Offspring overcome poor parenting by being better parents

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Abstract

The evolutionary repercussions of parental effects are often discussed as static effects that can have negative influences on offspring fitness that may even persist across generations. However, individuals are not passive recipients and may mitigate the persistence of parental effects through their behaviour. Here, we tested how the burying beetle *Nicrophorus orbicollis*, a species with complex parental care, responded to poor parenting. We manipulated the duration of parental care received and measured the impact on traits of both F1 and F2 offspring. As expected, reducing parental care negatively affected traits that are ecologically important for burying beetles, including F1 offspring development time and body size. However, F1 parents that received reduced care as larvae spent more time feeding F2 offspring than parents that received full care as larvae. As a result, both the number and mass of F2 offspring were unaffected by the developmental experience of their parents. Our results show that flexible parental care may be able to overcome poor developmental environments and limit negative parental effects to a single generation.

1. INTRODUCTION

All parents influence the development of their offspring (Marshall & Uller, 2007, Roach & Wulff, 1987, Mousseau & Fox, 1998, Molinier et al., 2006, Royle et al., 2012), ranging from short-term impacts to effects that persist for multiple generations (Molinier et al., 2006, Cheverud & Moore, 1994, Yin et al., 2019). Adaptive parental effects are expected to evolve to help offspring overcome challenging environments by modifying offspring phenotypes or buffering (Badyaev & Uller, 2009, Mousseau & Fox, 1998, Wilson, 1975). In the former, parents prepare offspring to face a future environment (Potticary & Duckworth, 2020, Mousseau & Dingle, 1991, Dantzer et al., 2013, Burgess & Marshall, 2014), whereas in the latter, parents buffer offspring by compensating for unfavorable conditions that would otherwise be experienced during offspring development. As environments can change rapidly or unpredictably, parental effects on offspring development range from positive (Dantzer et al., 2013, Galloway & Etterson, 2007, Duckworth et al., 2015, Badyaev et al., 2006) to negative (Potticary & Duckworth, 2018, Gluckman et al., 2005, Sheriff & Love, 2013) depending on the environmental context (Bernardo, 1996, Bateson et al., 2014). Consequently, the evolutionary repercussions of parental effects are often viewed as static effects on offspring phenotype (Badyaev & Uller, 2009) that may even persist into subsequent generations. This is an assumption that should be tested (Badyaev & Uller, 2009) because individuals are not passive recipients of their environment. The persistence of a parental effect depends on the interactions between offspring and their environment later in life (Francis et al., 2002, Cheverud & Moore, 1994, Bernardo, 1996), which can also be mediated by flexibility in individual behaviour. For this reason, parental effects that are produced by behaviour, such as parental care, provide a unique opportunity to determine how variation in the behaviour of both parents and offspring influences the persistence of parental effects. Here, we specifically investigate how behaviour may impact the persistence of parental effects over time.

63 Parental care is expected to buffer offspring development from environmental threats (Wilson,
64 1975). Moreover, parental care by definition influences offspring development, and variation in
65 parental care can have cascading effects that impact multiple generations (Champagne, 2008, Rossiter,
66 1996). The goals of parents and offspring are often not aligned, as variation in parental care can be
67 driven by environmental conditions that change the benefits of allocating effort to current and future
68 reproductive opportunities (Stearns, 1989, Richardson et al., 2020). Thus, on one hand, parental care
69 is a coordinated strategy to buffer offspring from current threats, and on the other, variation in parental
70 care can induce transgenerational effects that may even be negative, depending on the ecological
71 context. This is particularly the case because parental care is not a single behaviour but a composite of
72 multiple behaviours that may buffer or modify offspring development in different ways. Despite the
73 long-standing hypothesis that parental care acts as a buffer (Wilson, 1975, Tallamy, 1984), it is unclear
74 how parental care may buffer offspring development within and between generations. One way to test
75 these ideas is to manipulate the parental care that young receive and determine how this impacts the
76 expression of parental care and F2 offspring phenotypes when these individuals are parents themselves.
77 Here, we investigated how variation in parental care influences the development and behaviour
78 of the burying beetle *Nicrophorus orbicollis* across generations. Specifically, we tested whether
79 individuals that received poor parenting can compensate for their own developmental environment
80 when they are parents or whether the developmental impact of poor parenting is perpetuated to the next
81 generation. Given that parental care is a complex behavioural trait, we further asked how the expression
82 of the behaviours composing parental care are affected by an individual's developmental experience.
83 We investigated this by manipulating the duration of parental care that mixed foster broods received
84 to produce larvae that experienced reduced (six hours) or full (approximately seven days) parental care.
85 Burying beetles require small animal carcasses to breed and provide complex care to their young, which
86 includes a suite of parenting behaviours (Robertson, 1993, Eggert & Müller, 1997, Scott, 1998). In
87 *Nicrophorus*, parents bury and defend carcasses, which in part acts to ensure that the carcass is

88 available for longer (Robertson, 1993, Trumbo & Sikes, 2021), eliminate bacterial and fungal
89 consumers to avoid putrefaction (Haberer et al., 2014, Arce et al., 2012), create cavities with
90 predigested carrion "soup" to support offspring self-feeding (Duarte et al., 2021, Capodeanu-Nägler et
91 al., 2018, Eggert & Müller, 1997), and directly provision food to larvae through regurgitation of carrion
92 (Eggert et al., 1998). Both direct provisioning and maintenance of the carcass are known to increase
93 larval size and growth rate in *Nicrophorus* (Lock, 2012, Lock et al., 2004, Walling et al., 2008).
94 Parental behaviour affects not only larval development but also has a significant impact on the traits of
95 those offspring when they become adults. Larvae do not feed after they leave the carcass until they
96 metamorphose into adults; thus, adult body size is largely determined by resource acquisition during
97 the larval period, similar to other insects (Nijhout, 2003). Body size is an important trait for
98 *Nicrophorus*, as larger beetles are more effective competitors for carcasses, which are ephemeral and
99 are expected to be a limited breeding resource (Otronen, 1988, Robertson, 1993, Royle & Hopwood,
100 2017). Thus, parental care is particularly important in this system because it creates a direct link
101 between a larva's development and competitive ability upon reaching adulthood.

102 Our study was designed to determine whether poor parenting begets poor parenting or if parents
103 can behaviourally buffer their offspring from their own developmental experience. Both hypotheses
104 predict that removing parental care will impact ecologically relevant F1 offspring traits. If poor
105 parenting in one generation leads to poor parenting in the next, then parents that receive reduced
106 parental care should show less care than individuals raised in full care environments, and this variation
107 in care should produce concomitant changes in F2 offspring phenotypes. Alternatively, if parents can
108 behaviourally buffer their offspring, then parents that received reduced care should differ in their
109 expression of care behaviours relative to full care parents, and there should be no difference in F2 larval
110 traits between treatments. As expected, we found that our experimental reduction of parental care
111 greatly reduced the body size of F1 offspring when they became adults. However, we also found that
112 when these individuals became parents, they altered the expression of key parental behaviours and,

113 consistent with buffering, raised broods of equal number and weight. These results suggest that parental
114 care produces an adaptive parental effect that ensures that the negative effects of poor parenting do not
115 persist. Thus, parental care may not only buffer offspring development within a generation but may
116 also act as a buffer that mitigates the legacy of environments past.
117
118 **2. MATERIALS AND METHODS**
119
120 2.1 Insect colony and husbandry
121
122 We used *Nicrophorus orbicollis* from an outbred colony maintained at the University of Georgia,
123 which is regularly supplemented with wild-caught beetles from Whitehall Forest in Athens, GA, USA.
124 All beetles were maintained in an incubator (Percival, model no. I4-66VL, Perry, IA) at 22 ± 0.1°C on
125 a 14:10 hours light:dark cycle that simulated summer breeding conditions. We bred all individuals used
126 here in the laboratory to ensure that parentage and age were known and to standardize rearing
127 conditions. We housed beetles individually at larval dispersal in 9-cm-diameter by 4-cm-deep circular
128 compostable deli containers (EP-RDP5, Eco-Products) filled with approximately two cm of organic
129 potting soil. The larvae had no further social interactions with other burying beetles until they were
130 randomly allocated into treatments. We defined individuals as day 1 of adulthood on the day that they
131 eclosed. Beetles were fed *ad libitum* with organic ground beef twice a week following adult eclosion,
132 i.e., the emergence of the adult beetle.
133
134 2.2 Effect of removing parental care on offspring development
135
136 We manipulated the duration of parental care to determine the effect of parental care on F1 offspring
137 traits. To do this, we set up 36 families, N = 18 in both the full and reduced care treatments. We

138 randomly mated focal virgins on days 13-19 of adulthood by placing an unrelated male and female in
139 a small plastic container and observing them to ensure that mating occurred. The pair were then moved
140 to a plastic breeding box filled with approximately two cm of potting soil moistened with distilled
141 water and containing a freshly thawed mouse 20.8 ± 0.6 g SD (max = 22.2, min = 20.0; Rodent Pro,
142 Inglefield, IN, U.S.A.). There was no difference in the size of the mice provided between treatments
143 (20.9 g in the full care treatment, 20.8 g in the reduced care treatment; $F_{1,34} = 0.153$, P = 0.698).
144 Breeding boxes were then returned to the incubator and kept on a darkened shelf to simulate
145 underground breeding conditions. We checked the breeding boxes for eggs every twenty-four hours.
146 Once we observed eggs, we moved the female and brood ball to a new breeding box and removed the
147 male. We left the eggs in the original breeding box with a small piece of ground beef. After 90 hours
148 had elapsed since egg laying, we checked the breeding box containing the eggs every hour to determine
149 when the larvae had hatched and crawled to the beef. After we observed that the focal females' larvae
150 had hatched, we provided the focal female with an unrelated, mixed brood of 10 larvae. Burying beetles
151 identify their offspring temporally and eat any larvae that arrive too early on the carcass (Oldekop et
152 al., 2007, Potticary et al., 2023); thus, we only provided females with larvae after her own larvae had
153 hatched. For the full care treatment, we allowed females to associate with larvae until at least two of
154 the larvae had dispersed from the breeding carcass (~7-8 days). For the reduced care treatment, we
155 allowed females to associate with larvae for 6 hours and then removed the females. There is significant
156 variation in the duration of care that parents provide in the wild (Eggert & Müller, 1997, Wilson &
157 Fudge, 1984), and parents can desert early in the post-hatching stage. We checked the experimental
158 treatments daily to determine the larval dispersal date and larval survival.

159 We measured several ecologically relevant offspring traits (Lock et al., 2004), including
160 duration of developmental periods, mass at dispersal, and adult size. We checked the treatments daily
161 to determine the number of surviving larvae and to measure the duration of each developmental stage.
162 The development of burying beetles occurs in discrete and easily scored periods: from hatching to

163 dispersal from the carcass, during which time the larvae are fed by the parents and feed from the carcass
164 (*hatch to dispersal*); non-feeding "wandering" larvae that have dispersed from the carcass and then
165 bury themselves at a distance from the consumed carcass where they form pupae (*dispersal to pupae*);
166 and pupal development to adult eclosion, where they emerge as winged teneral adults from the soil
167 (*pupae to eclosion*). After the larvae had dispersed from the carcass, we measured larval mass to the
168 nearest 0.1 mg and placed larvae into individual containers. We determined the sex and measured the
169 pronotum length of full and reduced care offspring on days 14 – 16 post-eclosion with calipers to the
170 nearest 0.01 mm.
171
172 2.3 Effect of variation in parental care environment on the next generation
173
174 To determine the effect of the experimental manipulation of parental care on F1 offspring when they
175 reached adulthood, we established 89 uniparental broods using the resulting offspring from the reduced
176 and full care treatments (26 full care males, 20 full care females, 24 reduced care males, and 17 reduced
177 care females). Individuals were randomly selected from the families within each treatment. On days
178 14 – 16 following eclosion, adult beetles that received full or reduced care as offspring were provided
179 with an unrelated, virgin mate from our stock colony and a 19.6 - 21.2 g thawed mouse carcass (mean
180 = 20.6 g). These were placed in a breeding container with approximately two cm of moistened soil. As
181 variation in parental care may manifest as variation in investment across the breeding cycle, we fitted
182 breeding boxes with an escape chamber to allow focal beetles to desert their breeding attempt. We
183 checked breeding boxes daily for eggs. We placed beetles that escaped into the escape chamber before
184 egg laying back into the breeding box, while we counted beetles that escaped after egg-laying and
185 remained in the escape chamber for more than 15 minutes as deserters and removed them. Once we
186 observed larvae, we removed the non-focal partner, which left the focal male or female in a uniparental

187 state to care for the offspring. If the focal beetle deserted a breeding attempt before larvae hatching,
188 we did not conduct any no further monitoring on that breeding attempt.

189 We measured the expression of parental care using behavioural observations twenty-four hours
190 after the larvae were first observed on the carcass. We moved the breeding boxes into an observation
191 room and allowed the focal beetle to acclimate for at least 30 minutes before observation. All
192 observations were conducted under red lights to ensure no light effects on individual care behaviour,
193 as *N. orbicollis* is nocturnal (Wilson et al., 1984). In each behavioural observation, we recorded both
194 direct and indirect parental care behaviours for 15 minutes (Walling et al., 2008). We defined direct
195 care as when the parent fed regurgitated carrion to the larvae. We defined indirect care as activities
196 related to carcass cleaning and maintenance, which allows for the preservation of the carcass for future
197 offspring provisioning and thus represents a type of parental care without direct interactions with
198 offspring. We also recorded the time spent showing "no care," defined as when the parent was on the
199 carcass but not engaged in direct or indirect care, or when the individual was off the carcass. We
200 recorded behaviours continuously as both a duration and frequency (e.g., the number of times an adult
201 fed larvae). This gave each individual three scores (direct care, indirect care, and no care) which
202 together summed to 15 minutes.

203 We did not manipulate the number of offspring in the F2 generation. To account for the fact
204 that rates of parental provisioning may be affected by the number of larvae (Rauter & Moore, 2004),
205 we also measured the amount of time larvae spent on the carcass as well as the number and mass of
206 larvae at dispersal for each larvae as a proxy for parental effort. Measurements of development times
207 and larval mass were the same as those used in the experimental manipulation of the duration of
208 parental care (see the previous section).

209

210 2.4 Statistical analyses

211

212 We performed all statistical analyses using JMP Pro (v. 16.0.0, http://jmp.com) and produced figures
213 in SigmaPlot (v. 14.5, http://www.sigmaplot.co.uk). Results are presented as the means ± standard
214 error (SEM) unless otherwise noted. All analysis of variance (ANOVA) tests were two-tailed.

215

216 2.4.1 Effect of removing parental care on offspring development

217

218 We used nested ANOVA to analyze the effect of care treatment (reduced or full parental care) on F1
219 larval mass, with care treatment and family as random factors. We used family as a nested term to
220 control for measures of multiple individuals in each family. We analyzed the duration of the *hatch to*
221 *dispersal* period as a single value for each family, as all larvae disperse from the carcass within hours
222 of each other. We analyzed the duration of *hatch to dispersal* with a Pearson chi-square goodness-of-
223 fit test, as these data were categorical (see Results). The other periods of development can vary between
224 individuals within a family (Lock, 2012); therefore, we analyzed each larva individually using nested
225 ANOVA with family nested under treatment. We included larval mass at dispersal as a random factor
226 when analyzing development off the carcass and total postembryonic development, including *dispersal*
227 *to pupae*, *pupae to eclosion*, and *hatch to eclosion*, as the duration of post-dispersal development can
228 be influenced by mass (Rauter & Moore, 2002). The relationship between an individual's mass at larval
229 dispersal and their body size in adulthood was analyzed using linear regression.

230

231 2.4.2 Effect of variation in parental care environment on the next generation

232

233 We tested the hypothesis that the parental care treatment (reduced or full care) received by F1 beetles
234 influences their behaviour when they become parents and the development of their offspring (F2
235 generation). We analyzed whether F1 parents raised with reduced or full care differed in their

236 abandonment of larvae using a chi-square test. We then analyzed the effect of F1 care treatment on the
237 behaviour of F1 towards F2 offspring using a two-way ANOVA with the sex of the F1 parent and care
238 treatment as factors, with the proportion of the behavioural observation that the parent spent a) on the
239 carcass (total time on the carcass, regardless of behaviour expressed), b) engaged in indirect care, or c)
240 engaged in direct care behaviour, as the dependent variable.

241 We tested the hypothesis that the parental care treatment received by F1 beetles influences the
242 development of their F2 offspring in several ways. First, we analyzed brood size and F2 larval mass
243 with a fully factorial two-way ANOVA with caregiver sex as a fixed factor and care treatment as a
244 random factor. We used the average F2 larval mass for the family reared by each F1 parent to control
245 for the fact that multiple individuals were measured in each family. We analyzed the duration of F2
246 larval development on the carcass with F1 caregiver sex, care treatment, and their interaction using a
247 GLM with a Poisson distribution, as these data are categorical because larvae within a family disperse
248 on the same day.

249 We next tested how F1 behaviour to their F2 offspring influenced the number of surviving larvae
250 and their mass at dispersal. Average larval mass and the number of offspring are strongly correlated
251 (see Results) because the rates of provisioning can be influenced by the number of begging offspring
252 and impact offspring growth (Lock, 2012, Lock et al., 2004, Smiseth et al., 2003). For this reason, we
253 used a principal component analysis to reduce them to a single variable. PC1 was retained because it
254 had an eigenvalue greater than one and explained 84% of the variance. We used Pearson correlation to
255 determine whether direct and indirect care behaviours were related to PC1.

256

257 **3. RESULTS**

258

259 3.1 Effect of reducing parental care on offspring development

260

261 Larval survival on the carcass was high for both the full and reduced care treatments; from an initial
262 ten fostered larvae placed on the carcass, three to ten larvae survived to dispersal from the mouse with
263 a mean of 8.3 ± 0.4 surviving larvae for full care families and 8.7 ± 0.4 for reduced care ($F_{1,34}$ = 0.430,
264 P = 0.516). Following dispersal, 12% (N = 35 of 304) of the total individuals died before adult eclosion,
265 including 11% of larvae that received full care (N = 16 of 148) and 12% of larvae that received reduced
266 care (N = 19 of 156).

267 The care treatment F1 larvae received - reduced or full care - affected both the duration of
268 development on the carcass and larval mass at dispersal, but the duration of developmental periods that
269 occurred once larvae had left the carcass was primarily influenced by larval mass. F1 larvae that
270 received full care completed development on the carcass faster than larvae that received reduced care
271 (*hatch to dispersal*; χ^2 = 18.857, df = 2, p < 0.001, N = 36 families). Larvae in the full care treatment
272 dispersed in 7 or 8 days, whereas reduced care larvae dispersed in 8 or 9 days. F1 larval mass at
273 dispersal was strongly and positively affected by the duration of parental care received. Controlling for
274 the significant effect of variation among families ($F_{34,269}$ = 10.155, P < 0.0001), larvae that received
275 full care were larger than those that received reduced care ($F_{1,34}$ = 45.358, P < 0.0001; **Figure 1*A***).
276 Controlling for variation amongst families, larvae that received full care weighed an average of 500.4
277 ± 4.4 mg, whilst larvae that received reduced care weighed an average of 373.4 ± 4.2 mg. F1 larval
278 mass at dispersal was positively and strongly correlated with their final adult body size (r = 0.91, N =
279 248).

280 The time that larvae spent in the *dispersal to pupation* stage showed statistically significant
281 variation attributable to family ($F_{34,261}$ = 9.301, P < 0.0001) and larval mass at dispersal ($F_{1,261}$ = 34.501,
282 P < 0.0001), but not to care treatment ($F_{1,261}$ = 3.363, P = 0.073). The same was true for the duration
283 of the *pupae to adult eclosion* stage, with statistically significant variation arising from family ($F_{34,231}$
284 = 3.173, P < 0.0001) and larval mass at dispersal ($F_{1,231}$ = 55.586, P < 0.0001), but not to care treatment

($F_{1,34}$ = 0.488, P = 0.487). Even though larvae in the full care treatment developed faster on the carcass, the duration of total postembryonic development, *hatch to eclosion*, was influenced primarily by family ($F_{34,232}$ = 9.699, P < 0.0001) and larval mass at dispersal ($F_{1,232}$ = 77.283, P < 0.0001), and not to care treatment ($F_{1,34}$ = 1.311, P = 0.254; **Figure 1*B***).

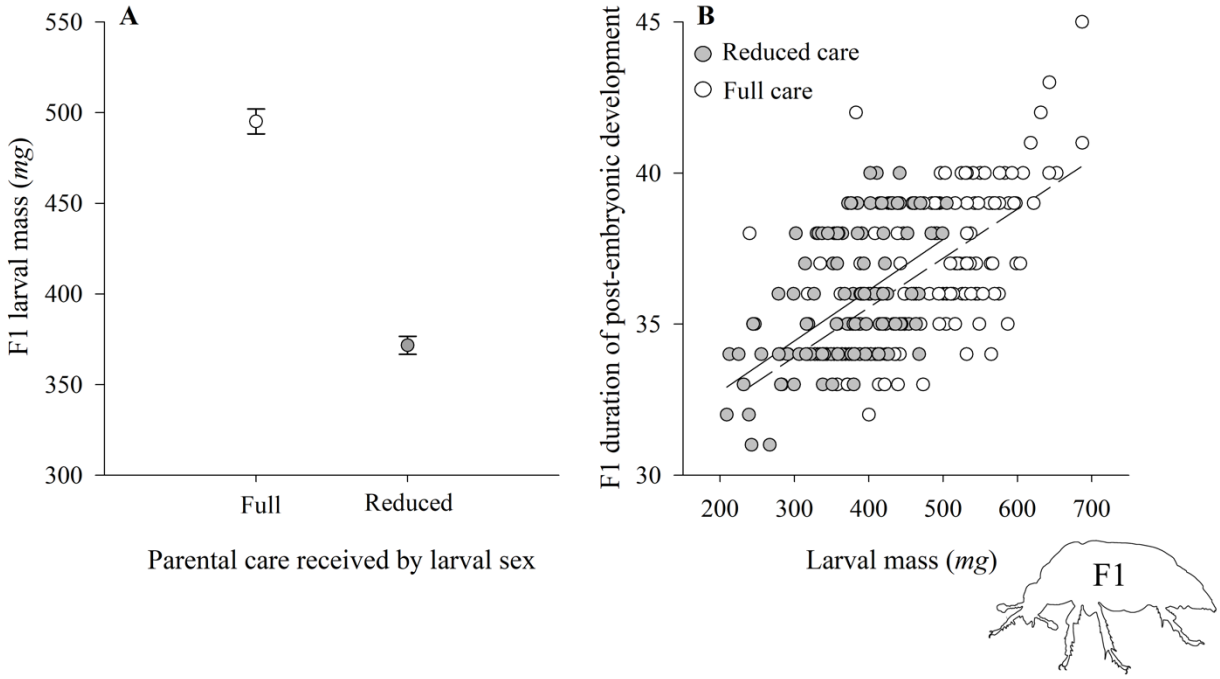

Figure 1. Care influences larval development time and size. Data from F1 beetles that experience full or reduced parental care; generation indicated in beetle graphic. ***(A)*** Larvae that received full care were larger than larvae that received reduced care. Error bars are mean ± SEM. ***(B)*** Duration of the larval period (in days) from larval hatch until ecolsion was statistically dependent on larval mass at dispersal but not on care treatment (N = 248). Grey circles and the solid line refer to larvae that received reduced care, while open circles and the dashed line refer to individuals that received full care.

3.2 Effect of variation in parental care environment on the next generation

There is no evidence that manipulation of care experiment (full or reduced care) influences whether parents abandoned the breeding attempt before showing parental care to the larvae. Focal male caretakers were more likely to abandon before eggs hatched (reduced care males: 11/24 breeding

302 attempts; full care males: 11/26 breeding attempts) than female caretakers (reduced care females: 2/17
303 breeding attempts; full care females 2/20 breeding attempts). Once the larvae hatched, most of the focal
304 parents stayed for the entire larval development period on the carcass; when caretakers did abandon
305 larvae, they were almost exclusively males (10/29 males, 1/33 females; $\chi^2 = 11.640$, df = 1, P = 0.0012).
306 The one female that abandoned early had received full care; for males, four had received full care and
307 six received reduced care.

308 There are strong differences between uniparental females and males in this species (**Figure 2**)
309 and F1 care treatment affected some parental care behaviours. Females spent more time on the carcass
310 during the observation period than males ($F_{1,55} = 17.610$, P < 0.001; **Figure 2*A***), regardless of the care
311 treatment they received as larvae ($F_{1,55} = 1.878$, P = 0.176), and there was not a statistically significant
312 interaction between care treatment and caretaker sex on the amount of time spent on the carcass ($F_{1,55}$
313 = 1.332, P = 0.253). The time that parents spent regurgitating to their larvae, or direct care, was
314 influenced by F1 sex and care treatment, where caretakers that received reduced care provided more
315 direct care themselves. Females provided more direct care than males under both treatments ($F_{1,55}$ =
316 12.699, P = 0.0008; **Figure 2*B***), and direct care was strongly influenced by care treatment ($F_{1,55}$ =
317 6.761, P = 0.012). This pattern was consistent for both males and females, but there was no interaction
318 between caretaker sex and care treatment ($F_{1,55} = 0.111$, P = 0.741). In contrast, indirect care, or time
319 spent maintaining the carcass, was not affected by caretaker sex ($F_{1,55} = 3.072$, P = 0.085; **Figure 2*C***)
320 or care treatment ($F_{1,55} = 1.181$, P = 0.282), nor was there an interaction between sex and care treatment
321 ($F_{1,55} = 0.705$, P = 0.405).

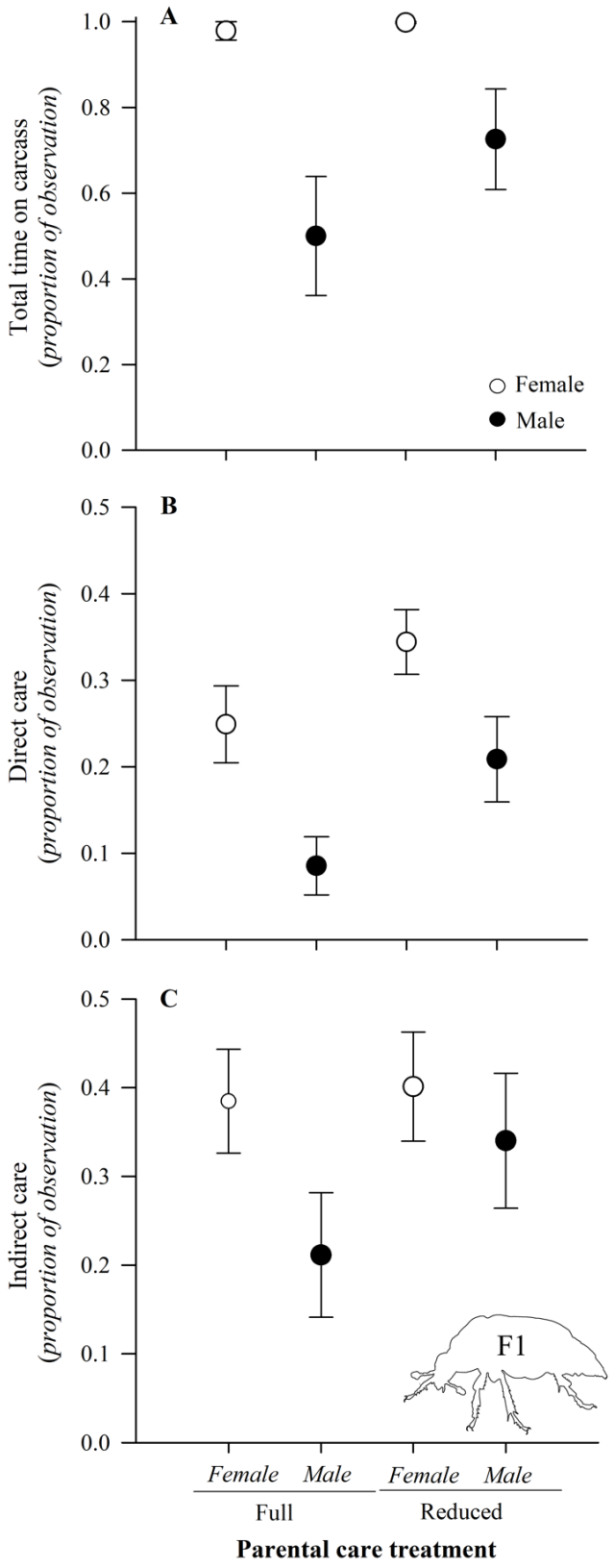

Figure 2. Parental behaviour of F1 parents is affected by the care received as larvae. Data from F1 beetles that experience full or reduced parental care; generation indicated in beetle graphic. N = 15 observations for all treatments except full care males, where N = 14 observations. ***(A)*** Females spent more time on the carcass during the observation period than males regardless of how much care they received. In addition, ***(B)*** both females and males that experienced reduced care increased the amount of direct care given to their offspring but ***(C)*** not indirect care. Error bars are mean ± SEM.

The effects of the reduced and full care treatments experienced by the F1 generation did not carry over into the F2 generation. There was no statistically significant effect on the duration of the *hatch to dispersal* period arising from F1 caregiver sex ($\chi^2 = 0.405$, df = 1, P = 0.524), F1 care treatment ($\chi^2 = 0.004$, P = 0.949), or their interaction ($\chi^2 = 0.405$, P = 0.524). There were no significant effects on the average mass of dispersing F2 larvae arising from F1 caregiver sex ($F_{1,57} = 4.313$, P = 0.286), F1 care treatment ($F_{1,57} = 2.728$, P = 0.347), or their interaction ($F_{1,57} = 0.513$, P = 0.477; **Figure 3*A***). Female caregivers reared more larvae to dispersal than males ($F_{1,57} = 19.527$, P < 0.0001), but there was no effect of F1 care treatment ($F_{1,57} = 0.567$, P = 0.455), or their interaction ($F_{1,57} = 0.147$, P = 0.703; **Figure 3*B***).

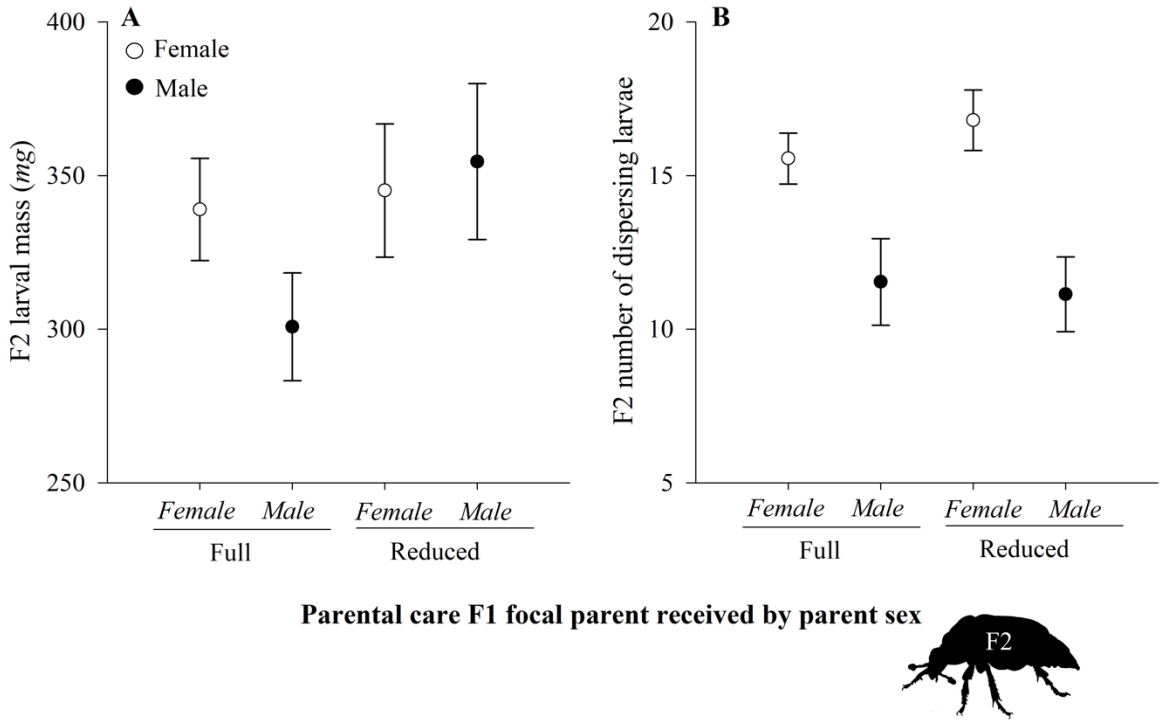

Figure 3. Parent sex influences offspring outcomes, but not the parental developmental environment. Data are from larvae in the F2 generation, whose F1 parents had experienced full or reduced parental care; generation is indicated in the beetle graphic. **(*A*)** There was no difference in the average larval mass of F2 offspring, regardless of whether the F1 parent had received full or reduced care. (***B)*** Females reared more offspring than males, but there was no effect of F1 care treatment on the number of F2 offspring reared. Female parents (N = 18 full care, N = 15 reduced care) are indicated

with open circles and male parents (N = 13 full care, N = 15 reduced care) with closed circles. Error bars are mean ± SEM.

The number of larvae that survived to dispersal was strongly and negatively associated with average larval mass at dispersal (linear regression; $r^2 = 0.47$, $P < 0.0001$, n = 60). For this reason, we created a composite variable, PC1, to reflect the tradeoff between the number of larvae and their mass. We found that parental care behaviours were strongly associated with offspring outcomes. There was a negative relationship between the amount of direct care F1 parents showed to F2 larvae and PC1 ($r = -0.55$, $P < 0.0001$; **Figure 4**), indicating that parents that showed more direct care had more larvae survive to dispersal, but parents with more larvae typically had relatively smaller larvae. There was no relationship between the indirect care that F1 parents showed to their larvae and PC1 ($r = -0.05$, $P = 0.73$), indicating that there was no relationship between the amount of time parents spent maintaining the carcass, larval mass, and the number of larvae to disperse.

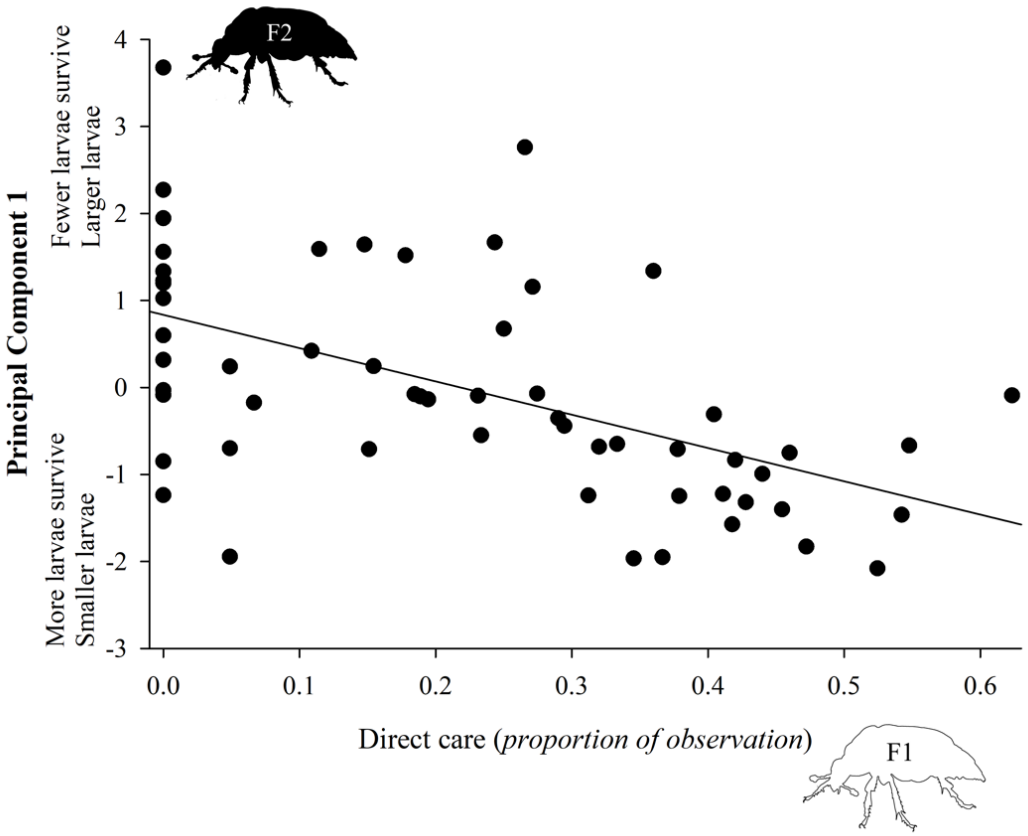

Figure 3. Parents that showed more direct care had more, but relatively smaller, larvae survive to dispersal. Data for F1 beetles expressing direct care toward F2 larvae; generation indicated in beetle graphics on each axis.

4. DISCUSSION

Adaptive parental effects are expected to evolve to help offspring overcome challenging environments by modifying offspring phenotypes or buffering (Mousseau & Fox, 1998, Wilson, 1975, Badyaev & Uller, 2009, West-Eberhard, 2003). We tested whether poor parenting begets poor parenting or if parents can compensate for their own poor developmental environment when they become parents themselves by changing their parental behaviour. Our manipulation of the parental care environment influenced the development of ecologically relevant offspring traits, including the duration of development on the carcass and body size (**Figure 1**). However, there was no difference in the size or number of offspring in the next generation when the affected individuals became parents themselves (**Figure 3**). Parents that received reduced parental care spent more time directly transferring nutrients to offspring, as opposed to parents that received full care, who invested relatively more in indirect mechanisms to enable nutrient acquisition by the larvae (i.e., maintenance of the food resource; **Figure 2**). Moreover, parents that spent more time in direct care had more larvae survive to dispersal (**Figure 4**), which is concordant with previous results demonstrating that direct provisioning increases offspring size or growth rate in *Nicrophorus* species (Lock, 2012, Lock et al., 2004). Together, these data suggest that parents may behaviourally compensate for their own poor developmental environment, an adaptive parental effect that may ensure that poor developmental environments do not persist.

Beetles that received full parental care were larger and developed faster on the carcass, although larger beetles overall took longer to develop into adult beetles. Why might the duration of development

402 on the carcass differ from other developmental periods? On a local level, developmental rates can be
403 directly influenced by variation in parental care. Parents regurgitate to larvae, and direct provisioning
404 has been shown to increase offspring size or growth rate across taxa (Naef-Daenzer & Keller, 1999,
405 Lock, 2012, Lock et al., 2004), and we similarly found a pattern between the time invested in direct
406 care and the number of surviving larvae (**Figure 4**). An ultimate explanation for rapid larval
407 development on the carcass may be that there is selection arising from competition or predation for
408 offspring to leave the carcass quickly. Studies of *N. orbicollis* in the wild find that nests are frequently
409 discovered by scavengers or taken over by conspecifics, which generally eat larvae and eggs during
410 successful takeovers (Robertson, 1993). High predation risk has been associated with rapid
411 development rates in nests across bird species (Martin et al., 2011). Thus, we suggest that local
412 provisioning rates serve to enhance offspring growth rates while on the carcass, reducing the risk at
413 this vulnerable stage while still allowing larvae to attain large adult size.

414 Our experimental reduction of parental care resulted in F1 larvae that took longer to develop
415 on the carcass, were smaller as adults, and invested more time in direct care of offspring than beetles
416 that received full parental care. However, despite an observed increase in direct provisioning by
417 reduced care beetles, there was no difference in the mass or number of F2 offspring between reduced
418 and full care parents. Why did the increase in direct provisioning by reduced care parents not result in
419 larger offspring than full care parents, which provisioned less? One possibility is that behaviour is not
420 the causative factor influencing offspring development, although this explanation is not parsimonious
421 given that a similar experiment in *N. vespilloides* found that reducing the parental care an individual
422 received resulted in poor parenting in later life (Kilner et al., 2015), removing parental care altered
423 both development on the carcass and body size (this study), and body size is associated with the extent
424 of parental care across *Nicrophorus* (Jarrett et al., 2017). Alternatively, the increase in direct
425 provisioning may allow parents to compensate for their developmental environment, particularly if
426 receiving reduced parental care impacts other traits, such as egg size. Steiger (2013) found that smaller

427 *N. vespilloides* females lay smaller eggs than larger females. A tradeoff between allocation to eggs and
428 to post-hatching care may explain why reduced care parents worked harder than full care parents and
429 yet had similar numbers and sizes of offspring survive to dispersal.

430 Parental effects range from short-term effects that dissipate when offspring pass a certain
431 developmental stage (Gluckman et al., 2005) to effects that persist for multiple generations, even for
432 the same traits (e.g., stress reactivity; Meaney, 2001, Mustoe et al., 2014, Sheriff et al., 2017). Previous
433 studies on other *Nicrophorus* species and taxa have documented such transgenerational effects of poor
434 parental care environments (e.g., Lock, 2012, Kilner et al., 2015, Champagne, 2008, Meaney, 2001).
435 An unanswered question is why the effects of a poor developmental environment are propagated across
436 generations under some circumstances but not in others. The persistence of parental effects likely
437 depends on both abiotic and biotic factors, such as the durability of the physiological mechanisms that
438 transmit them (Weaver et al., 2004, Horsthemke, 2018) and concordance between the parent and
439 offspring environments (Bateson et al., 2014, Marshall & Uller, 2007, Gluckman et al., 2005).
440 Moreover, the persistence of parental effects likely relies on whether the effects arise from an evolved
441 interaction between parents and offspring development or are merely a disruption of normal
442 development (Gluckman et al., 2005). Adaptive parental effects are predicted to induce variation in
443 offspring traits that are functionally related to the environment that triggers them (e.g., Sharda et al.,
444 2021, Duckworth et al., 2015, Tollrian, 1995, Sheriff et al., 2017). In our study, *N. orbicollis* that
445 received poor parenting altered their parental feeding behaviour, and there were no differences between
446 the F2 offspring of parents raised with reduced or full care. These data imply that parental care may
447 act as a developmental buffer between generations and indicate evolution for this purpose in *N.*
448 *orbicollis,* particularly given how important body size is for competitive interactions in burying beetles
449 (Robertson, 1993, Creighton, 2005, Schrempf et al., 2021). While many processes may alter the
450 persistence of parental effects, changes in the expression of parental care may enable the reversal of
451 poor developmental environments.

References

Arce, A. N., Johnston, P. R., Smiseth, P. T. & Rozen, D. E. (2012). Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *Journal of Evolutionary Biology* **25**: 930-937. doi:10.1111/j.1420-9101.2012.02486.x.

Badyaev, A. V., Hamstra, T. L., Oh, K. P. & Acevedo Seaman, D. A. (2006). Sex-biased maternal effects reduce ectoparasite-induced mortality in a passerine bird. *Proceedings of the National Academy of Sciences* **103**: 14406-11. doi:10.1073/pnas.0602452103.

Badyaev, A. V. & Uller, T. (2009). Parental effects in ecology and evolution: mechanisms, processes and implications. *Philosophical Transactions of the Royal Society B* **364**: 1169-1177. doi:10.1098/rstb.2008.0302.

Bateson, P., Gluckman, P. & Hanson, M. (2014). The biology of developmental plasticity and the Predictive Adaptive Response hypothesis. *Journal of Physiology* **592**: 2357-2368. doi:10.1113/jphysiol.2014.271460.

Bernardo, J. (1996). Maternal effects in animal ecology. *American Zoologist* **36**: 83-105.

Burgess, S. C. & Marshall, D. J. (2014). Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. *Oikos* **123**: 769-776. doi:10.1111/oik.01235.

Capodeanu-Nägler, A., Prang, M. A., Trumbo, S. T., Vogel, H., Eggert, A.-K., Sakaluk, S. K. & Steiger, S. (2018). Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles. *Frontiers in Zoology* **15**: 33. doi:10.1186/s12983-018-0278-5.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology* **29**: 386-397. doi:10.1016/j.yfrne.2008.03.003.

Cheverud, J. M. & Moore, A. (1994) Quantitative genetics and the role of the environment provided by relatives in behavioral evolution. In: *Quantitative genetic studies of behavioral evolution*, (Boake, C., ed.). pp. 68-100. University of Chicago Press, Chicago.

Creighton, J. C. (2005). Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behavioral Ecology* **16**: 1031-1036. doi:10.1093/beheco/ari084.

Dantzer, B., Newman, A. E. M., Boonstra, R., Palme, R., Boutin, S., Humphries, M. M. & McAdam, A. G. (2013). Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal. *Science* **340**: 1215-1217. doi:10.1126/science.1235765.

Duarte, A., Rebar, D., Hallett, A. C., Jarrett, B. J. M. & Kilner, R. M. (2021). Evolutionary change in the construction of the nursery environment when parents are prevented from caring for their young directly. *Proceedings of the National Academy of Sciences* **118**: e2102450118. doi:doi:10.1073/pnas.2102450118.

Duckworth, R. A., Belloni, V. & Anderson, S. R. (2015). Cycles of species replacement emerge from locally induced maternal effects on offspring behavior in a passerine bird. *Science* **347**: 875-877. doi:10.1126/science.1260154.

Eggert, A.-K. & Müller, J. K. (1997) Biparental care and social evolution in burying beetles: lessons from the larder. In: *The Evolution of Social Behaviour in Insects and Arachnids*, (Choe, J. C. & Crespi, B. J., eds.). pp. 216. Cambridge University Press, Cambridge.

Eggert, A.-K., Reinking, M. & Müller, J. K. (1998). Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour* **55**: 97-107. doi:10.1006/anbe.1997.0588.

496 Francis, D. D., Diorio, J., Plotsky, P. M. & Meaney, M. J. (2002). Environmental enrichment
497 reverses the effects of maternal separation on stress reactivity. *Journal of Neuroscience* **22**:
498 7840-7843. doi:10.1523/jneurosci.22-18-07840.2002.
499 Galloway, L. F. & Etterson, J. R. (2007). Transgenerational plasticity is adaptive in the wild. *Science*
500 **318**: 1134-1136.
501 Gluckman, P. D., Hanson, M. A., Spencer, H. G. & Bateson, P. (2005). Environmental influences
502 during development and their later consequences for health and disease: implications for the
503 interpretation of empirical studies. *Proceedings of the Royal Society B* **272**: 671-677.
504 doi:10.1098/rspb.2004.3001.
505 Haberer, W., Steiger, S. & Müller, J. K. (2014). Dynamic changes in volatile emissions of breeding
506 burying beetles. *Physiological Entomology* **39**: 153-164. doi:10.1111/phen.12059.
507 Horsthemke, B. (2018). A critical view on transgenerational epigenetic inheritance in humans.
508 *Nature Communications* **9**: 2973. doi:10.1038/s41467-018-05445-5.
509 Jarrett, B. J. M., Schrader, M., Rebar, D., Houslay, T. M. & Kilner, R. M. (2017). Cooperative
510 interactions within the family enhance the capacity for evolutionary change in body size.
511 *Nature Ecology and Evolution* **1**: 0178. doi:10.1038/s41559-017-0178.
512 Kilner, R. M., Boncoraglio, G., Henshaw, J. M., Jarrett, B. J. M., De Gasperin, O., Attisano, A. &
513 Kokko, H. (2015). Parental effects alter the adaptive value of an adult behavioural trait. *eLife*
514 **4**. doi:e0734010.7554/eLife.07340.
515 Lock, J. E. (2012). Transgenerational effects of parent and grandparent gender on offspring
516 development in a biparental beetle species. *Biology Letters* **8**: 408 - 411.
517 Lock, J. E., Smiseth, P. T. & Moore, A. J. (2004). Selection, inheritance, and the evolution of parent-
518 offspring interactions. *American Naturalist* **164**: 13-24. doi:10.1086/421444.
519 Marshall, D. J. & Uller, T. (2007). When is a maternal effect adaptive? *Oikos* **116**: 1957-1963.
520 Martin, T. E., Lloyd, P., Bosque, C., Barton, D. C., Biancucci, A. L., Cheng, Y.-R. & Ton, R. (2011).
521 Growth rate variation among passerine species in tropical and temperate sites: an antagonistic
522 interaction between parental food provisioning and nest predation risk *Evolution* **65**: 1607-
523 1622. doi:10.1111/j.1558-5646.2011.01227.x.
524 Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences
525 in stress reactivity across generations. *Annual Review of Neuroscience* **24**: 1161-1192.
526 doi:10.1146/annurev.neuro.24.1.1161.
527 Molinier, J., Ries, G., Zipfel, C. & Hohn, B. (2006). Transgeneration memory of stress in plants.
528 *Nature* **442**: 1046-9. doi:10.1038/nature05022.
529 Mousseau, T. A. & Dingle, H. (1991). Maternal effects in insect life histories. *Annual Review of*
530 *Entomology* **36**: 511-534. doi:10.1146/annurev.en.36.010191.002455.
531 Mousseau, T. A. & Fox, C. W. 1998. *Maternal effects as adaptations*. Oxford University Press,
532 Oxford.
533 Mustoe, A. C., Taylor, J. H., Birnie, A. K., Huffman, M. C. & French, J. A. (2014). Gestational
534 cortisol and social play shape development of marmosets' HPA functioning and behavioral
535 responses to stressors. *Developmental Psychobiology* **56**: 1229-1243. doi:10.1002/dev.21203.
536 Naef-Daenzer, B. & Keller, L. F. (1999). The foraging performance of great and blue tits (*Parus*
537 *major* and *P. caeruleus*) in relation to caterpillar development, and its consequences for
538 nestling growth and fledging weight. *Journal of Animal Ecology* **68**: 708-718.
539 doi:10.1046/j.1365-2656.1999.00318.x.
540 Nijhout, H. F. (2003). The control of body size in insects. *Developmental Biology* **261**: 1-9.
541 doi:10.1016/S0012-1606(03)00276-8.
542 Oldekop, J. A., Smiseth, P. T., Piggins, H. D. & Moore, A. J. (2007). Adaptive switch from
543 infanticide to parental care: how do beetles time their behaviour? *Journal of Evolutionary*
544 *Biology* **20**: 1998-2004. doi:10.1111/j.1420-9101.2007.01364.x.

Otronen, M. (1988). The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). *Annales Zoologici Fennici* **25**: 191-201.

Potticary, A. L. & Duckworth, R. A. (2018). Environmental mismatch results in emergence of cooperative behavior in a passerine bird. *Evolutionary Ecology* **32**: 215-229. doi:10.1007/s10682-018-9933-8.

Potticary, A. L. & Duckworth, R. A. (2020). Multiple environmental stressors induce an adaptive maternal effect. *American Naturalist* **196**: 487-500. doi:10.1086/710210.

Potticary, A. L., McKinney, E. C., Moore, P. J. & Moore, A. J. (2023). *takeout* gene expression is associated with temporal kin recognition. *Royal Society Open Science* **10**: 230860. doi:10.1098/rsos.230860.

Rauter, C. M. & Moore, A. J. (2002). Quantitative genetics of growth and development time in the burying beetle *Nicrophorus pustulatus* in the presence and absence of post-hatching parental care. *Evolution* **56**: 96-110.

Rauter, C. M. & Moore, A. J. (2004). Time constraints and trade-offs among parental care behaviours: effects of brood size, sex and loss of mate. *Animal Behaviour* **68**: 695-702. doi:10.1016/j.anbehav.2003.09.018.

Richardson, J., Stephens, J. & Smiseth, P. T. (2020). Increased allocation to reproduction reduces future competitive ability in a burying beetle. *Journal of Animal Ecology* **89**: 1918-1926. doi:10.1111/1365-2656.13242.

Roach, D. A. & Wulff, R. D. (1987). Maternal effects in plants. *Annual Reviews of Ecology and Systematics* **18**: 209-235. doi:10.1146/annurev.es.18.110187.001233.

Robertson, I. C. (1993). Nest intrusions, infanticide, and parental care in the burying beetle, *Nicrophorus orbicollis* (Coleoptera: Silphidae). *Journal of Zoology* **231**: 583-593. doi:10.1111/j.1469-7998.1993.tb01940.x.

Rossiter, M. (1996). Incidence and consequences of inherited environmental effects. *Annual Review of Ecology and Systematics* **27**: 451-476.

Royle, N., Smiseth, P. & Kölliker, M. 2012. *The Evolution of Parental Care*. Oxford University Press, Oxford.

Royle, N. J. & Hopwood, P. E. (2017) Covetable corpses and plastic beetles—the socioecological behavior of burying beetles. In: *Advances in the Study of Behavior*, Vol. 49 (Naguib, M., Podos, J., Simmons, L. W., Barrett, L., Healy, S. D. & Zuk, M., eds.). pp. 101-146. Academic Press.

Schrempf, S. D., Burke, K. W., Wettlaufer, J. D. & Martin, P. R. (2021). Behavioral dominance interactions between *Nicrophorus orbicollis* and *N. tomentosus* burying beetles (Coleoptera: Silphidae). *PeerJ* **9**: e10797. doi:10.7717/peerj.10797.

Scott, M. (1998). The ecology and behavior of burying beetles. *Annual Review of Entomology* **43**: 595-618. doi:10.1146/annurev.ento.43.1.595.

Sharda, S., Zuest, T., Erb, M. & Taborsky, B. (2021). Predator-induced maternal effects determine adaptive antipredator behaviors via egg composition. *Proceedings of the National Academy of Sciences* **118**. doi:10.1073/pnas.2017063118.

Sheriff, M. J., Bell, A., Boonstra, R., Dantzer, B., Lavergne, S. G., McGhee, K. E., MacLeod, K. J., Winandy, L., Zimmer, C. & Love, O. P. (2017). Integrating ecological and evolutionary context in the study of maternal stress. *Integrative and Comparative Biology* **57**: 437-449. doi:10.1093/icb/icx105.

Sheriff, M. J. & Love, O. P. (2013). Determining the adaptive potential of maternal stress. *Ecology Letters* **16**: 271-280. doi:10.1111/ele.12042.

Smiseth, P. T., Darwell, C. T. & Moore, A. J. (2003). Partial begging: an empirical model for the early evolution of offspring signalling. *Proceeding of the Royal Society B* **270**: 1773-7. doi:10.1098/rspb.2003.2444.

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional Ecology* **3**: 259-268. doi:10.2307/2389364.

Steiger, S. (2013). Bigger mothers are better mothers: disentangling size-related prenatal and postnatal maternal effects. *Proceeding of the Royal Society B* **280**: 20131225. doi:10.1098/rspb.2013.1225.

Tallamy, D. W. (1984). Insect parental care. *BioScience* **34**: 20-24. doi:10.2307/1309421.

Tollrian, R. (1995). Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. *Ecology* **76**: 1691-1705.

Trumbo, S. T. & Sikes, D. S. (2021). Resource concealment and the evolution of parental care in burying beetles. *Journal of Zoology* **315**: 175-182. doi:10.1111/jzo.12916.

Walling, C. A., Stamper, C. E., Smiseth, P. T. & Moore, A. J. (2008). The quantitative genetics of sex differences in parenting. *Proceedings of the National Academy of Sciences* **105**: 18430-18435.

Weaver, I. C. G., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, Jr., Dymov, S., Szyf, M. & Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience* **7**: 847-854. doi:10.1038/nn1276.

West-Eberhard, M. 2003. *Developmental plasticity and evolution*. Oxford University Press, New York.

Wilson, D. S. & Fudge, J. (1984). Burying beetles: intraspecific interactions and reproductive success in the field. *Ecological Entomology* **9**: 195-203. doi:10.1111/j.1365-2311.1984.tb00715.x.

Wilson, D. S., Knollenberg, W. G. & Fudge, J. (1984). Species packing and temperature dependent competition among burying beetles (Silphidae, *Nicrophorus*). *Ecological Entomology* **9**: 205-216. doi:10.1111/j.1365-2311.1984.tb00716.x.

Wilson, E. O. 1975. *Sociobiology: the new synthesis*. Harvard University Press, Cambridge.

Yin, J., Zhou, M., Lin, Z., Li, Q. Q. & Zhang, Y.-Y. (2019). Transgenerational effects benefit offspring across diverse environments: a meta-analysis in plants and animals. *Ecology Letters* **22**: 1976-1986. doi:10.1111/ele.13373.