



# THE UNIVERSITY *of* EDINBURGH

## Edinburgh Research Explorer

### **Females manipulate behavior of caring males via prenatal maternal effects**

**Citation for published version:**

Paquet, M & Smiseth, PT 2017, 'Females manipulate behavior of caring males via prenatal maternal effects' Proceedings of the National Academy of Sciences, vol. 114, no. 26, pp. 6800-6805. DOI: 10.1073/pnas.1619759114

**Digital Object Identifier (DOI):**

[10.1073/pnas.1619759114](https://doi.org/10.1073/pnas.1619759114)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Peer reviewed version

**Published In:**

Proceedings of the National Academy of Sciences

**General rights**

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



1 **Females manipulate behavior of caring males via prenatal**  
2 **maternal effects**

3

4 **Authors: Matthieu Paquet\* and Per T Smiseth**

5

6 **Affiliations:**

7 Institute of Evolutionary Biology, School of Biological Sciences, The University of  
8 Edinburgh, Charlotte Auerbach Road, Edinburgh, EH9 3FL, UK

9

10 \* correspondence to M. Paquet: [matthieu.paquet@outlook.com](mailto:matthieu.paquet@outlook.com)

11 **Abstract:**

12 In species with biparental care, there is sexual conflict as each parent is under  
13 selection to minimize its personal effort by shifting as much as possible of the  
14 workload over to the other parent. Most theoretical and empirical work on the  
15 resolution of this conflict has focused on strategies used by both parents, such as  
16 negotiation. However, because females produce the eggs, this might afford females  
17 with an ability to manipulate male behavior via maternal effects that alter offspring  
18 phenotypes. To test this hypothesis, we manipulated the prenatal conditions (i.e.,  
19 presence or absence of the male), performed a cross-fostering experiment, and  
20 monitored subsequent effects of prenatal conditions on offspring and parental  
21 performances in the burying beetle *Nicrophorus vespilloides*. We found that  
22 offspring were smaller at hatching when females laid eggs in presence of a male,  
23 suggesting that females invest less in eggs when expecting male assistance.  
24 Furthermore, broods laid in the presence of a male gained more weight during  
25 parental care, and they did so at the expense of male weight gain. Contrary to our  
26 expectations, males cared less for broods laid in the presence of a male. Our  
27 results provide experimental evidence that females can alter male behavior during  
28 breeding by adjusting maternal effects according to prenatal conditions. However,  
29 rather than increasing the male's parental effort, females appeared to suppress the  
30 male's food consumption, thereby leaving more food for their brood.

31 **Significance**

32 In biparental species, sexual conflict arises as each parent attempts to minimize its  
33 personal effort. Most work has focused on how this conflict is resolved through  
34 symmetrical decisions between parents. We investigated whether females can  
35 influence male decisions by altering the offspring's phenotype via the eggs. We  
36 manipulated the prenatal presence of the male, performed a cross-fostering  
37 experiment, and monitored the subsequent effects on offspring and parent  
38 performances. Offspring laid in presence of a male were smaller at hatching.  
39 Additionally, males lost more weight when with larvae laid in presence of a male.  
40 Our results show that females can manipulate male behavior, suggesting that  
41 prenatal maternal effects can play an important role in the resolution of sexual  
42 conflict between parents. *body*

43 **Introduction**

44 In species where parents cooperate to care for their joint offspring (as long as there  
45 is scope for divorce and/or re-mating following the partner's death), there will be  
46 sexual conflict over parental care with each parent being under selection to  
47 minimize its own effort and shift as much as possible of the workload to its partner  
48 (1). Previous empirical and theoretical work has focused mainly on three behavioral  
49 mechanisms that may mediate the resolution of this conflict (1, 2). First, incomplete  
50 compensation occurs when each parent increases its level of care in response to a  
51 reduction in its partner's contribution, but such that it does not fully correspond to its  
52 partner's reduction (3). Second, matching occurs when each parent adjusts its level  
53 of care to its partner's contribution by matching any increase or reduction in its  
54 partner's contribution in the same direction as its partner (4). Third, sealed bids  
55 models assume that each parent makes an initial fixed decision about how much  
56 care to provide irrespective of its partner's decision (5). There is some support for  
57 all three mechanisms from experimental studies on birds and other taxa (e.g.  
58 negotiation: 6, matching: 7, sealed bid: 8). However, a meta-analysis of mate  
59 removal and handicapping experiments on birds found overall support for  
60 negotiation (2).

61 Our current understanding of the resolution of sexual conflict suggest that  
62 males and females employ the same behavioral strategies for resolving conflict  
63 (e.g., negotiation) (9). However, given that females produce the eggs, they might  
64 use their control over egg production as a mechanism for biasing conflict resolution  
65 in their favor. In many species, females deposit hormones and/or nutrients into the  
66 eggs that alter the offspring's behavior or development (9-11), thereby providing a  
67 potential tool for manipulating the behavior of caring males. For example, by

68 producing smaller eggs, females could redirect the costs of parental care from the  
69 prenatal period where they pay the full costs of egg production towards the  
70 postnatal period where the costs of rearing young are shared with the male (9).  
71 Alternatively, females may deposit yolk androgens that modulate offspring begging  
72 behavior in a way that alters the male's perception of offspring need, thereby  
73 increasing male contributions towards care (9-11). Thus, if prenatal maternal effects  
74 influence the resolution of sexual conflict over care, this would introduce an  
75 asymmetry of power between the two sexes with the female gaining the upper  
76 hand.

77         Currently, we lack conclusive evidence as to whether females can use  
78 prenatal maternal effects to manipulate the behavior of caring males (12-16). A  
79 main reason for this is that it is difficult to demonstrate female manipulation due to  
80 several challenges. First, females could manipulate males via several mechanisms,  
81 including yolk androgens, other egg components, egg size and egg coloration (4).  
82 Thus, existing experimental designs that focus on specific mechanisms risk  
83 targeting the wrong mechanism. Second, to demonstrate that prenatal maternal  
84 effects alter male behavior, it is essential to separate the effects of prenatal  
85 conditions from those of postnatal conditions. Third, there may be a limited time  
86 window for maternal manipulation because maternal effects on offspring behavior  
87 often wane as offspring develop (17-19). Fourth, in addition to sexual conflict over  
88 parental care, there may be sexual conflict over food consumption from shared  
89 resources (20, 21). Finally, to demonstrate female manipulation, it is crucial to  
90 document fitness benefits to females and/or offspring and fitness costs to males (4).

91         Here we report an experiment on the burying beetle *Nicrophorus vespilloides*,  
92 an insect exhibiting facultative biparental care (22). This species is ideal for studying

93 female manipulation of male behavior because it allows us to address all five  
94 challenges listed above. First, we use a new experimental approach where we  
95 target prenatal environmental conditions expected to influence female decisions on  
96 maternal effects and then monitor downstream consequences for offspring size at  
97 hatching, male and female care, and male, female and offspring fitness (9). Here,  
98 we target a prenatal environmental condition that is essential for female  
99 manipulation of males: the presence or absence of the male during egg laying.  
100 Second, we conduct a cross-fostering experiment to separate pre- and postnatal  
101 effects by giving each pair of beetles an experimental brood of newly hatched larvae  
102 derived from one of our two treatment groups. In one treatment, larvae derived from  
103 eggs laid when the male was present during egg laying, while in the other  
104 treatment, larvae derived from eggs laid when the male was absent during egg  
105 laying. Third, we recorded parental behaviors over 3 consecutive days, covering the  
106 full duration of parental care from hatching until nutritional independence (23).  
107 Fourth, we investigate sexual conflict over both parental care and food consumption  
108 from a resource shared by both parents and their offspring (i.e., a small vertebrate  
109 carcass). To this end, we recorded the body mass of males and females before and  
110 after breeding. Finally, we monitored subsequent effects on post-breeding survival  
111 of males and females as well as offspring growth and survival across different life  
112 stages.

113

## 114 **Results**

115 We find evidence for prenatal maternal effects due to the presence or absence of  
116 the male during laying on offspring size at hatching. Females that laid eggs in the  
117 presence of a male partner produced larvae that were 3.4% lighter at hatching than

118 females that did so in the absence of a male (Fig. 1) (Estimate±SE = 1.85±0.826,  
119  $F_{1,59} = 5.02$ ,  $P = 0.0289$ ; table S1). There was no difference in the number of eggs  
120 laid by females in the presence or absence of a male partner ( $F_{1,142} = 0.431$ ,  $P =$   
121 0.512). Thus, our result provides evidence for an anticipatory maternal effect,  
122 whereby females reduce their prenatal investment in offspring when anticipating  
123 help from a male partner (24, 25).

124 We find evidence that females use maternal effects to manipulate the  
125 behavior of caring males, but maternal effects influenced male food consumption  
126 rather than male care. There was no evidence that males increased the amount of  
127 care they provided in response to prenatal maternal effects. Males did not adjust  
128 their direct care 1h after hatching ( $Z = 0.0716$ ,  $P = 0.943$ ; table S3), but in contrast  
129 to what we predicted, males provided *less* direct care 25h after hatching when  
130 caring for a foster brood derived from eggs laid in the presence of a male (Fig. 2A)  
131 (Estimate±SE = 1.02±0.477,  $Z = 2.15$ ,  $P = 0.0319$ ; table S3). We found that males  
132 gained less or lost more weight when caring for a foster brood derived from eggs  
133 laid in the presence of a male (Fig. 2B) (Estimate±SE = 0.0127±6.02e<sup>-04</sup>,  $F_{1,57} =$   
134 4.44,  $P = 0.0394$ , table S1). Males that cared more 25h after hatching also gained  
135 more weight suggesting that spending more time caring for larvae provided them  
136 with better access to the food resource (Fig. 2C) (lm, Estimate±SE = 1.58e<sup>-3</sup>±6.72e<sup>-</sup>  
137 4,  $F_{1,58} = 5.53$ ,  $P = 0.0222$ ). Finally, male weight gain decreased as the initial brood  
138 weight increased (Fig. 2D) (Estimate±SE = -2.67±0.925,  $F_{1,57} = 8.32$ ,  $P = 0.00552$ ;  
139 table S1), indicating that males also adjust the amount of carrion they eat based on  
140 the larvae's size. Our findings suggest that maternal effects provide females with a  
141 means to manipulate the behavior of caring males by suppressing male food  
142 consumption from the shared resource.



143 We find no evidence that maternal effects influenced the behavior of caring  
144 females. There were no effects of prenatal maternal effect on female direct care 25h  
145 (Fig. 2A) or 49h after hatching (respectively  $Z = 0.626$ ,  $P = 0.531$ ,  $Z = -0.365$ ,  $P =$   
146  $0.715$ ; table S3). As predicted under the hypothesis of female manipulation, females  
147 tended to care less 1h after hatching for larvae laid in presence of a male but this  
148 effect was marginally non-significant ( $Z = 1.93$ ,  $P = 0.0539$ ; table S3). In contrast to  
149 what we found for males, prenatal maternal effects did not affect female weight gain  
150 (Fig. 2B) ( $F_{1,58} = 0.437$ ,  $P = 0.511$  table S1). Thus, these results confirm that the  
151 prenatal maternal effect affected male behavior, but had no impact on the female's  
152 own behavior.

153 We find evidence of immediate fitness consequences of prenatal maternal  
154 effects mediated through the change in male behavior. Foster broods derived from  
155 eggs laid in the presence of a male were heavier at dispersal (i.e., the end of the  
156 parental care period) than foster broods derived from eggs laid in the absence of a  
157 male (Fig.3) (Estimate $\pm$ SE =  $-0.333\pm 0.144$ ,  $F_{1,57} = 5.35$ ,  $P = 0.0243$ ; table S1).  
158 There were no significant effects of prenatal maternal effects on either the number  
159 of dispersing larvae or average larval weight (respectively  $F_{1,58} = 2.04$ ,  $P = 0.158$ ;  
160  $F_{1,57} = 2.69$ ,  $P = 0.106$ , table S1), suggesting that the greater brood weight when  
161 eggs were laid in the absence of a male was due to a relatively small increase in  
162 both number of offspring and average larval weight. We also found that broods that  
163 were heavier at dispersal were heavier at hatching (Fig. 3) (Estimate $\pm$ SE =  
164  $50.3\pm 22.1$ ,  $F_{1,57} = 5.16$ ,  $P = 0.0269$ , table S1). Keeping in mind that foster broods  
165 derived from eggs laid in the presence of a male were lighter at hatching, this  
166 suggests that the maternal effects on postnatal brood weight were strong enough to  
167 override the initial differences in weight at hatching, which were in the opposite

168 direction from those at dispersal. We then added male weight gain to the model on  
169 brood mass to examine whether the maternal effects on male weight change  
170 explained why broods derived from eggs laid in the presence of a male were  
171 heavier at dispersal. We found that male weight change had a highly significant  
172 effect on brood weight (Estimate±SE = -9.93±2.81,  $F_{1,58} = 12.5$ ,  $P = 0.000810$ ; table  
173 S4), and when we included male weight change, the effect of treatment was no  
174 longer significant ( $F_{1,57} = 1.62$ ,  $P = 0.208$ ; table S4). There was also a significant  
175 effect of female weight change on brood weight (Estimate±SE = -7.58±2.75,  $F_{1,56} =$   
176 7.62,  $P = 0.00779$ ), but in contrast to what we found for males, the effect of  
177 treatment was still significant when female weight gain was added (Estimate±SE = -  
178 0.306±0.138,  $F_{1,56} = 5.01$ ,  $P = 0.0291$ ; table S4). Thus, our results suggest that the  
179 beneficial effects of prenatal maternal effects on offspring growth were mediated  
180 through the reduction in male food consumption from the shared resource.

181 Finally, we examined long-term fitness consequences of prenatal maternal  
182 effects by looking at offspring survival until eclosion as an adult, adult size as well  
183 as their lifespan post eclosion. There was a nonsignificant trend for offspring laid in  
184 the presence of a male to survive better from dispersal to eclosion (lm, eclosion  
185 rate:  $F_{1,57} = 3.80$ ,  $P = 0.0561$ ), and offspring laid in absence of a male lived longer  
186 as adults (Hazard coefficient±SE = -0.632±0.250,  $\chi^2 = 6.39$ ,  $P = 0.0114$ , table S2,  
187 Fig.S1). The longer adult lifespan of offspring laid in the absence of a male may be  
188 caused by selective disappearance if weaker offspring have higher mortality in the  
189 period before eclosion. There was a nonsignificant trend for males (but not females)  
190 to die sooner after caring for larvae derived from eggs laid in the presence of a male  
191 ( $Z_{1,57} = 1.83$ ,  $P = 0.0670$ , table S2, Fig. S2).

192

193 **Discussion**

194 Our results provide experimental evidence that females can manipulate the  
195 behavior of caring males through prenatal maternal effects and that females appear  
196 to suppress male food consumption from a resource that is shared by the two  
197 parents and their offspring. We found that females respond to male presence during  
198 laying by producing larvae that are lighter at hatching, suggesting that females  
199 redirect the costs of parental care from the prenatal period where they pay the full  
200 costs of egg production towards the postnatal period where the costs of care are  
201 shared with the male. We also found that males gained less weight when caring for  
202 broods derived from eggs laid in the presence of a male, that such broods gained  
203 more weight during parental care, and that the greater weight gain of these broods  
204 came at the expense of the male's weight gain. These findings suggest that the  
205 female's suppression of the male's food consumption was beneficial to the offspring  
206 as it enhanced their access to the shared resource. Our results indicate that female  
207 manipulation of male behavior was targeted towards sexual conflict over food  
208 consumption rather than conflict over parental care.

209 Previous work on sexual conflict between caring parents has mainly focused  
210 conflict over parental care (1, 2). Thus, our results suggest that sexual conflict over  
211 food consumption from shared resources may be more important than traditionally  
212 recognized. This suggestion is also consistent with theoretical predictions and prior  
213 empirical work on *Nicrophorus vespilloides*. Although explicit models of maternal  
214 effects as a tool for female manipulation of male behavior are lacking, they could be  
215 interpreted as a form of Stackleberg games where one parent (here the female)  
216 makes the initial decision about how much to contribute and the second parent  
217 consequently responds by deciding its contribution (26). In such game theoretical

218 models, the individual making the initial decision is expected to gain the upper hand  
219 (26). Thus, if sexual conflict is primarily over care, we should expect females to  
220 provide less care than males. This prediction is not supported in our system where  
221 females provide more care than males (27, 28). However, if sexual conflict occurs  
222 over food consumption from a shared resource, females are expected to consume  
223 more than males. This prediction is supported by our study as females gain more  
224 weight during breeding than males (paired t test:  $t_{59} = 3.37$ ,  $P = 0.00131$ ). Sexual  
225 conflict over consumption may be particularly important in our study species given  
226 that it breeds on carcass of small vertebrates that serve as food for both parents  
227 and developing larvae (20, 21). However, such conflict may also be important in  
228 other systems, such as birds, where the two parents find food for themselves and  
229 their offspring within a shared territory.

230         Although our study provides evidence for female manipulation of male  
231 behavior, it leaves an unanswered question as to what mechanisms are  
232 responsible. To be effective, such mechanisms must influence the offspring's  
233 phenotype, thereby altering the male's behavior in a way that increases the female's  
234 or the offspring's fitness at the expense of the male's fitness. Our results reveal that  
235 egg size (measured as larval weight at hatching) is not the mechanism responsible  
236 for female manipulation of male behavior. Although females reduced egg size in  
237 response to the male's presence, we found that males gained more weight when  
238 caring for lighter larvae. Thus, the effect of egg size on male weight change was in  
239 the opposite direction of the effect due to prenatal conditions. Nevertheless, this  
240 result demonstrates that females respond to the presence of the male by adjusting  
241 offspring size at hatching, suggesting that females might adjust other maternal  
242 effect mechanisms. One potential such mechanism is deposition of maternal

243 hormones in the eggs (9, 10). There is good evidence that that female birds deposit  
244 testosterone into the eggs and that maternal testosterone stimulates nestling  
245 begging and growth, although it is debated whether this provides a mechanism for  
246 female manipulation of male behavior (10-12). Insects have a different hormonal  
247 system from vertebrates, but there is evidence that females deposit juvenile  
248 hormones or ecdysone into the eggs (29, 30). Potentially, these maternal hormones  
249 might influence larval behavior or development, thereby altering male behavior.  
250 Males might also respond to prenatal maternal effect indirectly, by responding to the  
251 female's response to the offspring phenotype. We found no evidence for this  
252 suggestion, as females did not respond to prenatal maternal effects in our study.

253         Our study raises a key question: why should males allow themselves to be  
254 manipulated by females? After all, if females use maternal effects to enhance their  
255 own or their offspring's fitness at the expense of male fitness, we should expect  
256 males to be under selection to evolve a counterstrategy to such manipulation (11).  
257 In a recent paper, we distinguish between deception, where maternal effects  
258 somehow bias the male's behavior away for his own optimum, and incentivization,  
259 where maternal effects somehow alter the cost/benefit function of male behavior (9).  
260 It is unlikely that deception would be evolutionarily stable because males should  
261 simply evolve to ignore manipulating maternal effects. In contrast, incentivization  
262 might be evolutionarily stable because maternal effects alter the benefits and/or  
263 costs of male behaviors, inducing a change in the male's optimal behavior (9, 31).  
264 For example, by depositing hormones into eggs, females might alter the offspring's  
265 physiology and growth trajectory, thereby incentivizing males to consume less food  
266 for themselves to achieve their cost/benefit optimum.

267

## 268 **Conclusion**

269 Until now, most theoretical and empirical work on the resolution of sexual conflict  
270 has assumed symmetry of power between males and females with the same  
271 mechanisms of conflict resolution applying to both parents (3-5). Our study adds to  
272 our understanding of sexual conflict between caring parents by showing that  
273 maternal effects provide females with a greater power over their partner's behavior  
274 that allow them to manipulate male behavior. Therefore, we urge future work to  
275 consider the potential importance of prenatal maternal effects in the resolution of  
276 sexual conflict between parents.

277

## 278 **Methods**

### 279 **General procedure**

280 We used virgin beetles from an outbred laboratory population maintained at the  
281 University of Edinburgh. Beetles were housed individually in clear plastic boxes  
282 (124 x 82 x 22 mm) containing moist soil, kept at  $21 \pm 2$  °C under constant lighting  
283 and fed small pieces of organic beef twice a week. The beetles were aged 13–24  
284 days post-eclosion at the start of the experiment.

285

### 286 **Cross-fostering procedure**

287 We weighed males and females at the beginning of the experiments to record their  
288 prebreeding mass. We then placed each pair into a plastic box (110 x 110 x 30mm)  
289 with 10mm of moist soil for about 24h to allow all experimental females to be  
290 fertilized by a male. We randomly placed either both parents (n = 72) or females  
291 only (n = 72) in a larger box (170 x 120 x 60 mm) filled with a 10–20mm layer of soil  
292 and provided with a freshly defrosted mouse (21.4–23.7g, supplied from Livefoods

293 Direct Ltd, Sheffield, UK). In the interval between the end of egg laying and the start  
294 of hatching (62–63 h after providing the carcass), we moved the parents and their  
295 prepared carcass to a new box with fresh soil. Females breeding alone and their  
296 prepared carcass were discarded from the rest of the experiment and only boxes  
297 with both parents were used as foster parents. At this stage, we counted the  
298 number of eggs visible at the bottom of the box as an estimation of clutch size (32).  
299 The eggs from both treatments were left to develop in the original box.

300 Larvae hatching from eggs left in the original containers were used to  
301 generate experimental foster broods. As soon as possible after their own larvae  
302 began to hatch, we provided pairs of breeding beetles with experimental foster  
303 broods that differed with respect to whether they hatched from eggs laid in the  
304 presence or absence of a male. All experimental broods were comprised of 20  
305 larvae from at least 2 different donor pairs that were not the larvae's foster parents.  
306 We gave caring parents 20 larvae to match the mean brood size in this species  
307 (33). We weighed the larvae before placing them on the carcass as a measure of  
308 prenatal maternal investment (34). From the 72 potential receiver pairs, we  
309 excluded 5 pairs because their own eggs failed to hatch and 6 other pairs because  
310 we did not obtain enough larvae to set up foster broods. Thus, in total, we set up 61  
311 experimental pairs. One experimental pair was subsequently excluded from further  
312 analyses as one of the parents died during the period of parental care. As we had  
313 no prior expectation on effect sizes, we aimed for large and predefined sample size  
314 of 30 successful pairs per treatment. The total sample size in the experiment was  $n$   
315 = 60 (31 pairs raising broods laid in presence of a male and 29 pairs raising broods  
316 laid in absence of a male).

317

318 **Behavioral observations**

319 In order to cover the whole period of parental care (23), we conducted three  
320 observations on each pair. We first conducted behavioral observations of parents  
321 1h ( $\pm 15$ min) after generating the experimental brood, which is when maternal  
322 effects are likely to be most pronounced (32, 35). We then conducted observations  
323 after 25h ( $\pm 15$ min), which corresponds to the peak in parental care and offspring  
324 begging (23). Finally, we conducted observations after 49h ( $\pm 15$ min), which is just  
325 before larvae become nutritionally independent (23). We used instantaneous  
326 sampling, scoring behaviors every 1min for 30min in accordance with established  
327 protocols (33). We scored the number of scans each parent spent providing direct  
328 care, defined as regurgitation of food to the larvae, manipulation of carrion, or  
329 regurgitation of carrion within the crater (36, 37).

330

331 **Offspring and parental fitness**

332 Parents were left undisturbed until the larvae dispersed from the carcass 8–10 days  
333 after pairing, at which point we recorded the number of larvae and weighed the  
334 brood. Parents were weighed, placed in individual boxes and checked for survival  
335 twice a week as we fed them small pieces of organic beef. We obtained measures  
336 of lifespan for  $n = 57$  females (excluding 3 females that escaped from their boxes)  
337 and  $n = 60$  males. We placed the dispersed larvae in a box (170 x 120 x 60mm)  
338 filled with soil to allow them to pupate and eclose as adults. We then recorded the  
339 number and sex of the eclosed offspring. We randomly selected one male and one  
340 female offspring from each brood, kept them in individual boxes and fed them twice  
341 a week to record their lifespan. Once dead, we measured pronotum width of adult  
342 parents and offspring using a Mitutoyo Absolute Digimatic calliper. To minimize



343 observer bias, all observations and measurements were done blind with respect to  
344 the treatment by allocating a two-letters code to each experimental pair.

345

### 346 **Statistical analyses**

347 All statistical analyses were conducted using R (38). We used linear models (lm  
348 function in stats) except for the behavioral data where we used generalized linear  
349 models for zero inflated negative binomial distributions (glmmadmb function in  
350 glmmADMB) and for the offspring's adult size where we used linear mixed models  
351 (lme function in nlme) given that we measured one male and one female per brood.  
352 For survival analyses we used survival models (function survreg and coxph in the  
353 package survival). As parametric tests (when an appropriate distribution is  
354 available) are statistically more powerful and give more accurate estimates than  
355 semiparametric and nonparametric tests (39), we first tried to fit the different  
356 available survival distributions to our data and selected the best distribution (log  
357 logistic for parents' survival) based on AIC comparison and confirmed graphically  
358 that the model fitted our data (see Figure S2). As offspring survival presented an  
359 odd distribution (see Figure S1), we used semiparametric Cox proportional Hazard  
360 regression models as the effect of the treatment met the assumption of proportional  
361 hazards ( $\chi^2 = 0.622$ ,  $P = 0.430$ ). For all analyses, we included the effect of  
362 treatment (presence or absence of a male partner before hatching) as well as a  
363 small set of pertinent explanatory variables in the full models (see supplementary  
364 text). We then applied a backward-stepwise procedure to remove non-significant  
365 variables. Statistics of the nonsignificant variables presented in the tables were  
366 obtained by sequentially reintroducing each of them into the minimal model (40).

367

368 **Data Availability**

369 If accepted, the results from this paper will be made available through Dryad

370

371 **Acknowledgments:**

372 This project was supported by Association for the Study of Animal Behaviour  
373 research grant and Matthieu Paquet was funded by FYSSSEN. We thank Lucy Ford,  
374 Athina Georgiou-Shippi, Edward Ivimey-Cook, Lisa Kopsieker, Daniel Sieber,  
375 Ashleigh Whiffin and Ben Whittaker, for their help with the maintenance of the  
376 laboratory population and their advice. We are very thankful to Maarit Mäenpää for  
377 her advice regarding the experimental set up and the behavioral observations. We  
378 also thank Jacob Moorad, Loeske Kruuk, Charlotte Regan, Natalie  
379 Pilakouta, Joshua Moatt, the editor and two anonymous reviewers for their useful  
380 feedback.

381

382 **References**

- 383 1. Houston AI, Szekely T, & McNamara JM (2005) Conflict between parents  
384 over care. *Trends Ecol. Evol.* 20(1):33-38.
- 385 2. Harrison F, Barta Z, Cuthill I, & Székely T (2009) How is sexual conflict over  
386 parental care resolved? A meta-analysis. *J Evol Biol* 22(9):1800-1812.
- 387 3. McNamara JM, Gasson CE, & Houston AI (1999) Incorporating rules for  
388 responding into evolutionary games. *Nature* 401(6751):368-371.
- 389 4. Johnstone RA & Hinde CA (2006) Negotiation over offspring care—how  
390 should parents respond to each other's efforts? *Behav. Ecol.* 17(5):818-827.
- 391 5. Houston A & Davies N (1985) The evolution of cooperation and life history in  
392 the Dunnock, *Prunella modularis*.

- 393 6. Wright J & Cuthill I (1989) Manipulation of sex-differences in parental care.  
394 *Behav Ecol Sociobiol* 25(3):171-181.
- 395 7. Hinde C (2006) Negotiation over offspring care?—a positive response to  
396 partner-provisioning rate in great tits. *Behav. Ecol.* 17(1):6-12.
- 397 8. Schwagmeyer P, Mock DW, & Parker GA (2002) Biparental care in house  
398 sparrows: negotiation or sealed bid? *Behav. Ecol.* 13(5):713-721.
- 399 9. Paquet M & Smiseth PT (2016) Maternal effects as a mechanism for  
400 manipulating male care and resolving sexual conflict over care. *Behav Ecol.*
- 401 10. Moreno-Rueda G (2007) Yolk androgen deposition as a female tactic to  
402 manipulate paternal contribution. *Behav. Ecol.* 18(2):496-498.
- 403 11. Müller W, Lessells CM, Korsten P, & von Engelhardt N (2007) Manipulative  
404 signals in family conflict? On the function of maternal yolk hormones in birds.  
405 *Am. Nat.* 169(4):E84-E96.
- 406 12. Müller W, Boonen S, Groothuis TGG, & Eens M (2010) Maternal yolk  
407 testosterone in canary eggs: toward a better understanding of mechanisms  
408 and function. *Behav. Ecol.* 21(3):493-500.
- 409 13. Noguera JC, Kim S-Y, & Velando A (2013) Maternal testosterone influences  
410 a begging component that makes fathers work harder in chick provisioning.  
411 *Horm Behav* 64(1):19-25.
- 412 14. Ruuskanen S, *et al.* (2009) Yolk androgens do not appear to mediate sexual  
413 conflict over parental investment in the collared flycatcher *Ficedula albicollis*.  
414 *Horm Behav* 55(4):514-519.
- 415 15. Laaksonen T, Adamczyk F, Ahola M, & Möstl E (2011) Yolk hormones and  
416 sexual conflict over parental investment in the pied flycatcher. *Behav Ecol*  
417 *Sociobiol* 65(2):257-264.

- 418 16. Tschirren B & Richner H (2008) Differential effects of yolk hormones on  
419 maternal and paternal contribution to parental care. *Anim. Behav.* 75:1989-  
420 1994.
- 421 17. Schwabl H (1996) Maternal testosterone in the avian egg enhances postnatal  
422 growth. *Comp. Biochem. Phys. A* 114(3):271-276.
- 423 18. Barnett CA, Clairardin SG, Thompson CF, & Sakaluk SK (2011) Turning a  
424 deaf ear: a test of the manipulating androgens hypothesis in house wrens.  
425 *Anim. Behav.* 81(1):113-120.
- 426 19. Paquet M, Covas R, & Doutrelant C (2015) A cross-fostering experiment  
427 reveals that prenatal environment affects begging behaviour in a cooperative  
428 breeder. *Anim. Behav.* 102(0):251-258.
- 429 20. Pilakouta N, Richardson J, & Smiseth PT (2016) If you eat, I eat: resolution  
430 of sexual conflict over consumption from a shared resource. *Anim. Behav.*  
431 111:175-180.
- 432 21. Boncoraglio G & Kilner RM (2012) Female Burying Beetles Benefit from Male  
433 Desertion: Sexual Conflict and Counter-Adaptation over Parental Investment.  
434 *PLoS ONE* 7(2):e31713.
- 435 22. Scott MP (1998) The ecology and behavior of burying beetles. *Annual review*  
436 *of entomology* 43(1):595-618.
- 437 23. Smiseth PT, Darwell CT, & Moore AJ (2003) Partial begging: an empirical  
438 model for the early evolution of offspring signalling. *Proceedings of the Royal*  
439 *Society B-Biological Sciences* 270(1526):1773-1777.
- 440 24. Savage JL, Russell AF, & Johnstone RA (2015) Maternal allocation in  
441 cooperative breeders: should mothers match or compensate for expected  
442 helper contributions? *Anim. Behav.* 102:189-197.

- 443 25. Russell AF, Langmore NE, Cockburn A, Astheimer LB, & Kilner RM (2007)  
444 Reduced egg investment can conceal helper effects in cooperatively  
445 breeding birds. *Science* 317(5840):941-944.
- 446 26. McNamara JM, Houston AI, Barta Z, & Osorno J-L (2003) Should young ever  
447 be better off with one parent than with two? *Behav. Ecol.* 14(3):301-310.
- 448 27. Pilakouta N, Richardson J, & Smiseth P (2015) State-dependent cooperation  
449 in burying beetles: parents adjust their contribution towards care based on  
450 both their own and their partner's size. *Journal of Evolutionary Biology*  
451 28(11):1965-1974.
- 452 28. Smiseth PT & Moore AJ (2004) Behavioral dynamics between caring males  
453 and females in a beetle with facultative biparental care. *Behav. Ecol.*  
454 15(4):621-628.
- 455 29. De Loof A, Boerjan B, Ernst UR, & Schoofs L (2013) The mode of action of  
456 juvenile hormone and ecdysone: Towards an epi-endocrinological paradigm?  
457 *Gen Comp Endocr* 188:35-45.
- 458 30. Nijhout HF (1998) *Insect hormones* (Princeton University Press).
- 459 31. Paquet M & Smiseth PT (2016) Maternal effects and female manipulation: a  
460 response to comments on Paquet and Smiseth. *Behav. Ecol.* 27(3):698-699.
- 461 32. Monteith K, Andrews C, & Smiseth P (2012) Post-hatching parental care  
462 masks the effects of egg size on offspring fitness: a removal experiment on  
463 burying beetles. *Journal of evolutionary biology* 25(9):1815-1822.
- 464 33. Smiseth PT & Moore AJ (2002) Does resource availability affect offspring  
465 begging and parental provisioning in a partially begging species? *Anim.*  
466 *Behav.* 63(3):577-585.

- 467 34. Ford L & Smiseth P (2016) Asynchronous hatching provides females with a  
468 means for increasing male care but incurs a cost by reducing offspring  
469 fitness. *Journal of evolutionary biology* 29(2):428-437.
- 470 35. Krist M (2011) Egg size and offspring quality: a meta-analysis in birds. *Biol*  
471 *Rev* 86(3):692-716.
- 472 36. Jenkins EV, Morris C, & Blackman S (2000) Delayed benefits of paternal  
473 care in the burying beetle *Nicrophorus vespilloides*. *Anim. Behav.* 60(4):443-  
474 451.
- 475 37. Walling CA, Stamper CE, Smiseth PT, & Moore AJ (2008) The quantitative  
476 genetics of sex differences in parenting. *Proceedings of the National*  
477 *Academy of Sciences* 105(47):18430-18435.
- 478 38. Team RC (2013) R: A language and environment for statistical computing.
- 479 39. Zhou M (2007) Use software R to do survival analysis and simulation. a  
480 tutorial. (Department of Statistics, University of Kentucky).
- 481 40. Crawley MJ (2002) Statistical computing: An introduction to data analysis  
482 using R.

483

484

485

## 486 **Figures legends**

487 **Fig.1. Maternal effect of the presence of the male before hatching on brood**  
488 **weight at hatching.** Raw data are shown for brood weight of larvae laid in absence  
489 of a male (black open circles) N=30 and brood weight of larvae laid in presence of a  
490 male (red filled circles) N=31 as well as associated means  $\pm$ SE. The inset  
491 represents the predicted means $\pm$ SE from the final model. Experimental broods were  
492 all comprised of 20 larvae mixed from different donor pairs of the same pre-hatching  
493 treatment that were not the larvae's foster parents. We weighed the 20 larvae  
494 together before placing them on the carcass as a measure of prenatal maternal  
495 investment.

496

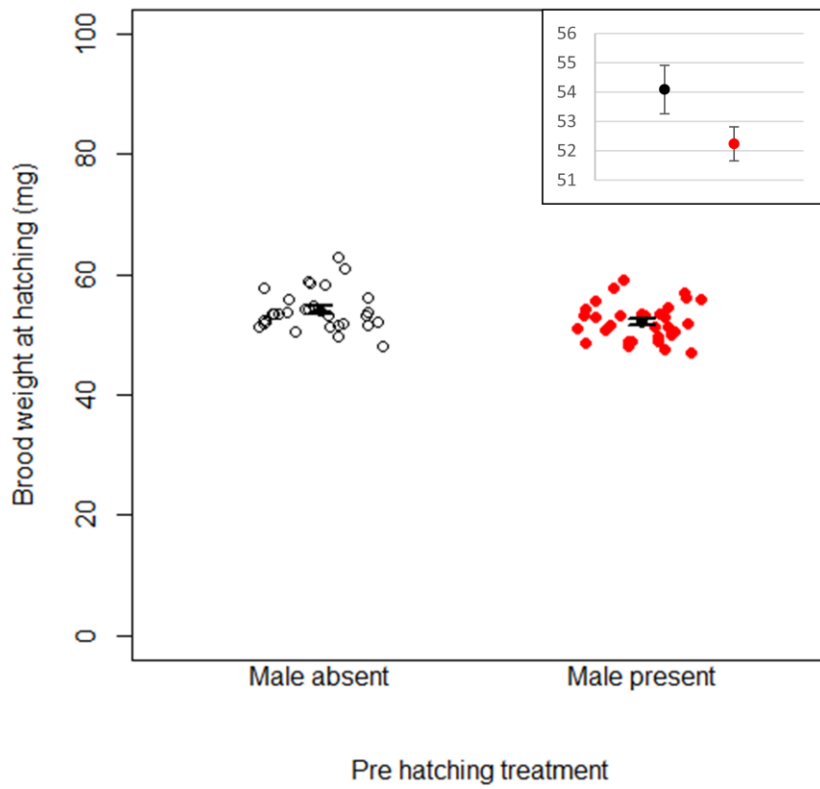
497 **Fig. 2. Prenatal maternal effects on parental care and weight change.** Raw data  
498 are shown for the time females and males spent providing direct care 25 hours after  
499 hatching **(A)** and females and males weight change from mating to larval dispersal  
500 **(B)** when caring for larvae laid in absence of a male (N=29) or in presence of a  
501 male (N=31) as well as associated means  $\pm$ SE. The insets represent the predicted  
502 means $\pm$ SE from the final models. **(C)** Male care 25 hours after hatching was  
503 positively associated with male weight change. The dashed lines depict the  
504 regression line from the model. Brood weight at hatching and larvae laid in  
505 presence of a male have negative effects on male weight change **(D)**. The dashed  
506 lines depict the regression lines from the final model for each pre-hatching  
507 treatment.



508 **Fig.3. Effect of brood weight at hatching and the prenatal conditions (i.e.,**  
509 **presence or absence of a male during laying) on brood weight at dispersal.**

510 Raw data are shown (N=60) and the dashed lines depicts the regression lines from  
511 the final model for each pre-hatching treatment.

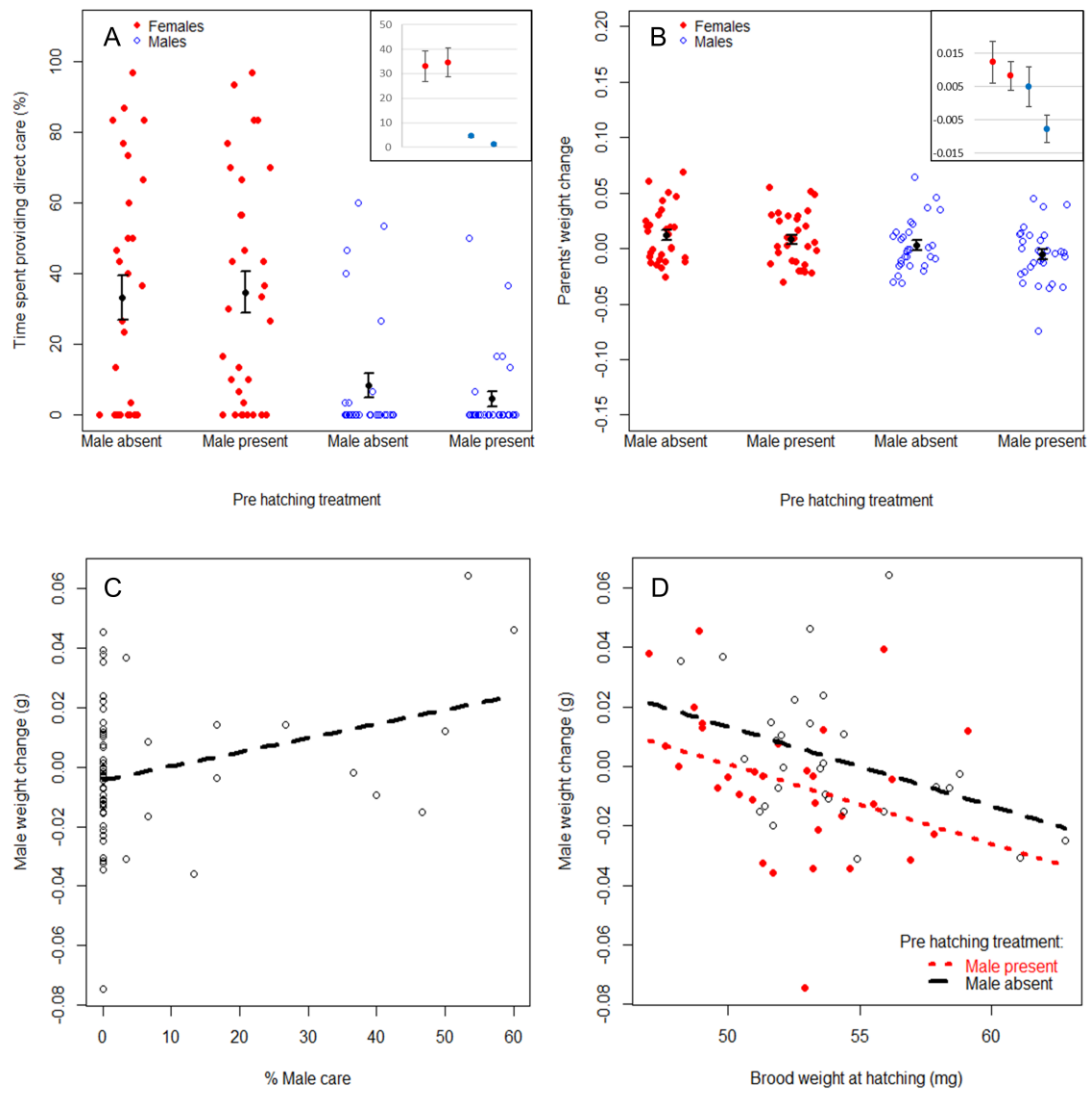
512



513

514 Fig.1

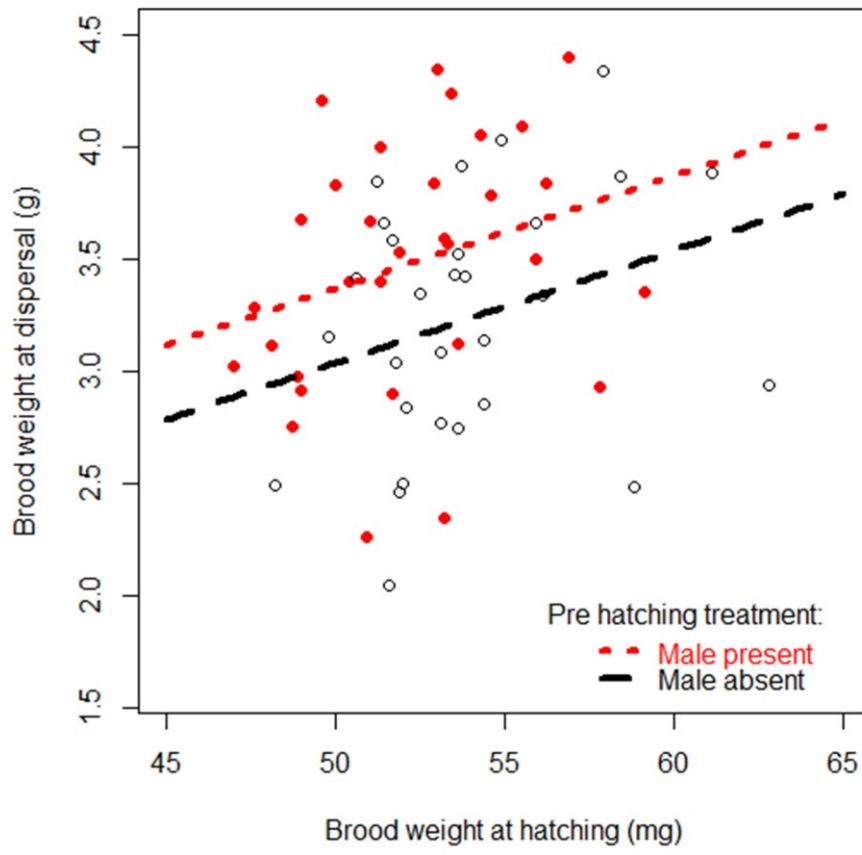
515



516

517 Fig.2

518



519

520 Fig.3