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

Authors: Matthew Schrader1*, Benjamin J. M. Jarrett1, and Rebecca M. Kilner1

1 Department of Zoology, University of Cambridge

Downing Street

Cambridge

CB2 3EJ

*corresponding author’s email address: mss63@cam.ac.uk

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

*Nicrophorus vespilloides, interacting phenotypes
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.
Introduction

All animals provision their young in some form. This provisioning may come as yolk deposited in eggs, nutrients transferred between mothers and embryos via a placenta, or as resources provided to dependent young after birth or hatching. Understanding why animals vary in the mode by which they provision their young, and the amount of resources they provide, has been a major focus of evolutionary and behavioral ecology (Clutton-Brock 1991; Roff 1992, 2002). Much of this work has considered parental provisioning as an adult life-history trait that is likely to experience different strengths or forms of selection in different environments (Smith and Fretwell 1974; Roff 1992, 2002). If levels of parental provisioning are heritable, then selection for different levels of parental provisioning in different populations can result in divergence in traits such as egg size or offspring size at birth (e.g. Schwarzkopf et al. 1999; Czesak and Fox 2003; Heath et al. 2003; Fischer et al. 2006). Indeed, several studies have found associations between environmental variables and egg size or size at birth suggesting that parental provisioning is often locally adapted (e.g. Reznick and Endler 1982; Johnston and Leggett 2002; Heath et al. 2003; Schrader and Travis 2012).

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

ing conflict and coadaptation. For example, parent-offspring conflict is predicted to favor adaptations in offspring that allow them to extract more care from parents than is optimal for parents to provide and models of coadaptation predict that selection on offspring will result in the joint evolution of traits expressed in parents (e.g. supply of resources) and offspring (e.g. demand for resources) that interact to influence offspring fitness (Trivers 1974; Wolf and Brodie 1998; Wolf 2000; Hinde et al. 2010). Support for this assumption comes mainly from quantitative genetic estimates of social epistasis or genetic correlations between traits involved in parental supply of resources and offspring demand for these resources (reviewed in Kölliker et al. 2012). The presence of such correlations suggests that selection has favored specific combinations of these traits (i.e. there has been correlational selection on parent-offspring interactions). In most cases however, it is not known how these genetic correlations became established and explanations for the forces that generated them are inferred from the direction of the correlation (Agrawal et al. 2001; Kölliker et al. 2005; Lock et al. 2004; reviewed in Kölliker et al. 2012). For example, Agrawal et al. (2001) found a negative genetic correlation between parental provisioning and offspring begging in Burrower bugs (*Sehirus cinctus*) and the direction of this correlation is consistent with predictions of parent-offspring coadaptation theory that assume the presence of stabilizing selection on offspring phenotype (Wolf and Brodie 1998; Agrawal et al. 2001). In short, previous work suggests that traits involved in parent-offspring interactions should coevolve and some studies have uncovered genetic correlations between traits expressed in parents and offspring consistent with such coevolution. However, no studies have measured whether a change in parental behavior causes an evolved change in traits expressed by the offspring.
Here we describe an experiment in which we examine directly how populations of burying beetles, *Nicrophorus vespilloides* adapt to a change in parental provisioning. Like all species in the genus, *N. vespilloides* breeds on the carcasses of small vertebrates. Upon encountering a carcass, parents mate and prepare the carcass for their young to feed upon. Carcass preparation involves shaving the fur or feathers from the carcass, rolling it into a ball, and smearing the surface of the carcass with anal exudates that delay decomposition (Scott 1998). The eggs, which are laid near the carcass, hatch and the larvae migrate to the carcass where they feed. *N. vespilloides* larvae exhibit begging behaviors and parents respond to these behaviors by regurgitating predigested carrion that larvae consume. Post-hatching parental care is facultative in *N. vespilloides*. Although larvae are able to complete development with no post-hatching care, measures of breeding success and larval performance are typically higher when parents are allowed to provision larvae than when they are not (Eggert et al. 1998).

We took advantage of the facultative nature of post-hatching care in this species to ask how populations adapt to the complete removal of post-hatching care. To do this we used experimental evolution. This approach involves establishing replicate experimental populations, exposing these populations to different environments for many generations, and then comparing traits between populations that have evolved in these different environments. Experimental evolution has been used to study how populations adapt to changes in environmental variables such as predation risk (e.g. Reznick et al. 1997) as well as social aspects of the environment such as the mating system (e.g. Hollis and Kawecki 2014) but has not yet been used to study the evolution of parent-offspring interactions. We allowed *N. vespilloides* populations to evolve for several generations with and without post-hatching parental
care (Full Care and No Care populations respectively). Then, for each experimental population, we measured offspring performance with and without post-hatching parental care. We predicted that adaptation to the absence of post-hatching parental care would reduce larval dependence upon care such that, in the absence of parental care, larvae from No Care populations would perform better than larvae from Full Care populations.

Methods

Establishment and maintenance of experimental populations

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

The experimental populations were descended from beetles collected in 2012 from two wild populations (Byron’s Pool and Wicken Fen) in Cambridgeshire, UK. These populations were interbred over the course of four generations, with 40 pairs breeding each generation. We then interbred this population with a laboratory population for a single generation (breeding 160 pairs) to create a large, genetically diverse stock population. From this stock population, we created two populations that
were maintained without post-hatching parental care (No Care 1 and 2) and two populations that were maintained with full parental care (Full Care 1 and 2).

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

After measuring brood size and larval mass, we placed the larvae in 25-celled eclosion boxes (box dimensions, length x width x depth: 10 cm x 10 cm x 1.8 cm) with one larva in each cell (individual cell dimensions: 2 cm x 2 cm x 1.8 cm), covered them with damp peat, and left them to pupate for 17 days. Most individuals had eclosed by 17 days. However, those that had not were allowed more time to pupate (usually an additional day). After eclosion we sexed and photographed each beetle and then placed individual beetles in plastic boxes (box dimensions, length x width x depth: 12 cm x 8 cm x 2 cm), with damp compost and a small amount of
ground beef. Adult beetles remained in these boxes for two weeks and were fed ground beef twice per week. Two weeks after eclosion, we bred beetles from each population as described above. The number of pairs bred in each generation varied. In No Care 1 (NC1) we bred between 64 and 120 pairs per generation (mean = 110.75) and in No Care 2 we bred between 39 and 120 pairs per generation (mean = 82.7).

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

**Block One**

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

In the sixth generation, we bred beetles from the NC1 and FC1 populations with and without post-hatching parental care (see Figure 1). To do this we randomly paired unrelated adult males and females from within each experimental population (n = 47 pairs from the NC1 population; n = 69 pairs from the FC1 population). In the NC1 population we bred 22 pairs without post-hatching parental care and 25 pairs with post-hatching parental care. In the FC1 population, we bred 41 pairs without
post-hatching parental care and 28 pairs with post-hatching parental care. Eight days after pairs were bred, we recorded several measures of larval performance. First we recorded two proxies of larval survival: whether the breeding attempt produced at least one dispersing larva (breeding success, scored as a binary variable), and for pairs that bred successfully, the number of larvae at dispersal (brood size). We also recorded two measures of larval performance: whether the larvae had consumed the entire breeding carcass (carcass consumption) and the average mass of dispersing larvae (larval mass). Carcass consumption was scored as a binary variable. If there was no flesh remaining on the carcass at the time of larval dispersal we considered the carcass to be completely consumed, otherwise, we scored the carcass as unconsumed (as in Rauter and Moore 2002). The average mass of dispersing larvae was calculated as the total brood mass at dispersal divided by the number of larvae in the brood.

**Block Two**

We replicated the experiment described above using NC2 and FC2 populations. These populations had been maintained for seven generations and were each passed through an eighth generation in which larvae were reared with full parental care (Figure 1). The resulting offspring were used as the parents in the ninth generation. In the ninth generation, we bred beetles from the NC2 and FC2 populations with and without post-hatching parental care (Figure 1). Our methods were identical to those used in block one although sample sizes were different. In the NC2 population we bred 50 pairs without post-hatching parental care and 30 pairs with post-hatching parental care. In the FC2 population, we bred 50 pairs without post-hatching parental care and 30 pairs with post-hatching parental care.
Our experimental design was a 2 x 2 factorial with two population types (No Care and Full Care) and two levels of environment (No Care and Full Care). The entire experiment was replicated twice using independent No Care populations that had been evolving without parental care for different amounts of time (4 generations in block one and 7 generations in block two). To account for this, we included block as an additional factor in all analyses. When interactions involving block were significant we analyzed the data from each block separately.

We examined the effect of population, environment, block, and their interactions on breeding success using two complementary analyses. We first scored each breeding attempt as being either a success or a failure and analyzed the data using a GLM with a binomial error term. For this analysis, we scored pairs that laid eggs but did not produce any dispersing larvae as failures and pairs that had at least one dispersing larva as successes. Second, for pairs that bred successfully, we examined the effect of population, environment, block, and their interactions on the number of dispersing larvae (brood size) using a GLM with a Gaussian error term. Although brood size only takes on integer values, we chose to use a Gaussian error term instead of a Poisson error term because the overall mean brood size was fairly high (14.19) and the residuals from a Gaussian model and a quasipoisson model (the Poisson model was overdispersed) behaved similarly. We note however that using a quasipoisson error term did not qualitatively affect our results. Carcass mass did not influence brood size in our experiments so we did not include it as a covariate in this
We restricted our analysis of carcass consumption to the No Care environment since parents also feed upon the carcass making it impossible to attribute carcass consumption to offspring when parents are also present (we note however that carcass consumption was > 90% in all treatments where parents were allowed to remain with their larvae). We examined the effect of population (Full Care or No care), brood size, block, and their interactions on carcass consumption using a GLM with a binomial error term.

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

Results

Breeding success, measured as the proportion of pairs producing at least one dispersing larva, was lower without post-hatching parental care than with post-hatching parental care. However, the magnitude of this difference varied between the No Care and Full Care populations (Figure 2, Table 1). In the absence of post-
hatching care the No Care populations had higher breeding success than the Full Care populations (50% versus 34% in block one, 70% versus 52% in block two). In the presence of post-hatching care, breeding success was lower when the beetles were from the No Care populations than when they were from the Full Care populations (80% versus 93% in block one, 88% versus 97% in block two).

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

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

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

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

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

Discussion

Changes in parental provisioning are predicted to drive evolutionary changes in offspring, however few studies have directly examined how offspring adapt to a
change in a parental effect. In this experiment we used experimental evolution to investigate how populations of *N. vespilloides* evolve in response to the elimination of post-hatching parental care. We found that breeding success in the absence of post-hatching care was higher when the beetles had evolved in the No Care selection regime than when they evolved in the Full Care selection regime. This observation is consistent with rapid adaptation to the absence of post-hatching parental care. However, other components of larval performance related to resource use did not differ consistently between populations evolving with and without care.

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

The second route involves evolutionary change in traits expressed in the parents. It is well-known from earlier studies that parental investment in burying beetles involves activities before and after hatching (Scott 1998). Furthermore, there is individual variation in the relative magnitude of the pre- and post-hatching components of parental care (Lock et al. 2004; Lock et al. 2007; Steiger 2013). By
eliminating post-hatching parental care in one of our experimental evolution
treatments, it is possible that we selected for increased pre-hatching care. In other
words, larvae from the No Care populations may be less dependent on post-hatching
care because their parents have invested more heavily in pre-hatching care.

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

A second possibility is that selection in the No Care populations has favored
increased maternal investment into individual eggs. In many organisms, egg size or
size at birth is positively correlated offspring performance (e.g. Sinervo 1990;
Takakura 2004; Bashey 2006; Boivin and Gauvin 2009; Monteith et al. 2012;
reviewed in Fox and Czesak 2000). In *N. vespilloides*, the relationship between egg
size and larval performance depends upon the presence of post-hatching care
(Monteith et al. 2012). When parents are allowed to provision larvae, there is no
relationship between egg size and larval growth, but when post-hatching parental care
is removed this relationship is positive. These results suggest that an increase in egg
size can partially compensate for the absence of post-hatching parental care. It is
unclear however, whether the benefits of increased egg size observed by Monteith et
al (2012) would be realized in our experiment. For example, Monteith et al. (2012)
removed parents 72 hours after pairing (around the time of larval hatching). By this
time parents have usually created the feeding cavity in the carcass. In our experiment,
parents were removed well before they begin creating this cavity (as described
above). In addition, Monteith et al.’s (2012) No Care treatment involved cutting a
hole in the carcass with a razor blade immediately after removing the parents. This
was done to facilitate larval entrance into the breeding carcass. Our No Care
treatment did not involve cutting a hole in the carcass. Thus, larvae had to chew their
way in on their own. Whether offspring from larger eggs are better at chewing their
way into the carcass remains unknown.
We have suggested ways in which removing post-hatching care may have led to the evolution of traits in either offspring or parents. However, we wish to stress that these hypotheses are not mutually exclusive. Adaptation to the absence of post-hatching parental care may have involved the joint evolution of traits expressed in offspring and parents (i.e. coadaptation). For example, selection in the No Care populations may have favored parents that make small holes in the carcass and larvae that are able to exploit these holes effectively. Whatever traits may be involved, the rapid evolution of reduced offspring dependence on post-hatching care in the No Care lines suggests that there is extensive genetic variation for traits affecting larval survival in the absence of post-hatching care. It is possible that such variation persists cryptically in *N. vespilloides* because post-hatching parental care typically shields it from natural selection.

Similar to a previous study (Eggert et al. 1998), we found that post-hatching parental care positively affected the number of dispersing larvae. This effect did not differ between the Full Care and No Care lines however. This suggests that adaptation to the No Care selection regime has not involved traits that regulate brood size after larvae become established on the carcass, either because selection at this stage has been weak or because there is little heritable variation for the larval performance at this stage. Our experiment does not allow us to distinguish between these two possibilities. Although the effect of post-hatching parental care on brood size did not differ between the Full Care and No Care lines, we note that in the absence of care, the average brood size at dispersal in the No Care lines was 34-65 % higher than the average brood size in the Full Care lines (Figure 3). It is possible that further adaptation to the No Care selection regime will lead to greater divergence in brood size.
Although adaptation to the No Care selection regime reduced offspring dependence on parental care it did not lead to consistent differences in the sensitivity of larval mass to post-hatching care. In one block of the experiment post-hatching care increased larval mass, but only when the beetles were descended from the Full Care population. In the other block of the experiment, there was no effect of care on larval mass in beetles descended from either population. The absence of a consistent effect of post-hatching care on larval mass is somewhat surprising as previous studies of *Nicrophorus* beetles have found that post-hatching care increases larval mass (Rauter and Moore 1997; Eggert et al. 1998, Monteith et al. 2012). However, these previous studies examined the effect of parental care under different environmental conditions than we did. For example, Rauter and Moore (1997) and Eggert et al. (1998) measured the impact of post-hatching care in broods whose size had been standardized to minimize variation in larval mass caused by variation in brood size. We did not manipulate brood size and were unable to control for variation in brood size statistically because the presence or absence of post-hatching care substantially changed the relationship between brood size and larval mass (see above). Second, although Monteith et al. (2012) measured the effect of care on larval growth without manipulating brood size, they used larger carcasses than we did and their No Care treatment involved cutting a hole in the breeding carcass. Finally, all previous studies examining the impact of parental care on larval mass have removed care at larval hatching or very soon after. In contrast, our experiment involved removing parents well before larvae hatch.

Despite these methodological issue, the lack of divergence between lines in larval mass isn’t entirely surprising for two reasons. First, the No Care and Full Care selection regimes probably did not involve consistent differential selection on larval
mass. There was no selection on larval mass in the Full Care lines (mating was random with respect to adult size, which is positively correlated with larval mass). In the No Care lines, there was a history of selection for either large or small adults but any history of this selection was likely erased by the protocol we used to create the experimental populations (see supplemental methods). Second, we know from previous studies that the total heritability of adult body size in *N. vespilloides* (which is strongly positively correlated with larval mass at dispersal) is very low suggesting that a response to even very strong selection on body size is unlikely to result in evolutionary change over the short-term (Head et al. 2012; Schrader et al. unpublished data).

Perhaps more importantly than the individual results described above, our study suggests that experimental evolution can be applied to the study of parental care. Much of what we know about the evolution of parent-offspring interactions comes from quantitative genetic estimates of correlations between parental and offspring traits (reviewed in Kölliker et al. 2012). While these studies have been tremendously valuable, they can only be used inferentially to understand how selection has shaped parent-offspring interactions. We suggest that combining quantitative genetic approaches with experimental evolution will provide more direct insights into how parent-offspring interactions coevolve in response to different selection regimes. For example, by manipulating the presence of post-hatching parental care over many generations it might be possible to study not only how offspring adapt to a change in parental care but also whether such adaptation changes the direction or magnitude of genetic correlations between parental and offspring behaviors. Similarly, it might be possible to manipulate the extent of parent-offspring conflict in experimental populations (e.g. through manipulating the mating system).
and ask whether correlations between parental and offspring traits evolve in response
to antagonistic selection. Insects with flexible patterns of parental care and rapid
generation times, such as *N. vespilloides*, are ideal species for such experiments.

Acknowledgements

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


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.

<table>
<thead>
<tr>
<th>Factor</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>2.9</td>
<td>0.08</td>
</tr>
<tr>
<td>Treatment</td>
<td>44.5</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Population x Treatment</td>
<td>6.50</td>
<td>0.011</td>
</tr>
<tr>
<td>Block</td>
<td>7.61</td>
<td>0.0075</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Factor</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>0.016</td>
<td>1, 177</td>
<td>0.89</td>
</tr>
<tr>
<td>Treatment</td>
<td>32.90</td>
<td>1, 177</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Population x Treatment</td>
<td>2.35</td>
<td>1, 177</td>
<td>0.13</td>
</tr>
<tr>
<td>Block</td>
<td>8.80</td>
<td>1, 177</td>
<td>0.0034</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Factor</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>1.97</td>
<td>0.16</td>
</tr>
<tr>
<td>Brood Size</td>
<td>57.80</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Block</td>
<td>1.51</td>
<td>0.22</td>
</tr>
</tbody>
</table>

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.

**Block one**

<table>
<thead>
<tr>
<th>Factor</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>3.018</td>
<td>1, 66</td>
<td>0.087</td>
</tr>
<tr>
<td>Treatment</td>
<td>8.39</td>
<td>1, 66</td>
<td>0.0051</td>
</tr>
<tr>
<td>Population X Treatment</td>
<td>3.71</td>
<td>1, 66</td>
<td>0.058</td>
</tr>
<tr>
<td>Carcass mass</td>
<td>10.77</td>
<td>1, 66</td>
<td>0.0017</td>
</tr>
</tbody>
</table>

**Block two**

<table>
<thead>
<tr>
<th>Factor</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>2.19</td>
<td>1,107</td>
<td>0.14</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.45</td>
<td>1,107</td>
<td>0.51</td>
</tr>
<tr>
<td>Population X Treatment</td>
<td>0.14</td>
<td>1,107</td>
<td>0.71</td>
</tr>
</tbody>
</table>
Figure Legends

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

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

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

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

Figure 5. Mean larval mass (± 1 S. E. M.) of beetles descended from the No Care (gray squares and dashed lines) and Full Care (black squares and solid lines) populations in the absence or presence of post-hatching care. Data in the top panel are from block one and data in the bottom panel are from block two.
Figure 1.
Figure 2
Figure 3
Figure 4

Carcass consumption

Block one

Full Care

No Care

Block two

Full Care

No Care

Figure 4
Figure 5.

Block one

No Care | Full Care

Mean larval mass (g)

Block two

No Care | Full Care

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

Block 1

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

**Block 2**

We replicated the experiment described above using a second pair of experimental laboratory populations that differed with respect to the presence of post-hatching parental care in the previous seven generations. The first population, No Care 2 (NC2), was created by combining the NCL2 and NCS2 subpopulations 7 generations after establishing the experimental populations (Figure S1B). At this time, there was no difference between the NCL2 and NCS2 populations in pronotum width (mean pronotum width in generation seven: NCL1 = 4.45, mm, n = 417; NCS1 = 4.49 mm, n = 188; t_{442.165} = -1.10, P = 0.271). To combine these populations we conducted reciprocal hybrid crosses between the two lines (n = 10 in each direction) and within subpopulation crosses involving unrelated individuals (NCL2 x NCL2, n = 10 and NCS2 x NCS2, n = 10). At the same time, we created a corresponding full care population, Full Care 2 (FC2). This population was created by combining the FCC1 and FCC2 subpopulations 7 generations after establishing the experimental populations from the stock. To combine these populations we conducted reciprocal hybrid crosses between the two lines (n = 10 in each direction) and within subpopulation crosses involving unrelated individuals (FCC1 x FCC1, n = 10 and FCC2 x FCC2, n = 10). The individuals crossed to create the NC2 and FC2 populations were chosen randomly with respect to pronotum width and the crosses were conducted with full parental care to minimize differences between lines in
transgenerational effects of care. The offspring from these crosses were used as the parents in the experimental generation.
Figure S1. A detailed schematic showing the creation of the experimental lines used in block one (A) and block two (B) of the experiment. Dashed lines indicate the No Care environment and solid lines indicate the Full Care environment. Abbreviations for each line follow those in the text above. All populations were passed through a single generation with full parental care to minimize differences between lines in transgenerational effects (indicated by solid lines enclosed in gray rectangles). The resulting offspring were then bred with or without post-hatching parental care (solid and dashed arrows respectively). For each pair we measured breeding success, brood size at dispersal, mean larval mass, and carcass consumption.
Figure S2. The relationship between mean larval mass and brood size in the presence (left) or absence of post-hatching parental care. Data from block 1 are in the top row and data from block 2 are in the bottom row.