

1 **Title:** Using experimental evolution to study adaptations for life within the  
2 family

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4 **Authors:** Matthew Schrader<sup>1\*</sup>, Benjamin J. M. Jarrett<sup>1</sup>, and Rebecca M. Kilner<sup>1</sup>

5 <sup>1</sup> Department of Zoology,

6 University of Cambridge

7 Downing Street

8 Cambridge

9 CB2 3EJ

10

11 \*corresponding author's email address: [mss63@cam.ac.uk](mailto:mss63@cam.ac.uk)

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14 **Keywords:** parental care, experimental evolution, coadaptation, burying beetle,

15 *Nicrophorus vespilloides*, interacting phenotypes

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## Abstract

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Parents of many species provision their young and the extent of parental provisioning constitutes a major component of the offspring's social environment. Thus a change in parental provisioning, whether evolved or plastic, can alter the form or strength of selection on offspring and result in the coevolution of parental provisioning and traits expressed in offspring. Although this reasoning is central to our evolutionary understanding of family life, there is little direct evidence that selection by parents causes evolutionary change in their young. Here we use experimental evolution to examine directly how populations of burying beetles, *Nicrophorus vespilloides*, adapt to a change in post-hatching parental provisioning. We measured the performance of larvae descended from independent lab populations that had been propagated for several generations with and without post-hatching parental care (Full Care and No Care populations respectively). We found that adaptation to the absence of post-hatching care led to rapid and consistent changes in larval survival in the absence of care. Specifically, larvae descended from No Care populations had higher survival in the absence of care than larvae descended from Full Care populations. Other measures of larval performance, such as the ability of larvae to consume a breeding carcass and larval mass at dispersal did not differ consistently between the Full Care and No Care populations. Nevertheless, our results show that populations can adapt rapidly to a change in the extent of parental care and that experimental evolution can be used to study such adaptation.

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## Introduction

44

45 All animals provision their young in some form. This provisioning may come  
46 as yolk deposited in eggs, nutrients transferred between mothers and embryos via a  
47 placenta, or as resources provided to dependent young after birth or hatching.

48 Understanding why animals vary in the mode by which they provision their young,  
49 and the amount of resources they provide, has been a major focus of evolutionary and  
50 behavioral ecology (Clutton-Brock 1991; Roff 1992, 2002). Much of this work has  
51 considered parental provisioning as an adult life-history trait that is likely to  
52 experience different strengths or forms of selection in different environments (Smith  
53 and Fretwell 1974; Roff 1992, 2002). If levels of parental provisioning are heritable,  
54 then selection for different levels of parental provisioning in different populations can  
55 result in divergence in traits such as egg size or offspring size at birth (e.g.  
56 Schwarzkopf et al. 1999; Czesak and Fox 2003; Heath et al. 2003; Fischer et al.  
57 2006). Indeed, several studies have found associations between environmental  
58 variables and egg size or size at birth suggesting that parental provisioning is often  
59 locally adapted (e.g. Reznick and Endler 1982; Johnston and Leggett 2002; Heath et  
60 al. 2003; Schrader and Travis 2012).

61 Parental provisioning is not only an important adult life history trait. In  
62 animals with parental care it also constitutes an important part of the offspring's  
63 social environment (Wolf et al. 1998). Therefore a change in parental provisioning,  
64 may alter the form or strength of selection on offspring phenotype resulting in the  
65 further evolution of traits expressed in offspring (Kirkpatrick and Lande 1989; Wolf  
66 et al. 1998; Lock et al. 2004; Moore et al. 1997). That offspring will adapt to  
67 variation in parental provisioning is a central assumption of models of both parent-

68 offspring conflict and coadaptation. For example, parent-offspring conflict is  
69 predicted to favor adaptations in offspring that allow them to extract more care from  
70 parents than is optimal for parents to provide and models of coadaptation predict that  
71 selection on offspring will result in the joint evolution of traits expressed in parents  
72 (e.g. supply of resources) and offspring (e.g. demand for resources) that interact to  
73 influence offspring fitness (Trivers 1974; Wolf and Brodie 1998; Wolf 2000; Hinde et  
74 al. 2010). Support for this assumption comes mainly from quantitative genetic  
75 estimates of social epistasis or genetic correlations between traits involved in parental  
76 supply of resources and offspring demand for these resources (reviewed in Kölliker et  
77 al 2012). The presence of such correlations suggests that selection has favored  
78 specific combinations of these traits (i.e. there has been correlational selection on  
79 parent-offspring interactions). In most cases however, it is not known how these  
80 genetic correlations became established and explanations for the forces that generated  
81 them are inferred from the direction of the correlation (Agrawal et al. 2001; Kölliker  
82 et al. 2005; Lock et al. 2004; reviewed in Kölliker et al. 2012). For example, Agrawal  
83 et al. (2001) found a negative genetic correlation between parental provisioning and  
84 offspring begging in Burrower bugs (*Sehirus cinctus*) and the direction of this  
85 correlation is consistent with predictions of parent-offspring coadaptation theory that  
86 assume the presence of stabilizing selection on offspring phenotype (Wolf and Brodie  
87 1998; Agrawal et al. 2001). In short, previous work suggests that traits involved in  
88 parent-offspring interactions should coevolve and some studies have uncovered  
89 genetic correlations between traits expressed in parents and offspring consistent with  
90 such coevolution. However, no studies have measured whether a change in parental  
91 behavior causes an evolved change in traits expressed by the offspring.

92           Here we describe an experiment in which we examine directly how  
93 populations of burying beetles, *Nicrophorus vespilloides* adapt to a change in parental  
94 provisioning. Like all species in the genus, *N. vespilloides* breeds on the carcasses of  
95 small vertebrates. Upon encountering a carcass, parents mate and prepare the carcass  
96 for their young to feed upon. Carcass preparation involves shaving the fur or feathers  
97 from the carcass, rolling it into a ball, and smearing the surface of the carcass with  
98 anal exudates that delay decomposition (Scott 1998). The eggs, which are laid near  
99 the carcass, hatch and the larvae migrate to the carcass where they feed. *N.*  
100 *vespilloides* larvae exhibit begging behaviors and parents respond to these behaviors  
101 by regurgitating predigested carrion that larvae consume. Post-hatching parental care  
102 is facultative in *N. vespilloides*. Although larvae are able to complete development  
103 with no post-hatching care, measures of breeding success and larval performance are  
104 typically higher when parents are allowed to provision larvae than when they are not  
105 (Eggert et al. 1998).

106           We took advantage of the facultative nature of post-hatching care in this  
107 species to ask how populations adapt to the complete removal of post-hatching care.  
108 To do this we used experimental evolution. This approach involves establishing  
109 replicate experimental populations, exposing these populations to different  
110 environments for many generations, and then comparing traits between populations  
111 that have evolved in these different environments. Experimental evolution has been  
112 used to study how populations adapt to changes in environmental variables such as  
113 predation risk (e.g. Reznick et al. 1997) as well as social aspects of the environment  
114 such as the mating system (e.g. Hollis and Kawecki 2014) but has not yet been used to  
115 study the evolution of parent-offspring interactions. We allowed *N. vespilloides*  
116 populations to evolve for several generations with and without post-hatching parental

117 care (Full Care and No Care populations respectively). Then, for each experimental  
118 population, we measured offspring performance with and without post-hatching  
119 parental care. We predicted that adaptation to the absence of post-hatching parental  
120 care would reduce larval dependence upon care such that, in the absence of parental  
121 care, larvae from No Care populations would perform better than larvae from Full  
122 Care populations.

123

## 124 **Methods**

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### 126 *Establishment and maintenance of experimental populations*

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128 The experimental populations used in this study were created as part of a  
129 larger experiment designed to test whether post-hatching parental care influences the  
130 response to selection on body size (Jarrett et al. in prep). For the purposes of this  
131 study we ignore selection on body size and focus on four experimental populations  
132 that differed in the presence of post-hatching parental care. A complete description of  
133 the protocols used to establish and maintain these populations as well as data on  
134 differences in body size between the populations is provided in the supplemental  
135 material.

136 The experimental populations were descended from beetles collected in 2012  
137 from two wild populations (Byron's Pool and Wicken Fen) in Cambridgeshire, UK.  
138 These populations were interbred over the course of four generations, with 40 pairs  
139 breeding each generation. We then interbred this population with a laboratory  
140 population for a single generation (breeding 160 pairs) to create a large, genetically  
141 diverse stock population. From this stock population, we created two populations that

142 were maintained without post-hatching parental care (No Care 1 and 2) and two  
143 populations that were maintained with full parental care (Full Care 1 and 2).

144 Each No Care (NC) population was initiated by breeding 60 pairs of beetles  
145 from the stock population. We placed each pair in a box with commercially bought  
146 compost and a thawed mouse carcass (8-14 g) and then placed these boxes in a dark  
147 cupboard to simulate underground conditions. We removed both parents from the  
148 breeding box 53 hours after pairing. This is enough time for females to complete egg  
149 laying and carcass preparation but is before eggs begin to hatch (Boncoraglio and  
150 Kilner 2012, Smiseth et al. 2006). After removing both parents, we returned the box  
151 to the cupboard where it remained until larval dispersal (8 days after pairing). On the  
152 eighth day we counted and removed all of the larvae from each breeding box,  
153 weighed each brood, and then calculated the average mass of larvae in each brood  
154 (total brood mass / brood size). We measured brood size and brood mass 8 days after  
155 pairing because by this time larvae have nearly always left the carcass (even in cases  
156 where some flesh remains) and are wandering in the soil. Furthermore, in our  
157 population, leaving the larvae for longer increases that probability that one of the  
158 parents will eat some of the wandering larvae.

159 After measuring brood size and larval mass, we placed the larvae in 25-celled  
160 eclosion boxes (box dimensions, length x width x depth: 10 cm x 10 cm x 1.8 cm)  
161 with one larva in each cell (individual cell dimensions: 2 cm x 2 cm x 1.8 cm),  
162 covered them with damp peat, and left them to pupate for 17 days. Most individuals  
163 had eclosed by 17 days. However, those that had not were allowed more time to  
164 pupate (usually an additional day). After eclosion we sexed and photographed each  
165 beetle and then placed individual beetles in plastic boxes (box dimensions, length x  
166 width x depth: 12 cm x 8 cm x 2 cm), with damp compost and a small amount of

167 ground beef. Adult beetles remained in these boxes for two weeks and were fed  
168 ground beef twice per week. Two weeks after eclosion, we bred beetles from each  
169 population as described above. The number of pairs bred in each generation varied.  
170 In No Care 1 (NC1) we bred between 64 and 120 pairs per generation (mean =  
171 110.75) and in No Care 2 we bred between 39 and 120 pairs per generation (mean =  
172 82.7)

173 The Full Care (FC) populations were each initiated by breeding 40 pairs of  
174 beetles and were treated in exactly the same way as the NC populations except that  
175 we allowed parents to remain with their larvae until larval dispersal. In the Full Care  
176 1 population (FC1) we bred 80 pairs per generation and in the Full Care 2 (FC2) we  
177 bred between 6 and 80 pairs per generation (mean = 75.7).

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### ***Block One***

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181 We maintained NC1 and FC1 for four generations as described above. We  
182 then passed both populations through a fifth generation in which larvae were reared  
183 with full parental care, following the protocol for the Full Care populations (Figure 1).  
184 This was done to minimize differences between lines in transgenerational effects of  
185 care. The resulting offspring were used as the parents in the sixth generation.

186 In the sixth generation, we bred beetles from the NC1 and FC1 populations  
187 with and without post-hatching parental care (see Figure 1). To do this we randomly  
188 paired unrelated adult males and females from within each experimental population ( $n$   
189 = 47 pairs from the NC1 population;  $n$  = 69 pairs from the FC1 population). In the  
190 NC1 population we bred 22 pairs without post-hatching parental care and 25 pairs  
191 with post-hatching parental care. In the FC1 population, we bred 41 pairs without



192 post-hatching parental care and 28 pairs with post-hatching parental care. Eight days  
193 after pairs were bred, we recorded several measures of larval performance. First we  
194 recorded two proxies of larval survival: whether the breeding attempt produced at  
195 least one dispersing larva (breeding success, scored as a binary variable), and for pairs  
196 that bred successfully, the number of larvae at dispersal (brood size). We also  
197 recorded two measures of larval performance: whether the larvae had consumed the  
198 entire breeding carcass (carcass consumption) and the average mass of dispersing  
199 larvae (larval mass). Carcass consumption was scored as a binary variable. If there  
200 was no flesh remaining on the carcass at the time of larval dispersal we considered the  
201 carcass to be completely consumed, otherwise, we scored the carcass as unconsumed  
202 (as in Rauter and Moore 2002). The average mass of dispersing larvae was calculated  
203 as the total brood mass at dispersal divided by the number of larvae in the brood.

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### ***Block Two***

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207 We replicated the experiment described above using NC2 and FC2  
208 populations. These populations had been maintained for seven generations and were  
209 each passed through an eighth generation in which larvae were reared with full  
210 parental care (Figure 1). The resulting offspring were used as the parents in the ninth  
211 generation. In the ninth generation, we bred beetles from the NC2 and FC2  
212 populations with and without post-hatching parental care (Figure 1). Our methods  
213 were identical to those used in block one although sample sizes were different. In the  
214 NC2 population we bred 50 pairs without post-hatching parental care and 30 pairs  
215 with post-hatching parental care. In the FC2 population, we bred 50 pairs without  
216 post-hatching parental care and 30 pairs with post-hatching parental care.

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### *Analysis*

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220           Our experimental design was a 2 x 2 factorial with two population types (No  
221 Care and Full Care) and two levels of environment (No Care and Full Care). The  
222 entire experiment was replicated twice using independent No Care populations that  
223 had been evolving without parental care for different amounts of time (4 generations  
224 in block one and 7 generations in block two). To account for this, we included block  
225 as an additional factor in all analyses. When interactions involving block were  
226 significant we analyzed the data from each block separately.

227           We examined the effect of population, environment, block, and their  
228 interactions on breeding success using two complementary analyses. We first scored  
229 each breeding attempt as being either a success or a failure and analyzed the data  
230 using a GLM with a binomial error term. For this analysis, we scored pairs that laid  
231 eggs but did not produce any dispersing larvae as failures and pairs that had at least  
232 one dispersing larva as successes. Second, for pairs that bred successfully, we  
233 examined the effect of population, environment, block, and their interactions on the  
234 number of dispersing larvae (brood size) using a GLM with a Gaussian error term.  
235 Although brood size only takes on integer values, we chose to use a Gaussian error  
236 term instead of a Poisson error term because the overall mean brood size was fairly  
237 high (14.19) and the residuals from a Gaussian model and a quasipoisson model (the  
238 Poisson model was overdispersed) behaved similarly. We note however that using a  
239 quasipoisson error term did not qualitatively affect our results. Carcass mass did not  
240 influence brood size in our experiments so we did not include it as a covariate in this

241 analysis [linear regression of carcass mass on brood size in both blocks pooled; slope  
242 ( $\pm$ SE) = 0.11 (0.46),  $R^2 = 0.0004$ ,  $F_{1,181} = 0.066$ ,  $P = 0.80$ ).

243 We restricted our analysis of carcass consumption to the No Care environment  
244 since parents also feed upon the carcass making it impossible to attribute carcass  
245 consumption to offspring when parents are also present (we note however that carcass  
246 consumption was > 90 % in all treatments where parents were allowed to remain with  
247 their larvae). We examined the effect of population (Full Care or No care), brood  
248 size, block, and their interactions on carcass consumption using a GLM with a  
249 binomial error term.

250 We intended to examine the effects of population, environment, and block on  
251 larval mass using a GLM with brood size as a covariate. We originally included the  
252 interaction between brood size and environment (Care versus No Care) in this  
253 analysis. This interaction was highly significant ( $P < 0.01$ ) in both blocks of the  
254 experiment indicating that the homogeneity of slopes assumption of the model was  
255 violated. Because the relationship between brood size and mean larval mass differs  
256 between the two parental care environments it is inappropriate to proceed with testing  
257 the significance of the main effects assuming a common slope. Below we report the  
258 results of the analysis of larval mass excluding brood size as a covariate.

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## Results

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262 Breeding success, measured as the proportion of pairs producing at least one  
263 dispersing larva, was lower without post-hatching parental care than with post-  
264 hatching parental care. However, the magnitude of this difference varied between the  
265 No Care and Full Care populations (Figure 2, Table 1). In the absence of post-

266 hatching care the No Care populations had higher breeding success than the Full Care  
267 populations (50 % versus 34 % in block one, 70 % versus 52 % in block two). In the  
268 presence of post-hatching care, breeding success was lower when the beetles were  
269 from the No Care populations than when they were from the Full Care populations  
270 (80 % versus 93 % in block one, 88 % versus 97 % in block two).

271 In both blocks of the experiment, post-hatching parental care led to an  
272 approximately 60 % increase in mean brood size at dispersal (pooling lines within  
273 each environment). The effect of parental care on average brood size did not differ  
274 between the No Care and Full Care populations (Figure 3, Table 2).

275 In both blocks of the experiment, the proportion of broods that successfully  
276 consumed the carcass appeared higher when the larvae were from the No Care  
277 populations than when they were from the Full Care populations (Figure 4). In block  
278 one carcass consumption was 4.6 times greater when beetles were descended from the  
279 No Care line than when they were from the Full Care line and in block two carcass  
280 consumption was 1.7 times greater when the beetles were descended from the No  
281 Care line than when they were from the Full Care line (Figure 4). However, after  
282 accounting for the effect of brood size on carcass consumption, there was no evidence  
283 for a difference between populations in carcass consumption (Table 3).

284 The analysis of larval mass was complicated by differences between the Full  
285 Care and No Care environments in the relationship between larval mass and brood  
286 size. In the Full Care environment there was a negative relationship between average  
287 offspring size and brood size while in the No Care environment this relationship was  
288 shallower and hump-shaped (Figure S2). We have observed the same relationship in  
289 other experiments and discuss its potential causes elsewhere (Schrader et al. in  
290 review). However, because the relationship between brood size and mean larval mass

291 differs between the Full Care and No Care environments we report the results of the  
292 analysis of larval mass excluding brood size as a covariate.

293 Initial analyses of the larval mass revealed a significant treatment by block  
294 interaction so we analyzed each block separately. In block one mean larval mass was  
295 significantly influenced by carcass mass [linear regression of mean larval mass on  
296 carcass mass; slope ( $\pm$  SE) = 0.008615 (0.00319),  $P = 0.0086$ ,  $R^2 = 0.086$ ,  $n = 71$ ] so  
297 we included carcass mass as a covariate in the analysis. After controlling for  
298 variation in carcass mass, we a found a significant effect of environment and  
299 marginally significant effects of population and the population by environment  
300 interaction on mean larval mass (Table 4). The presence of parental care increased  
301 larval mass, however this effect was more pronounced when beetles were from the  
302 Full Care population than the No Care population (Table 4, Figure 5).

303 In block two, the relationship between carcass mass and mean larval mass was  
304 marginally non-significant [linear regression of mean larval mass on carcass mass;  
305 slope ( $\pm$  SE) = 0.003386 (0.00182)  $P = 0.066$ ,  $R^2 = 0.022$ ,  $n = 110$ ]. Our results are  
306 qualitatively similar whether or not we include carcass mass as a covariate and for  
307 simplicity we present the results excluding carcass mass. In contrast to block one,  
308 there was no evidence that parental care increased mean larval mass, nor was there  
309 evidence that the Full Care and No Care populations differed in larval mass or the  
310 effects of parental care on larval mass (Table 4, Figure 5).

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## Discussion

313

314 Changes in parental provisioning are predicted to drive evolutionary changes  
315 in offspring, however few studies have directly examined how offspring adapt to a

316 change in a parental effect. In this experiment we used experimental evolution to  
317 investigate how populations of *N. vespilloides* evolve in response to the elimination of  
318 post-hatching parental care. We found that breeding success in the absence of post-  
319 hatching care was higher when the beetles had evolved in the No Care selection  
320 regime than when they evolved in the Full Care selection regime. This observation is  
321 consistent with rapid adaptation to the absence of post-hatching parental care.  
322 However, other components of larval performance related to resource use did not  
323 differ consistently between populations evolving with and without care.

324         Our results suggest that breeding success in the No Care populations has  
325 become less dependent upon post-hatching parental care, and can envisage two routes  
326 through which this may have evolved. The first possibility involves the evolution of a  
327 trait expressed in the larvae. Eggert et al. (1998) found that the presence of parents  
328 during the first 12 hours after larval hatching greatly increased larval survival and  
329 growth and suggested that this effect was due to parents chewing a hole in the carcass,  
330 thereby making it more accessible for the larvae. In our experiment, it may be that  
331 larvae from the No Care lines are better able to chew an opening in the breeding  
332 carcass themselves, whereas larvae from the Full Care lines are still dependent on  
333 their parents for this task. This change in the larvae could occur through either a  
334 behavioral (e.g. increased self feeding) or morphological (e.g. jaw structure)  
335 adaptation. We are currently examining these possibilities.

336         The second route involves evolutionary change in traits expressed in the  
337 parents. It is well-known from earlier studies that parental investment in burying  
338 beetles involves activities before and after hatching (Scott 1998). Furthermore, there  
339 is individual variation in the relative magnitude of the pre- and post-hatching  
340 components of parental care (Lock et al. 2004; Lock et al. 2007; Steiger 2013). By

341 eliminating post-hatching parental care in one of our experimental evolution  
342 treatments, it is possible that we selected for increased pre-hatching care. In other  
343 words, larvae from the No Care populations may be less dependent on post-hatching  
344 care because their parents have invested more heavily in pre-hatching care.

345         There are two general ways this might happen. First, parents from the No  
346 Care populations may have invested more in carcass preparation than parents from the  
347 Full Care populations, possibly making it easier for larvae to penetrate and use the  
348 breeding carcass. Around the time of hatching, *N. vespilloides* parents create a  
349 feeding cavity in the carcass that larvae recruit to after hatching. Adaptation to the No  
350 Care selection regime could theoretically have selected parents that create this  
351 depression earlier than usual (prior to parental removal at 53 hours after pairing).  
352 However, we think such a shift is unlikely to explain our results because we have  
353 never seen a feeding cavity (or the beginning of one) as early as 53 hours after  
354 pairing. Another possibility is that selection in the No Care environment has favored  
355 parents that create small holes in the carcass (as opposed to the rather large feeding  
356 cavity) prior to parental removal. This could happen as a byproduct of parents  
357 feeding on the carcass and neglecting to reseal the holes they create. We did not  
358 inspect the carcasses in this experiment for small holes. Nevertheless, some support  
359 for this idea comes from another experiment (Schrader et al. in revision). In that  
360 experiment, we removed parents at 53 hours post-pairing and manipulated brood sizes  
361 and inspected carcasses at larval hatching (70 hours post-pairing). At 70 hours post-  
362 pairing, we found that 13.75 % of prepared carcasses (11/80) did indeed have small  
363 holes visible. Although we cannot say with certainty that these holes were created by  
364 the parents, it is likely that at least some of them were. Nevertheless, this same  
365 experiment also found that larvae are able to penetrate the breeding carcass, even if

366 there is no preexisting hole. Determining whether adaptation to the No Care selection  
367 regime has involved a subtle change in carcass preparation will require more careful  
368 examination of carcasses.

369 A second possibility is that selection in the No Care populations has favored  
370 increased maternal investment into individual eggs. In many organisms, egg size or  
371 size at birth is positively correlated offspring performance (e.g. Sinervo 1990;  
372 Takakura 2004; Bashey 2006; Boivin and Gauvin 2009; Monteith et al. 2012;  
373 reviewed in Fox and Czesak 2000). In *N. vespilloides*, the relationship between egg  
374 size and larval performance depends upon the presence of post-hatching care  
375 (Monteith et al. 2012). When parents are allowed to provision larvae, there is no  
376 relationship between egg size and larval growth, but when post-hatching parental care  
377 is removed this relationship is positive. These results suggest that an increase in egg  
378 size can partially compensate for the absence of post-hatching parental care. It is  
379 unclear however, whether the benefits of increased egg size observed by Monteith et  
380 al (2012) would be realized in our experiment. For example, Monteith et al. (2012)  
381 removed parents 72 hours after pairing (around the time of larval hatching). By this  
382 time parents have usually created the feeding cavity in the carcass. In our experiment,  
383 parents were removed well before they begin creating this cavity (as described  
384 above). In addition, Monteith et al.'s (2012) No Care treatment involved cutting a  
385 hole in the carcass with a razor blade immediately after removing the parents. This  
386 was done to facilitate larval entrance into the breeding carcass. Our No Care  
387 treatment did not involve cutting a hole in the carcass. Thus, larvae had to chew their  
388 way in on their own. Whether offspring from larger eggs are better at chewing their  
389 way into the carcass remains unknown.



390           We have suggested ways in which removing post-hatching care may have led  
391 to the evolution of traits in either offspring or parents. However, we wish to stress  
392 that these hypotheses are not mutually exclusive. Adaptation to the absence of post-  
393 hatching parental care may have involved the joint evolution of traits expressed in  
394 offspring and parents (i.e. coadaptation). For example, selection in the No Care  
395 populations may have favored parents that make small holes in the carcass and larvae  
396 that are able to exploit these holes effectively. Whatever traits may be involved, the  
397 rapid evolution of reduced offspring dependence on post-hatching care in the No Care  
398 lines suggests that there is extensive genetic variation for traits affecting larval  
399 survival in the absence of post-hatching care. It is possible that such variation persists  
400 cryptically in *N. vespilloides* because post-hatching parental care typically shields it  
401 from natural selection.

402           Similar to a previous study (Eggert et al. 1998), we found that post-hatching  
403 parental care positively affected the number of dispersing larvae. This effect did not  
404 differ between the Full Care and No Care lines however. This suggests that  
405 adaptation to the No Care selection regime has not involved traits that regulate brood  
406 size after larvae become established on the carcass, either because selection at this  
407 stage has been weak or because there is little heritable variation for the larval  
408 performance at this stage. Our experiment does not allow us to distinguish between  
409 these two possibilities. Although the effect of post-hatching parental care on brood  
410 size did not differ between the Full Care and No Care lines, we note that in the  
411 absence of care, the average brood size at dispersal in the No Care lines was 34-65 %  
412 higher than the average brood size in the Full Care lines (Figure 3). It is possible that  
413 further adaptation to the No Care selection regime will lead to greater divergence in  
414 brood size.

415           Although adaptation to the No Care selection regime reduced offspring  
416 dependence on parental care it did not lead to consistent differences in the sensitivity  
417 of larval mass to post-hatching care. In one block of the experiment post-hatching  
418 care increased larval mass, but only when the beetles were descended from the Full  
419 Care population. In the other block of the experiment, there was no effect of care on  
420 larval mass in beetles descended from either population. The absence of a consistent  
421 effect of post-hatching care on larval mass is somewhat surprising as previous studies  
422 of *Nicrophorus* beetles have found that post-hatching care increases larval mass  
423 (Rauter and Moore 1997; Eggert et al. 1998, Monteith et al. 2012). However, these  
424 previous studies examined the effect of parental care under different environmental  
425 conditions than we did. For example, Rauter and Moore (1997) and Eggert et al.  
426 (1998) measured the impact of post-hatching care in broods whose size had been  
427 standardized to minimize variation in larval mass caused by variation in brood size.  
428 We did not manipulate brood size and were unable to control for variation in brood  
429 size statistically because the presence or absence of post-hatching care substantially  
430 changed the relationship between brood size and larval mass (see above). Second,  
431 although Monteith et al. (2012) measured the effect of care on larval growth without  
432 manipulating brood size, they used larger carcasses than we did and their No Care  
433 treatment involved cutting a hole in the breeding carcass. Finally, all previous studies  
434 examining the impact of parental care on larval mass have removed care at larval  
435 hatching or very soon after. In contrast, our experiment involved removing parents  
436 well before larvae hatch.

437           Despite these methodological issue, the lack of divergence between lines in  
438 larval mass isn't entirely surprising for two reasons. First, the No Care and Full Care  
439 selection regimes probably did not involve consistent differential selection on larval

440 mass. There was no selection on larval mass in the Full Care lines (mating was  
441 random with respect to adult size, which is positively correlated with larval mass). In  
442 the No Care lines, there was a history of selection for either large or small adults but  
443 any history of this selection was likely erased by the protocol we used to create the  
444 experimental populations (see supplemental methods). Second, we know from  
445 previous studies that the total heritability of adult body size in *N. vespilloides* (which  
446 is strongly positively correlated with larval mass at dispersal) is very low suggesting  
447 that a response to even very strong selection on body size is unlikely to result in  
448 evolutionary change over the short-term (Head et al. 2012; Schrader et al. unpublished  
449 data).

450         Perhaps more importantly than the individual results described above, our  
451 study suggests that experimental evolution can be applied to the study of parental  
452 care. Much of what we know about the evolution of parent-offspring interactions  
453 comes from quantitative genetic estimates of correlations between parental and  
454 offspring traits (reviewed in Kölliker et al. 2012). While these studies have been  
455 tremendously valuable, they can only be used inferentially to understand how  
456 selection has shaped parent-offspring interactions. We suggest that combining  
457 quantitative genetic approaches with experimental evolution will provide more direct  
458 insights into how parent-offspring interactions coevolve in response to different  
459 selection regimes. For example, by manipulating the presence of post-hatching  
460 parental care over many generations it might be possible to study not only how  
461 offspring adapt to a change in parental care but also whether such adaptation changes  
462 the direction or magnitude of genetic correlations between parental and offspring  
463 behaviors. Similarly, it might be possible to manipulate the extent of parent-offspring  
464 conflict in experimental populations (e.g. through manipulating the mating system)

465 and ask whether correlations between parental and offspring traits evolve in response  
466 to antagonistic selection. Insects with flexible patterns of parental care and rapid  
467 generation times, such as *N. vespilloides*, are ideal species for such experiments.

468

#### 469 Acknowledgements

470

471 The authors were supported by a Consolidator's Grant from the European Research  
472 Council (310785 Baldwinian Beetles). Research was funded by the Natural  
473 Environment Research Council UK (NE/H019731/1), the European Research  
474 Council, and the Department of Zoology at the University of Cambridge. We thank  
475 the associate editor and two anonymous reviewers for comments that greatly  
476 improved the quality of the manuscript. We are also grateful to the other members of  
477 the burying beetle group and K. McGhee for helpful discussions and to S. Aspinall  
478 and C. Swannack for help in the lab.

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Tables

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Table 1. Results of a general linear model of the effects of population (No Care or Full Care), treatment (No Care or Full Care), the population by treatment interaction, and experimental block on breeding success. See Figure 2 for sample sizes in each treatment.

<b>Factor</b>	<b><math>X^2</math></b>	<b><i>P</i></b>
Population	2.9	0.08
Treatment	44.5	<0.00001
Population x Treatment	6.50	0.011
Block	7.61	0.0075

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Table 2. Results of analyses of variance of the effects of population, treatment, the population by treatment interaction, and experimental block on average brood size.

<b>Factor</b>	<b><i>F</i></b>	<b>df</b>	<b><i>P</i></b>
Population	0.016	1, 177	0.89
Treatment	32.90	1,177	<0.00001
Population x Treatment	2.35	1,177	0.13
Block	8.80	1,177	0.0034

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Table 3. Results of a general linear model of the effects of population, brood size, and block on carcass consumption. See Figure 4 for sample sizes in each treatment.

<b>Factor</b>	<b><math>X^2</math></b>	<b><i>P</i></b>
Population	1.97	0.16
Brood Size	57.80	<0.0001
Block	1.51	0.22

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Table 4. Results of analyses of variance of the effects of population, treatment, and their interaction on average larval mass for both blocks of the experiment considered separately. Carcass mass was included as a covariate in block one of the experiment but was not included in the analysis of block two.

<b>Block one</b>			
<b>Factor</b>	<b><i>F</i></b>	<b>df</b>	<b><i>P</i></b>
Population	3.018	1, 66	0.087
Treatment	8.39	1, 66	0.0051
Population X Treatment	3.71	1, 66	0.058
Carcass mass	10.77	1, 66	0.0017
<b>Block two</b>			
<b>Factor</b>	<b><i>F</i></b>	<b>df</b>	<b><i>P</i></b>
Population	2.19	1,107	0.14
Treatment	0.45	1,107	0.51
Population x Treatment	0.14	1,107	0.71

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## Figure Legends

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603 Figure 1. A simplified schematic of the experimental populations and design.  
604 Dashed lines and solid lines represent populations that had been propagated without  
605 and with post-hatching parental care respectively. Numbers to the left of each arrow  
606 are the number of generations that each population had been maintained without or  
607 with post-hatching parental care. For example, NC1 had been propagated for 4  
608 generations with no post-hatching parental care. All populations were passed through  
609 a single generation with full parental care to minimize differences between lines in  
610 transgenerational effects (indicated by solid lines enclosed in gray rectangles). The  
611 resulting offspring were then bred with or without post-hatching parental care (solid  
612 and dashed arrows respectively). For each pair we measured breeding success, brood  
613 size at dispersal, mean larval mass, and carcass consumption. A complete description  
614 of the establishment and maintenance of the experimental populations can be found in  
615 the supplemental material.

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618 Figure 2. Breeding success of beetles descended from No Care (gray squares and  
619 dashed line) and Full Care (black squares and solid line) populations in the absence or  
620 presence of post-hatching care (x axis). Breeding success is the proportion of  
621 breeding attempts producing at least one dispersing larva. Numbers above each  
622 symbol are sample sizes. Data in the top panel are from block one and data in the  
623 bottom panel are from block two.

624

625 Figure 3. Mean brood size ( $\pm 1$  S. E. M.) of beetle pairs descended from No Care  
626 (gray squares and dashed lines) and Full Care populations (black squares and solid  
627 lines) in the absence or presence of post-hatching care (x axis). Data in the top panel  
628 are from block one and data in the bottom panel are from block two.

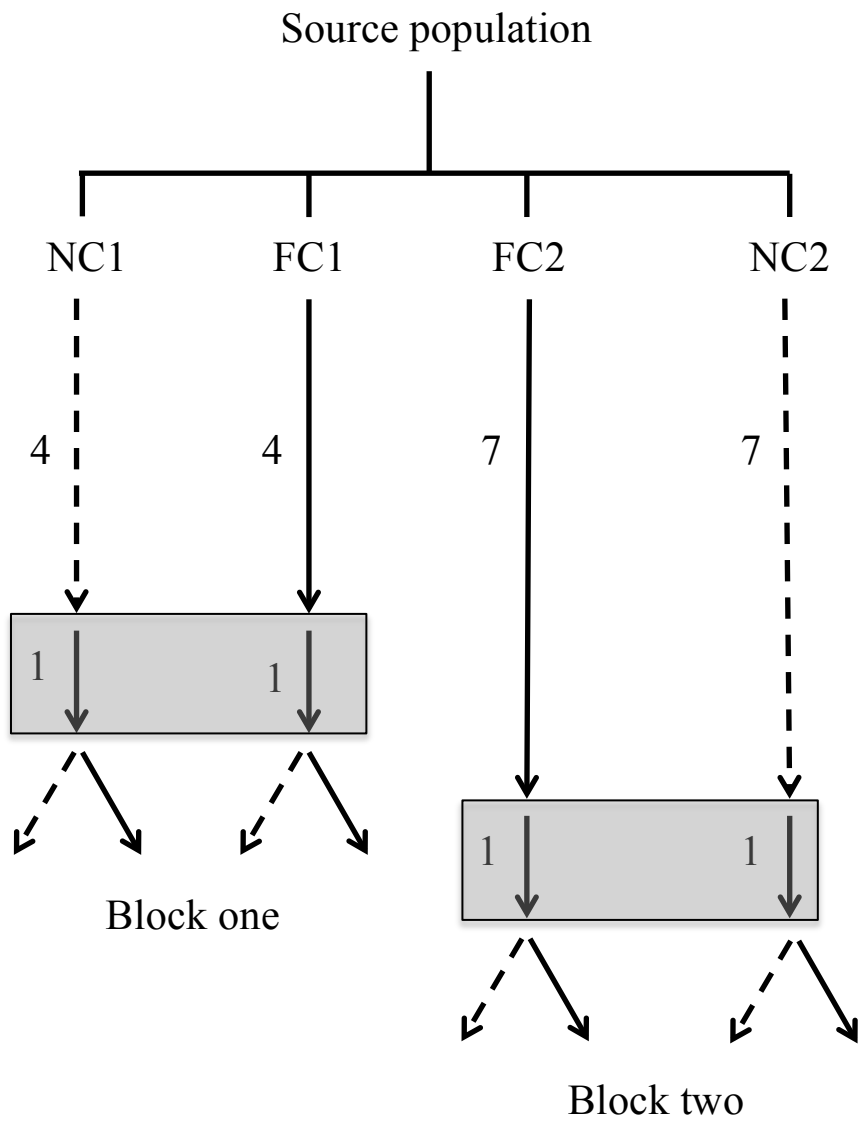
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630 Figure 4. The proportion of successful breeding bouts without parental care in which  
631 the breeding carcass was completely consumed when beetle pairs were descended  
632 from No Care (gray bars) and Full Care populations (black bars) in each experimental  
633 block. The sample size is indicated over each bar.

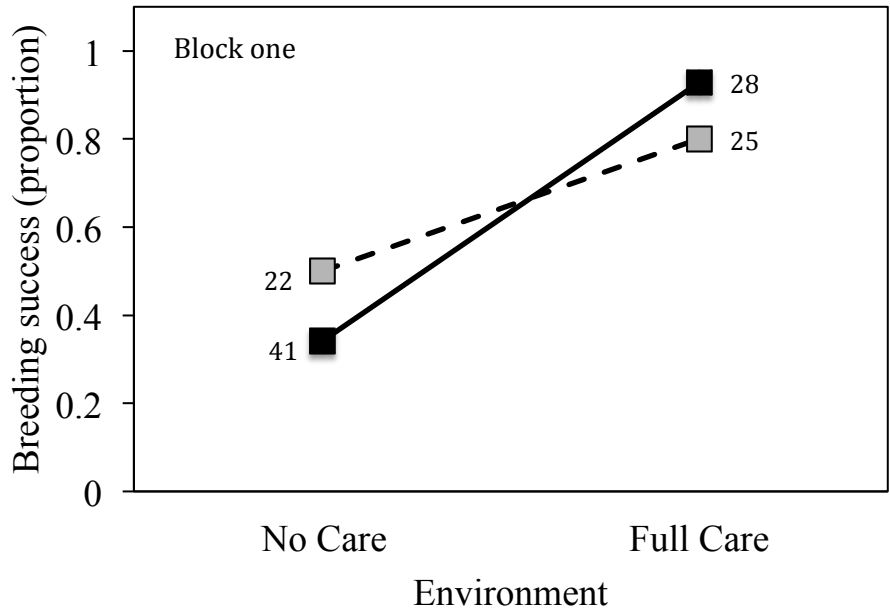
634 Figure 5. Mean larval mass ( $\pm 1$  S. E. M.) of beetles descended from the No Care  
635 (gray squares and dashed lines) and Full Care (black squares and solid lines)  
636 populations in the absence or presence of post-hatching care. Data in the top panel are  
637 from block one and data in the bottom panel are from block two.

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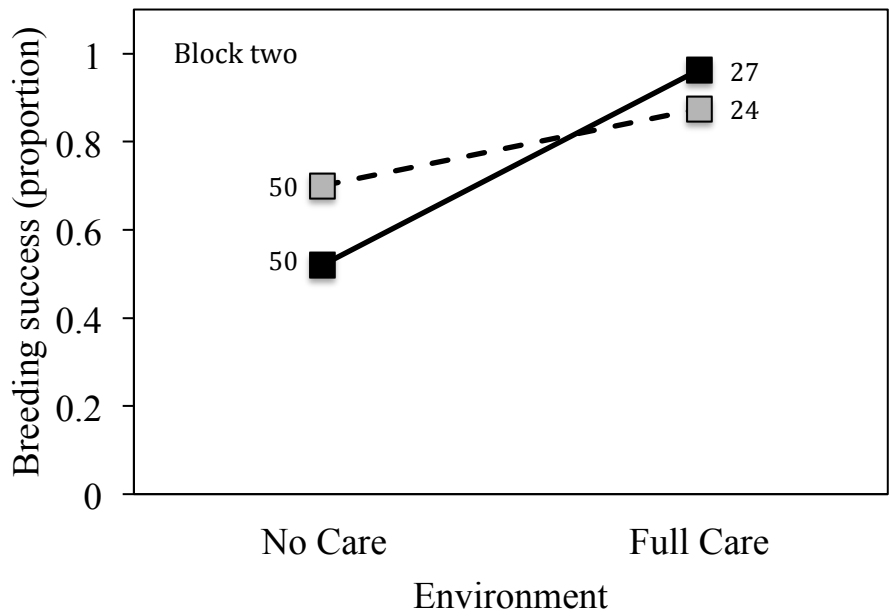
640 Figure 1.



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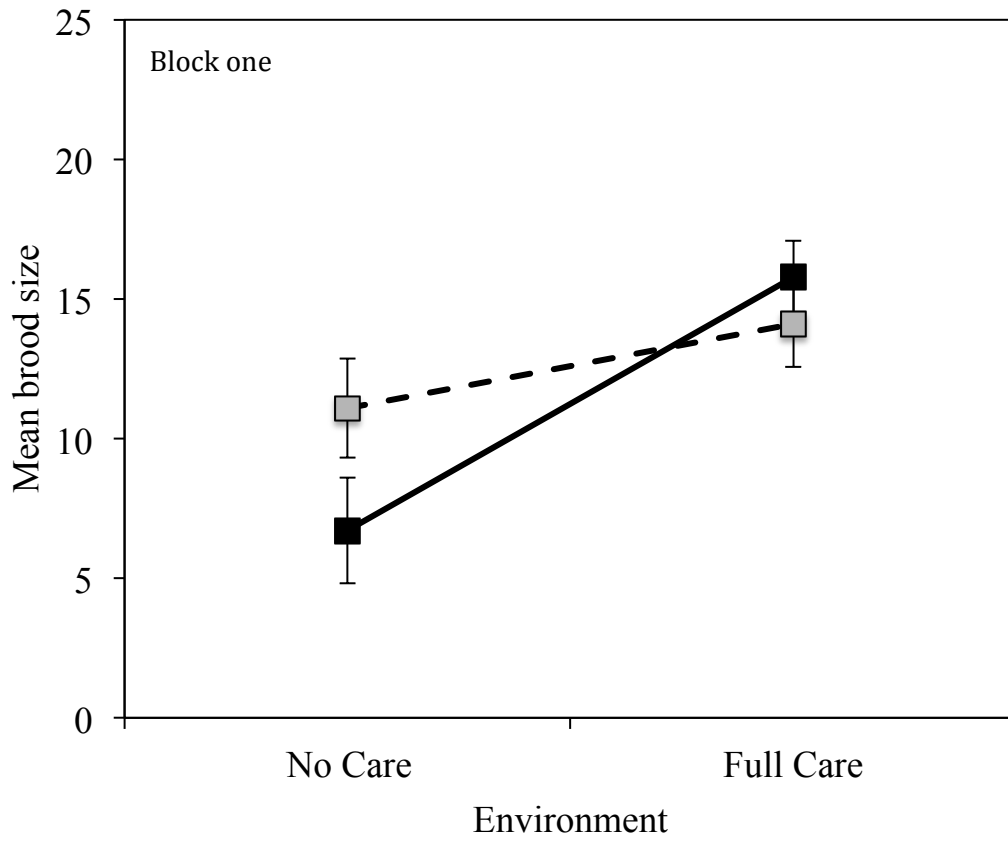


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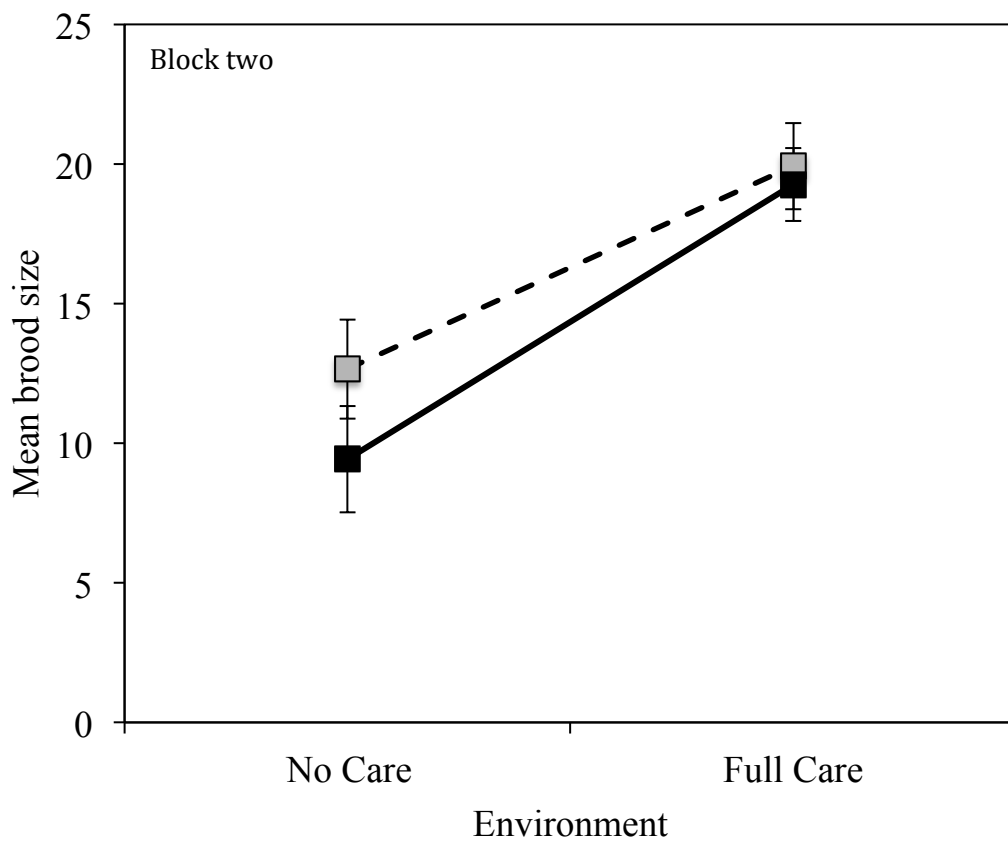


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Figure 2

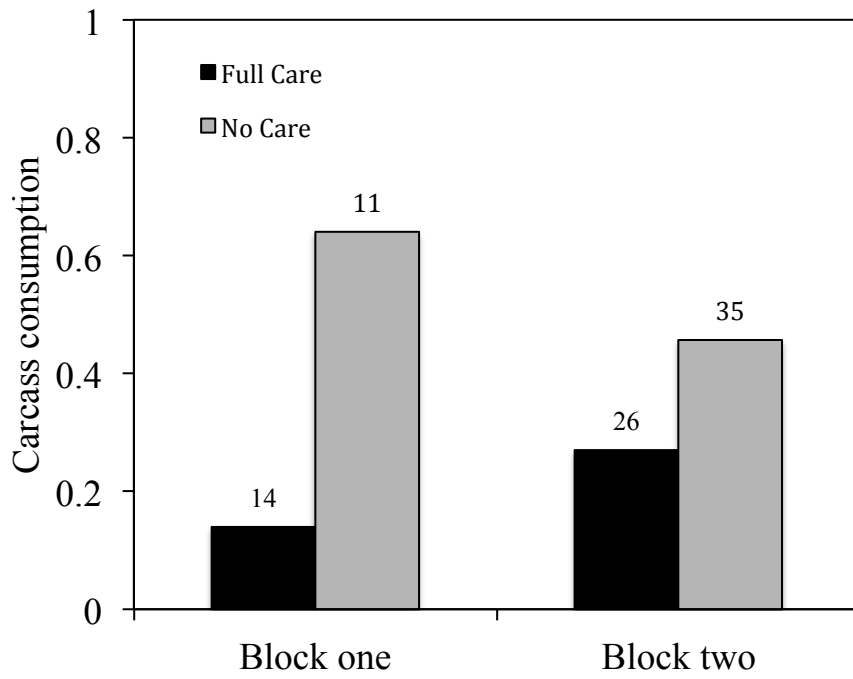


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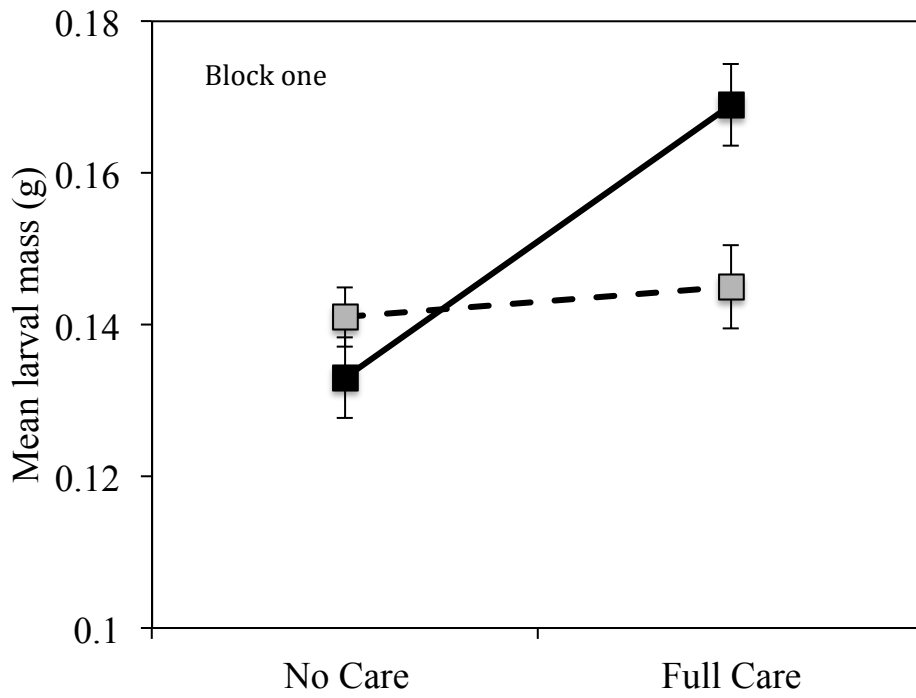
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Figure 3

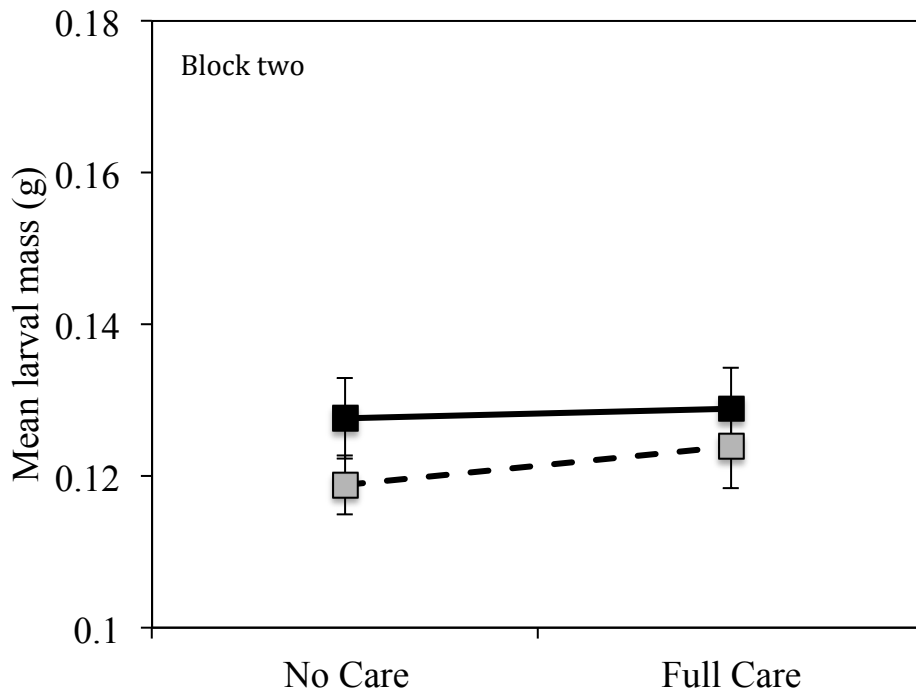


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Figure 4



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Figure 5.

## Supplemental Methods

The populations used in our experiment were created as part of an experiment in which we artificially selected on adult body size (pronotum width) in populations maintained with either full parental care (Full Care) or no post-hatching parental care (No Care). The goal of this experiment was to test whether the presence of parental care affects the response to selection (Jarrett et al. in prep). Below we describe the selection protocol for the different experimental lines and how these lines were combined to create the experimental populations.

### *Selection on adult size*

We selected for large body size in two independent Full Care populations [Full Care Large 1 (FCL1) and Full Care Large 2 (FCL2)] and two independent No Care populations [No Care Large 1 (NCL1) and No Care Large 2 (NCL2)]. In these lines we paired unrelated individuals in the largest 1/3 of the population to use as breeding stock each generation. We selected for small body size in two independent Full Care populations [Full Care Small 1 (FCS1) and Full Care Small 2 (FCS2)] and two independent No Care populations [No Care Small 1 (NCS1) and No Care Small 2 (NCS2)]. In these lines we paired unrelated individuals in the smallest 1/3 of the population to use as breeding stock each generation. We also maintained two replicate Full Care populations without selection on body size as controls [Full Care Control 1 (FCC1) and Full Care Control 2 (FCC2)]. In the control lines, we paired unrelated individuals randomly with respect to body size to use as breeding stock each generation. Our experiment utilized beetles descended from the four No Care

26 populations (NCL1, NCL2, NCS1, NCS2) and the two Full Care Control populations  
27 (FCC1 and FCC2). The Full Care Large / Full Care Small populations were not  
28 included in the experiment because the lines were staggered temporally making it  
29 impossible to breed beetles of similar ages to establish the experimental lines required  
30 for the experiment. For the purposes of this study, we ignore differences between  
31 lines in selection on adult size but note that there was no significant difference  
32 between large and small selected lines used in the experiment (see below).

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### *Block 1*

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36 Four generations after establishing the experimental populations we combined  
37 the NCL1 and NCS1 populations into a single population [No Care 1 (NC1)] (see  
38 Figure S1A). This was necessary to avoid inbreeding in one of the populations. At  
39 this time there was no significant difference between NCL1 and NCS1 in pronotum  
40 width (mean pronotum width in generation four: NCL1 = 4.52, mm, n = 239; NCS1 =  
41 4.56 mm, n = 150;  $t_{365.24} = -0.772$ ,  $P = 0.447$ ). To create the NC1 population, we  
42 conducted reciprocal hybrid crosses between NCL1 and NCS1 populations (n = 5 in  
43 each direction) and within population crosses involving unrelated individuals (NCL1  
44 x NCL1, n = 3 and NCS1 x NCS1, n = 7). At the same time, we created a  
45 corresponding full care population (FC1) by conducting reciprocal hybrid crosses  
46 between the FCC1 and FCC2 populations (n = 5 in each direction) and mating  
47 unrelated individuals from within each subpopulation (FCC1 x FCC1 and FCC2 x  
48 FCC2, n = 5 each). These populations were crossed to be consistent with the protocol  
49 described above for the NC1 population. The individuals crossed to create the NC1  
50 and FC1 populations were chosen randomly with respect to pronotum width and the



51 crosses were conducted with full parental care to minimize differences between lines  
52 in transgenerational effects of care. The offspring from these crosses were used as the  
53 parents in the experimental generation.

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### *Block 2*

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57 We replicated the experiment described above using a second pair of  
58 experimental laboratory populations that differed with respect to the presence of post-  
59 hatching parental care in the previous seven generations. The first population, No  
60 Care 2 (NC2), was created by combining the NCL2 and NCS2 subpopulations 7  
61 generations after establishing the experimental populations (Figure S1B). At this  
62 time, there was no difference between the NCL2 and NCS2 populations in pronotum  
63 width (mean pronotum width in generation seven: NCL1 = 4.45, mm, n = 417; NCS1  
64 = 4.49 mm, n = 188;  $t_{442.165} = -1.10$ ,  $P = 0.271$ ). To combine these populations we  
65 conducted reciprocal hybrid crosses between the two lines (n = 10 in each direction)  
66 and within subpopulation crosses involving unrelated individuals (NCL2 x NCL2, n =  
67 10 and NCS2 x NCS2, n = 10). At the same time, we created a corresponding full  
68 care population, Full Care 2 (FC2). This population was created by combining the  
69 FCC1 and FCC2 subpopulations 7 generations after establishing the experimental  
70 populations from the stock. To combine these populations we conducted reciprocal  
71 hybrid crosses between the two lines (n = 10 in each direction) and within  
72 subpopulation crosses involving unrelated individuals (FCC1 x FCC1, n = 10 and  
73 FCC2 x FCC2, n = 10). The individuals crossed to create the NC2 and FC2  
74 populations were chosen randomly with respect to pronotum width and the crosses  
75 were conducted with full parental care to minimize differences between lines in

76 transgenerational effects of care. The offspring from these crosses were used as the

77 parents in the experimental generation.

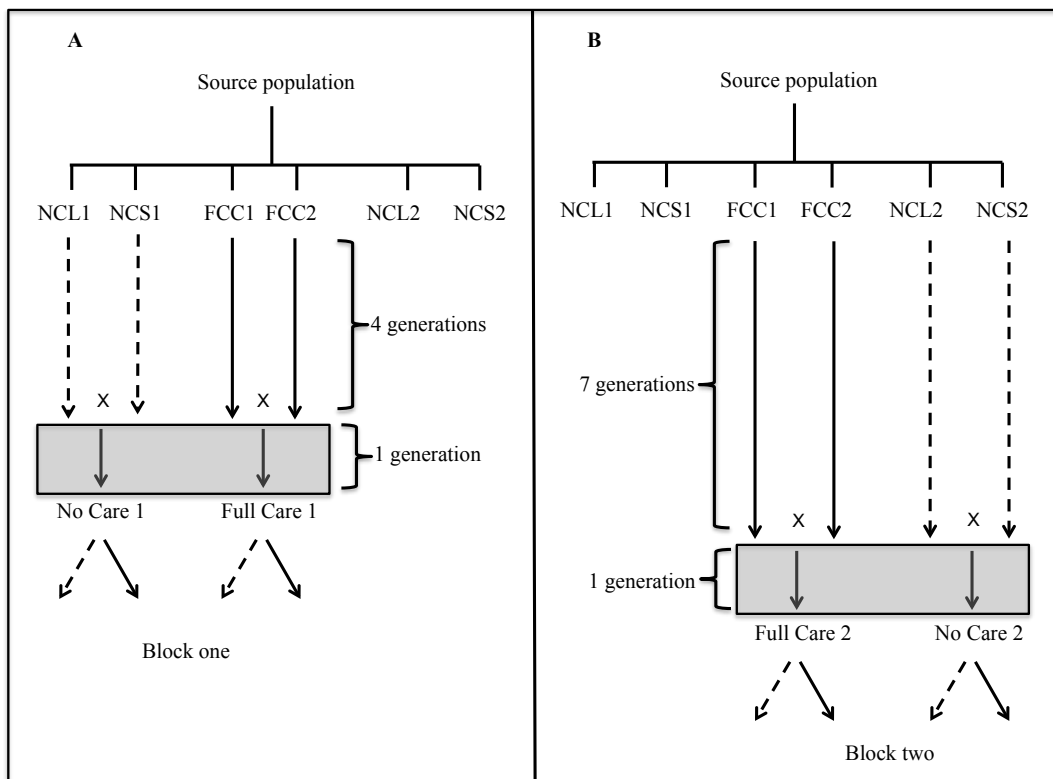
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### Supplemental Figures

81 Figure S1. A detailed schematic showing the creation of the experimental lines used  
82 in block one (A) and block two (B) of the experiment. Dashed lines indicate the No  
83 Care environment and solid lines indicate the Full Care environment. Abbreviations  
84 for each line follow those in the text above. All populations were passed through a  
85 single generation with full parental care to minimize differences between lines in  
86 transgenerational effects (indicated by solid lines enclosed in gray rectangles). The  
87 resulting offspring were then bred with or without post-hatching parental care (solid  
88 and dashed arrows respectively). For each pair we measured breeding success, brood  
89 size at dispersal, mean larval mass, and carcass consumption.

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97 Figure S2. The relationship between mean larval mass and brood size in the presence  
98 (left) or absence of post-hatching parental care. Data from block 1 are in the top row  
99 and data from block 2 are in the bottom row.

