full datasets can be found in Supplementary Table 3.  
524 Post-hatching parental care was classified as ‘facultative’ or ‘obligate’ using data  
525 from the published literature and from personal communication with other burying  
526 beetle researchers ( $N = 14$  species, Supplementary Table 4). We searched Web of  
527 Science and Google Scholar for information about parental care using the species  
528 name and ‘parental care’, in conjunction with either ‘facultative’ or ‘obligate’ as  
529 search terms. We contacted researchers that have worked on species without a  
530 classification and requested the information. ‘Obligate’ parental care was defined as  
531 the failure of larvae to survive to the third instar when parents were removed.  
532  
533 We used a phylogenetic generalised least squares regression (PGLS) to analyse the  
534 relationship between body size and parental care using R version 3.3.0<sup>36</sup> with  
535 packages *ape*<sup>42</sup>, *picante*<sup>43</sup> and *caper*<sup>44</sup>. Care was coded with a dummy variable that  
536 was treated as a factor in (1 = obligate post-hatching parental care, 0 = facultative  
537 post-hatching parental care). Species without a parental care classification were coded  
538 NA.  
539  
540 We removed data obtained through personal communication systematically and  
541 repeated the analysis to check whether these data affected our conclusions. They did

542 not. We removed *N. americanus* (N = 13, est =  $0.88 \pm 0.35$ ,  $t_{11} = 2.54$ , P = 0.028), *N.*  
543 *marginatus* (N = 13, est =  $1.72 \pm 0.72$ ,  $t_{11} = 2.40$ , P = 0.035), and *N. nepalensis* (N =  
544 13, est =  $1.52 \pm 0.71$ ,  $t_{11} = 2.13$ , P = 0.056) from our analysis separately, and without  
545 all three species (N = 11, est =  $0.85 \pm 0.42$ ,  $t_9 = 2.05$ , P = 0.07). The results without *N.*  
546 *nepalensis*, and without all three species, were still marginally significant. More  
547 importantly, a large effect size in the same direction was retained: that is, larger  
548 species have obligate care (see Main Text).

549

550 Data availability

551 Data are available in the Supplementary Materials.

552

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688

689 **Author contributions:**

690 BJMJ and RMK co-designed the selection experiment. BJMJ and MS carried it out,  
691 collected the associated data and analysed them. BJMJ and TMH co-designed the  
692 quantitative genetic experiment and analysed the data together. DR helped carry out  
693 the quantitative genetic experiment. RMK conceived the project and oversaw the  
694 analyses. BJMJ and RMK co-wrote the manuscript, with contributions from MS,  
695 TMH and DR.

696

697 **Declaration of competing interests:**

698 The authors declare no competing interests.

699

700 **Figure Legends**

701 Figure 1

702 The realised heritability of body size, as a function of the different selection regimes  
703 and social environments. The realised heritability is given by the regression slopes,  
704 forced through the intercept. For each treatment the gradient of these regression lines  
705  $\pm$  S.E are: Full Care Large,  $0.09 \pm 0.02$ ; Full Care Small,  $-0.01 \pm 0.02$ ; No Care  
706 Large,  $0.01 \pm 0.03$ ; No Care Small,  $-0.11 \pm 0.03$ . The cumulative selection  
707 differential is the difference between the population mean and the mean of the  
708 retained subset of the population. This is summed across the seven generations. The  
709 cumulative response to selection is the difference between the mean of the population  
710 and the mean of the population in the subsequent generation, and is also summed. The  
711 two replicates for each treatment were pooled for the regression, as they did not differ  
712 (see Supplementary Materials).

713

714 Figure 2

715 The effect of the social environment on the response to selection, in each of the  
716 experimental treatments. (A) and (B) show how the social environment enhances the  
717 capacity for evolutionary change; (C) and (D) show how the social environment could  
718 prevent evolutionary change. (A) Full Care Large: large beetles lay many eggs, but  
719 are more likely to cannibalize larvae and so have relatively small broods that yield  
720 large larvae, which mature in large adults. (B) No Care Small: small beetles lay fewer  
721 eggs, which yield a small brood of small larvae that mature into small adults. (C) No  
722 Care Large: large beetles lay many eggs, which yield a larger brood of small larvae  
723 that mature into small adults and are selected out of the experimental population; and  
724 (D) Full Care Small: small beetles lay fewer eggs which yield a small brood of large

725 larvae that mature into small adults and are selected out of the experimental  
726 population.  
727  
728 Figure 3  
729 Adult pronotum width of burying beetle species mapped on an existing molecular  
730 phylogeny<sup>31</sup>. Black circles indicate species with obligate post-hatching parental care;  
731 open circles indicate facultative post-hatching parental care. Species with missing  
732 data for parental care have no symbols. Body size data can be found in Supplementary  
733 Table 2. Information regarding parental care can be found in Supplementary Table 3.