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## Separating differential allocation by females from direct effects of male condition in a beetle

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1 **Separating differential allocation by females from direct**  
2 **effects of male condition in a beetle.**

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5

6 **Lay summary**

7 Female burying beetles had fewer larvae after mating with a food-deprived male. This  
8 shows that females adjust their reproductive allocation to the condition of their mate, a  
9 phenomenon known as differential allocation. Our experimental design excluded direct  
10 effects of male condition. Furthermore, there was a positive relationship between the  
11 number and size of larvae when females mated with a food-deprived male. Our results  
12 suggest that differential allocation could explain variation in life history trade-offs.

13

## 14 **Abstract**

15 Differential allocation is the adjustment of reproductive allocation, typically by a female, in  
16 response to the quality of her male partner. A recent theoretical model suggests that  
17 differential allocation may influence trade-offs between reproductive traits within a  
18 breeding attempt. Furthermore, it is often difficult to distinguish differential allocation from  
19 direct effects of male condition. We address these gaps using a novel cross-fostering design  
20 to exclude direct effects of male condition and to test whether differential allocation affects  
21 trade-offs between and within breeding attempts. This design detects differential allocation  
22 as effects of a female's mating partner and direct effects of male condition as effects of the  
23 larvae's sire. We used the burying beetle *Nicrophorus vespilloides*, a species which adjusts  
24 reproductive allocation by culling some larvae after hatching. We used food-deprivation to  
25 manipulate the nutritional condition of both the female's mating partner and the larvae's  
26 sire. We find clear evidence for differential allocation as females mating with food-deprived  
27 males had fewer larvae than females mating with control males. There was a trade-off  
28 between number and size of larvae when females mated with control males, but a positive  
29 relationship when females mated with food-deprived males. Thus, differential allocation  
30 influenced relationships between reproductive traits within a breeding attempt, but not  
31 necessarily through trade-offs. Instead, we suggest that there may be cryptic heterogeneity  
32 in quality among females or their mating partners that was only exposed when females  
33 mated with a male in poor condition.

34

35 **Keywords:** burying beetle; differential allocation; infanticide; male condition; reproductive  
36 trade-offs.

37

## 38 Introduction

39 Differential allocation is the adjustment of reproductive allocation, typically by a female, in  
40 response to the quality, attractiveness, or condition of her current mate (Burley 1986;  
41 Burley 1988). Positive differential allocation refers to greater allocation in offspring when  
42 paired with an attractive or high-quality male, while negative differential allocation  
43 (sometimes termed “reproductive compensation”) refers to greater allocation when paired  
44 with an unattractive or low-quality male (Sheldon 2000; Gowaty 2008; Ratikainen & Kokko  
45 2010). There is evidence for differential allocation from a number of studies focusing on  
46 various reproductive traits including: probability of breeding (Drickamer et al. 2003), time  
47 until onset of egg laying (Alonso-Alvarez et al. 2012), size of eggs or offspring (Cunningham  
48 & Russell 2000; Kolm 2001; Osorno et al. 2006; Loyau et al. 2007; Bolund et al. 2009; Bonato  
49 et al. 2009), clutch size (Reyer et al. 1999; Head & Brooks 2006; López-Rull & Gill 2009), egg  
50 components such as proteins and hormones (Gil 1999; Saino et al. 2002; Navara et al. 2006;  
51 Goncalves et al. 2010), number of broods per season (de Lope & Møller 1993), and offspring  
52 sex-ratios (Pike & Petrie 2005; Sardell & DuVal 2014). This prior work demonstrates that  
53 females respond to male quality by adjusting their allocation to reproductive traits in the  
54 current breeding attempt with likely consequences for future reproduction (Burley 1986;  
55 Sheldon 2000). However, few studies have examined whether differential allocation  
56 influences trade-offs to multiple different reproductive traits *within* a breeding attempt.  
57 This is an important knowledge gap as highlighted by a recent theoretical model (Ratikainen  
58 et al. 2018), predicting that mate quality can affect trade-offs within a breeding attempt,  
59 such as that between the number and size of offspring.

60 Differential allocation is expected to influence reproductive trade-offs within a  
61 breeding attempt because the quality, attractiveness or condition of the female’s current

62 mate can change the optimal amount of investment per offspring (Kindsvater & Alonzo  
63 2014; Ratikainen et al. 2018). For example, if offspring produced when mating with a high-  
64 quality male are more likely to produce grand-offspring, females may gain higher returns on  
65 their investment by increasing their allocation to each offspring when paired with a high-  
66 quality male. However, because individuals have access to a limited pool of resources  
67 (Stearns 1992), any increase in allocation to individual offspring (e.g. by increasing offspring  
68 size) should lead to a corresponding reduction in allocation to other reproductive traits,  
69 such as number of offspring. For example, in the freshwater crayfish *Austropotamobius*  
70 *pallipes*, females adjust egg size in response to male chelae size. However, this shift in  
71 allocation is associated with a trade-off between the number and size of eggs, which means  
72 that females lay larger but fewer eggs when mating with males with larger claws, and  
73 smaller but more numerous eggs when mating with males with smaller claws (Galeotti et al.  
74 2006). Thus, one way to improve our understanding of differential allocation is for more  
75 empirical studies to examine how male quality influences reproductive trade-offs within a  
76 breeding attempt rather than just those between current and future reproduction.

77 Another persistent limitation is that it can be difficult to distinguish differential  
78 allocation by females from direct effects of male quality. For example, if females mated to a  
79 high-quality male produce more offspring than females mated to a low-quality male, this  
80 could reflect changes in allocation by females (i.e. positive differential allocation). However,  
81 it could also reflect direct effects of male quality if low-quality males produce fewer or lower  
82 quality sperm (e.g. Rahman et al. 2013; O’dea et al. 2014), if male quality is associated with  
83 sperm-mediated epigenetic effects (e.g. Zajitschek et al. 2014; Gasparini et al. 2017), or if  
84 male quality influences the size or quality of nuptial gifts in species where males offer such  
85 gifts to females during mating (Wedell 1994; Simmons et al. 1999). This issue is particularly

86 challenging because differential allocation by females and direct effects of male quality on  
87 offspring are not mutually exclusive (Watson & Simmons 2012). One approach for  
88 distinguishing between differential allocation by females and direct effects of male quality is  
89 to manipulate male attractiveness independently of male quality. For example, studies in  
90 birds manipulate male attractiveness by fitting males with coloured leg rings that females  
91 find attractive or unattractive (e.g. Burley et al. 1982). However, this approach has limitation  
92 because it requires prior knowledge of which male traits females find attractive and how to  
93 manipulate them, and because manipulating male attractiveness may inadvertently alter  
94 male behaviour or physiology through feedback from social interactions with conspecifics  
95 (Rubenstein & Hauber 2008; Royle & Pike 2010). Thus, an additional gap in our  
96 understanding of differential allocation is how to separate its effects from direct effects of  
97 male quality or condition.

98         Here, we use a novel experimental approach to address these gaps in our current  
99 understanding of differential allocation. We first manipulated male condition and then use a  
100 cross-fostering design to separate between differential allocation by females and direct  
101 effects of male condition. In our experiment, females mated with a male that was in either  
102 good or poor condition (hereafter termed “mating partner”). These females then received a  
103 brood of hatched offspring that had been sired by a male that was in either good or poor  
104 condition (hereafter termed “sire”). This approach detects differential allocation as effects  
105 due to the condition of the mating partner and direct effects of male condition as effects  
106 due to the condition of the sire. We then monitored the subsequent effects on post-  
107 hatching reproductive allocation within a breeding attempt – focusing on the trade-off  
108 between number and size of offspring.

109           We used the burying beetle *Nicrophorus vespilloides* as our study system. This  
110 species rears broods of larvae on small, vertebrate carcasses. Females can adjust their  
111 reproductive allocation before hatching by changing the number, size, and contents of their  
112 eggs during egg laying. For example, females allocate fewer hormones to their eggs when  
113 mated to heavier males (Paquet et al. 2020). Females usually lay more eggs than the carcass  
114 can support (Bartlett 1987), and they must therefore adjust their reproductive allocation  
115 after hatching by culling excess larvae via filial cannibalism (Bartlett 1987; Bartlett &  
116 Ashworth 1988; Müller et al. 1990). Culling is therefore an important mechanism for  
117 adjusting reproductive allocation, allowing females to match the number of larvae to the  
118 size of the carcass. Given that the carcass is a limited resource, culling of excess larvae is  
119 important to ensure that the remaining larvae have access to sufficient amounts of carrion  
120 to develop into competitive adult beetles (Otronen 1988). Furthermore, parents do not  
121 recognise their own larvae (Oldekop et al. 2007). This is important as it allows us to use  
122 cross-fostering to separate differential allocation by females from direct effects of the  
123 condition of the larvae's sire.

124           Our aim was to test for effects of differential allocation by females on the trade-off  
125 between number and size of offspring within a breeding attempt. To this end, we generated  
126 males in good or poor condition by manipulating their nutritional state prior to mating. We  
127 focused on this aspect of male condition because females discriminate between control and  
128 food-deprived males during mate choice (Richardson & Smiseth 2019a), suggesting that  
129 females perceive a male's nutritional state as an indicator of his quality. We mated females  
130 with a food-deprived or a control male and later provided them with a cross-fostered brood  
131 of 20 larvae that had been sired by another food-deprived or control male in a fully crossed  
132 design. We then tested for subsequent effects on post-hatching reproductive allocation by

133 recording the number and size of larvae at dispersal. If there was differential allocation, we  
134 expected an effect of the condition of a female's mating partner on the number of larvae  
135 reared by the female. We predicted that having fewer larvae would allow females to  
136 increase their allocation of resources towards individual larvae due to the trade-off between  
137 the number and size of larvae (Smiseth et al. 2014). This has important consequences for  
138 larval fitness as heavier larvae develop into larger and more competitive adult beetles  
139 (Bartlett & Ashworth 1988; Otronen 1988). We did not have an *a priori* prediction about the  
140 direction of differential allocation. This is because females could respond to the condition of  
141 their mating partner either by reducing the number of larvae and therefore producing  
142 fewer, larger larvae, or by increasing the number of larvae reared and therefore producing  
143 more, smaller larvae. If there were direct effects of male condition on offspring, we  
144 expected that the condition of the sire would affect the number and/or size of larvae at  
145 dispersal. Finally, we provided females with a second reproductive attempt to determine  
146 how differential allocation and/or direct effects of male condition influenced the trade-off  
147 between current and future reproduction.

148

## 149 **Methods**

### 150 *Study system*

151 Burying beetles breed on carcasses of small vertebrates, which they bury underground.  
152 Parents prepare the carcass by removing any fur or feathers, rolling it into a ball, and  
153 applying antimicrobial secretions that suppress bacterial and fungal growth (Scott 1998; Arce  
154 et al. 2012). Males mate repeatedly and frequently with females as a paternity guard given  
155 the risk of sperm competition (Müller & Eggert 1989; House et al. 2008). However, there is  
156 no evidence that females receive direct benefits, such as nuptial gifts, from males during



157 mating (House et al. 2008). Females lay their eggs in the soil surrounding the buried carcass.  
158 After hatching, larvae crawl to the carcass and start feeding in a crater created by the  
159 parents. Larvae can self-feed, but parents also provision larvae with pre-digested carrion. In  
160 this species, parents cannot distinguish between their own and unrelated larvae using  
161 chemical cues. Instead, parents use temporal cues to predict when their own larvae are  
162 expected to hatch, and they will kill any larvae they encounter before this time (Müller &  
163 Eggert 1990). It is therefore possible to cross-foster larvae in this species, as long as foster  
164 parents are allocated foster broods after their own eggs have started hatching (Oldekop et  
165 al. 2007).

166

#### 167 *Beetle husbandry*

168 Our study used unmated beetles from an outbred laboratory stock population maintained at  
169 the University of Edinburgh. We used 5<sup>th</sup>–7<sup>th</sup> generation beetles descending from wild-  
170 caught individuals collected in Blackford Hill, Edinburgh. We kept all beetles at 20°C under a  
171 16:8 h light:dark cycle. Adults in the stock population were housed individually from  
172 eclosion in transparent plastic containers (12 x 8 x 2 cm) filled with moist soil and were fed  
173 twice a week on small pieces of raw beef (approximately 0.3g).

174

#### 175 *Experimental design*

176 Our experiment examined evidence for differential allocation and/or direct effects of male  
177 condition on reproductive trade-offs within a breeding attempt. To this end, we investigated  
178 how the condition of a female's mating partner and the condition of the sire of her larvae  
179 influenced the number and size of larvae at dispersal. We mated females with either a food-  
180 deprived or a control male mating partner (i.e. a male in poor or good condition,

181 respectively). We next provided each female with a cross-fostered brood of 20 larvae that  
182 had been sired by either a food-deprived or a control male in a fully crossed design. The  
183 larvae were pooled from across multiple females that had mated with either a food-  
184 deprived or a control male. As described earlier, this cross-fostering design allowed us to  
185 separate differential allocation by the female in response to the condition of her mating  
186 partner from direct effects of the condition of the larvae's sire. We subsequently monitored  
187 the number and size of larvae at dispersal to examine evidence for differential allocation  
188 and/or direct effects of male condition on post-hatching reproductive allocation. Our  
189 experiment included the following four treatments: (1) females mating with a control male  
190 and receiving a brood of larvae sired by a control male ( $n = 24$ ); (2) females mating with a  
191 control male and receiving a brood of larvae sired by a food-deprived male ( $n = 21$ ); (3)  
192 females mating with a food-deprived male and receiving a brood of larvae sired by a food-  
193 deprived male ( $n = 22$ ); and (4) females mating with a food-deprived male and receiving a  
194 brood of larvae sired by a control male ( $n = 25$ ). In addition, we recorded the number, size,  
195 and hatching success of the eggs laid by females. These measures provide information on  
196 reproductive allocation at the egg stage. However, we cannot separate between differential  
197 allocation and direct effects of male condition for traits measured prior to cross-fostering.  
198 Finally, we provided each female with a second breeding attempt to examine how  
199 differential allocation and/or male condition influenced the trade-off between current and  
200 future reproduction. Our experimental design is summarised in Figure 1.

201

### 202 *Manipulation of male condition*

203 We generated males that were in good or poor condition by manipulating their nutritional  
204 state such that it was different at the time of mating. We focused on this attribute of male

205 condition because previous work shows that females discriminate between control males  
206 and males that have been food-deprived after they became adults (Richardson & Smiseth  
207 2019a), suggesting that females perceive and respond to variation in male nutritional  
208 condition. Seven days prior to mating, we randomly assigned males to each treatment.  
209 Food-deprived males (n = 47) received no food for seven days prior to mating with a female,  
210 whereas control males (n = 45) were fed twice during this period. We began the food-  
211 deprivation treatment 10 days after eclosion, which is after sexual maturity, to ensure that  
212 our treatment did not delay sexual maturation (Hopwood et al. 2013). We used seven days  
213 of food deprivation because deprivation for this length of time leads to significant weight  
214 loss without causing a detectable increase in mortality (Hopwood et al. 2013; Gray et al.  
215 2018; Richardson & Smiseth 2019a, Richardson et al. 2019). There was no difference in the  
216 body mass of the food-deprived and control males before the start of food deprivation  
217 (estimate  $\pm$  SE =  $-0.011 \pm 0.012$ ,  $t = -0.94$ ,  $p = 0.35$ ; mean  $\pm$  SE for control males =  $0.2540 \pm$   
218  $0.0098$  g; mean  $\pm$  SE for food-derived male =  $0.2424 \pm 0.0075$ ). We weighed all males before  
219 mating to verify that food deprivation had the intended effect of causing substantial  
220 variation in male condition (see Results).

221

### 222 *Female reproductive allocation*

223 We used unmated females from our stock population in our experiment. We paired each  
224 female at random with either a food-deprived or a control male mating partner. We placed  
225 each pair in a transparent plastic container (11 x 11 x 3 cm) lined with 0.5 cm of moist soil  
226 for a period of 24 hours. We did this to ensure that all females had mated with their male  
227 partner, thereby allowing them to lay eggs once provided with a carcass (Botterill-James et  
228 al. 2017). After mating, we transferred females to a larger transparent plastic container (17

229 x 12 x 6 cm) lined with 1 cm of moist soil. At this time, we discarded all males to remove any  
230 potential confounding effects of male behaviour (e.g. infanticide or parental care by the  
231 male) on reproductive traits. To initiate breeding, we provided each female with a freshly  
232 thawed mouse carcass (Livefoods Direct Ltd., Sheffield, UK) that weighed 6–9 g (mean  $\pm$  SE =  
233  $7.90 \pm 0.081$  g). *Nicrophorus vespilloides* breeds on carcasses ranging in size from 1–40 g  
234 (Müller et al. 1990; Smiseth & Moore 2002). We chose this size range because females  
235 regulate brood size to match carcass size to a greater extent when breeding on carcasses  
236 smaller than 10 g (Bartlett 1987; Bartlett & Ashworth 1988). We weighed all females prior to  
237 giving them a carcass, using this as a measure of their pre-breeding mass to later calculate  
238 their mass change during breeding (see below).

239 We checked for the presence of eggs in the soil twice daily from the day we provided  
240 females with a carcass until two days after we recorded the presence of the first eggs in a  
241 clutch. Once females had finished egg laying, and immediately before the first eggs were  
242 expected to hatch (which takes 59 h from time of laying at 20°C; Smiseth et al. 2006), we  
243 scanned the bottom of each container using a flat-bed scanner (Canon CanoScan 9000F  
244 Mark II, Canon Inc., Tokyo, Japan). This allowed us to record the number and size of eggs  
245 laid by each female (Ford & Smiseth 2016). For each scanned image, we counted the  
246 number of visible eggs. Because each container has only a very thin layer of soil, the number  
247 of eggs visible at the bottom of the container is strongly correlated with the actual clutch  
248 size (Monteith et al. 2012). In addition, we measured the size of five randomly chosen eggs  
249 using ImageJ (<https://imagej.nih.gov/ij/>). For each egg, we measured length and width in  
250 three times using this information to calculate a prolate spheroid volume ( $V$ ) for each  
251 egg as  $V = (1/6) \pi w^2 L$ , where  $w$  is egg width and  $L$  egg length, respectively (Berrigan  
252 1991; Ford & Smiseth 2016). We continued to check the boxes after hatching (see below) to

253 record the number of unhatched eggs. We estimated hatching success by dividing the  
254 number of hatched eggs by clutch size.

255         Once we had scanned a given container, we moved the female and the carcass to a  
256 new container with fresh, moist soil. We left the eggs in the soil in the original container.  
257 Once the eggs started hatching, we used the newly hatched larvae to generate experimental  
258 broods for use in our cross-fostering design. We generated experimental broods by pooling  
259 larvae hatching from eggs laid by multiple different females. We collected newly hatched  
260 larvae sired by control males and newly hatched larvae sired by food-deprived males  
261 separately and kept them in different holding boxes. Once we had 20 larvae in a given  
262 holding box, we allocated them at random to a female that had previously mated with a  
263 control or a food-deprived male mating partner. Thus, all experimental broods comprised 20  
264 larvae, and all experimental broods were comprised of larvae that had been sired by either  
265 a control or a food-deprived male. We standardised the number of larvae to account for any  
266 potential differences at the egg laying stage (e.g. due to differences in clutch size or  
267 hatching success) between females mating with a control or a food-deprived male. This is  
268 important because our experimental design cannot separate between differential allocation  
269 by females and direct effects of male condition prior to hatching. We chose a brood size of  
270 20 larvae because it is within the range observed in this species (2–45 larvae; Smiseth &  
271 Moore 2002), but is slightly higher than the average number of larvae produced on smaller  
272 carcasses (Smiseth & Moore 2002). Thus, our design mimicked the overproduction of larvae  
273 that occurs naturally in this species, thus encouraging all females to cull some of their larvae  
274 to match the number of larvae to the amount of available resources. As parents kill any  
275 larvae that arrive on the carcass before their own eggs are expected to hatch (Müller &

276 Eggert 1990), we only provided experimental females with a brood of larvae once their own  
277 eggs had hatched.

278 Females were left to rear their larvae until the larvae dispersed from the carcass  
279 approximately seven days later. When all larvae dispersed from the carcass, we recorded  
280 the number of dispersing larvae and the total brood mass. We calculated mean larval mass  
281 at dispersal by dividing total brood mass by the number of larvae. At the time of dispersal,  
282 we also weighed each female to measure her post-breeding mass. We then calculated mass  
283 change during breeding for each female by subtracting her pre-breeding mass from her  
284 post-breeding mass. We recorded mass change as this is a proxy for allocation to future  
285 reproduction in species within the genus *Nicrophorus* (Creighton et al. 2009; Billman et al.  
286 2014). Experimental females were then transferred to individual containers (12 x 8 x 2 cm)  
287 filled with moist soil and left undisturbed for 24 hours.

288 Finally, we recorded reproductive success in a second breeding attempt to  
289 investigate effects on the trade-off between current versus future reproduction. To this end,  
290 we mated each female from the first part of our experiment with a second, unrelated,  
291 unmated male from the stock population (i.e. a control male). The second breeding attempt  
292 followed the same protocol as the first attempt described above with the exception that  
293 that we did not cross-foster the larvae. For the second breeding attempt, we recorded the  
294 number, size and hatching success of eggs and the number and mean mass of larvae at  
295 dispersal. Of the 92 females used in the first part of our experiment, we excluded four  
296 females that died before the start of the second breeding attempt.

297

298 *Statistical analyses*

299 Statistical analyses were conducted using R version 3.6.0 (R Core Team, 2019) loaded with  
300 the packages *car* (Fox & Weisberg 2019), and *MASS* (Venables & Ripley 2002). We analysed  
301 our data using a combination of univariate and bivariate models. We did this because  
302 differential allocation could influence trade-offs between traits in two ways – by increasing  
303 allocation to one trait at the expense of another, or by changing the direction or strength of  
304 the relationship between two traits. First, we used univariate models to examine evidence  
305 for differential allocation when focusing on single reproductive traits. These models tested  
306 whether the condition of the female’s mating partner and/or the condition of the larvae’s  
307 sire caused an increase or decrease in allocation to a particular trait. Next, we used bivariate  
308 models to examine evidence for differential allocation when focusing on the relationship  
309 between reproductive traits in a trade-off. These models tested whether the condition of  
310 the female’s mating partner and/or the sire of the larvae influenced the relationship  
311 between the number and size of larvae and between current and future reproduction.

312 For our univariate analyses, we used linear models for traits with normally  
313 distributed errors (egg size, number of larvae at dispersal, mean larval mass, female mass  
314 change), and generalised linear models for count data with Poisson errors (number of eggs)  
315 or proportional data with binomial errors corrected for overdispersion (hatching success).  
316 Models for egg laying traits (egg size, number of eggs, hatching success) included the  
317 condition of the female’s mating partner as a fixed effect. All subsequent models (number  
318 of dispersing larvae, mean larval mass, female mass change) included the condition of the  
319 female’s mating partner and the condition of the larvae’s sire as fixed effects. Including the  
320 interaction between the condition of a female’s mating partner and the condition of the sire  
321 had no significant effect on any traits, and we therefore excluded this interaction from the

322 final models. The size of the carcass was included as a covariate in all models to control for  
323 potential effects of resource availability.

324 For our bivariate analysis of the trade-off between number and size of larvae, we  
325 included both the number of dispersing larvae and mean larval mass as response variables.  
326 Meanwhile, for the bivariate analysis of the trade-off between current and future  
327 reproduction, we included brood mass in the first breeding attempt and brood mass in the  
328 second breeding attempt as response variables – using these measures as proxies for  
329 allocation to current and future reproduction, respectively. Both analyses included the  
330 condition of the female's mating partner and the condition of the sire as fixed effects.

331

## 332 **Results**

### 333 *Food deprivation treatment*

334 As intended, our food deprivation treatment generated variation among males with respect  
335 to condition. Males that had been food-deprived for seven days lost mass ( $t_{46} = -12.34$ ,  $p$   
336  $<0.001$ ), whilst control males that had not been food-deprived during this time did not ( $t_{44} =$   
337  $-0.92$ ,  $p = 0.35$ ). Food-deprived males lost, on average, 11.35% of their initial mass  
338 compared to a loss of just 1.05% for control males. As a consequence, there was a  
339 significant difference in weight between control and food-deprived males at the time of  
340 mating (estimate  $\pm$  SE =  $-0.033 \pm 0.0088$ ,  $t = -3.79$ ,  $p = 0.00027$ ) with food-deprived males  
341 weighing, on average, 13.67% less than control males.

342

### 343 *Number and size of eggs*

344 There was no difference in the number or size of eggs laid by females that mated with a  
345 food-deprived or a control male (Table 1). However, females mating with a food-deprived



346 male laid eggs with a lower hatching success than females mating with a control male (Table  
347 1), with the former female's eggs being, on average, 2.5% less likely to hatch. The size of the  
348 carcass had no effect on clutch size, egg size or hatching success (Table 1).

349

#### 350 *Number and size of larvae*

351 Our univariate analyses found evidence for positive differential allocation. Females that had  
352 mated with a control male had more dispersing larvae than females that had mated with a  
353 food-deprived male (Table 1; Figure 2A). In contrast, there was no difference in mean larval  
354 mass between females that had mated with a food-deprived or a control male (Table 1;  
355 Figure 2B). We found no evidence for direct effects of male condition on larvae, as there  
356 was no difference in either the number of dispersing larvae or mean larval mass between  
357 broods sired by a food-deprived or a control male (Table 1). Females had both a larger  
358 number of dispersing larvae and heavier larvae when breeding on larger carcasses (Table 1).

359 Our bivariate analysis found evidence that differential allocation influenced  
360 relationships between reproductive traits as the condition of the female's mating partner  
361 had a significant effect on the relationship between the number of dispersing larvae and  
362 mean larval mass (Pillai's trace = 0.10,  $F_{2, 86} = 5.19$ ,  $p = 0.007$ ; Figure 3). This effect occurred  
363 because there was a negative relationship (i.e. a trade-off) between the number of  
364 dispersing larvae and mean larval mass for females mating with a control male (Pearson's  
365 correlation:  $r = -0.54$ ,  $t = -4.29$ ,  $p < 0.001$ ; Figure 3), whilst there was a positive relationship  
366 between the number of dispersing larvae and mean larval mass for females mating with a  
367 food-deprived male (Pearson's correlation:  $r = 0.48$ ,  $t = 3.64$ ,  $p < 0.001$ ; Figure 3). There was  
368 no evidence for direct effects of male condition on this trade-off as the condition of the

369 male that had sired the larvae had no effect on the relationship between the number of  
370 dispersing larvae and mean larval mass (Pillai's trace = 0.022,  $F_{2, 86} = 1.00$ ,  $p = 0.37$ ; Figure 3).

371         Based on prior work, we anticipated a negative relationship (i.e. a trade-off)  
372 between the number of dispersing larvae and mean larval mass. Thus, the positive  
373 relationship when females mated with a food-deprived mating partner (Figure 3) warranted  
374 further investigation. Visual inspection of our data suggested that this positive relationship  
375 was driven by some females that had mated with a food-deprived male having only a very  
376 small number of very small larvae at dispersal (Figure 3). This pattern could potentially arise  
377 due to heterogeneity in the genetic or phenotypic quality of females or their mating  
378 partner, where low-quality females and/or females that mated with particularly low-quality  
379 males are more likely to abandon the breeding attempt. To investigate this possibility, we  
380 performed two post-hoc analyses. Our post-hoc analyses consisted of general linear models  
381 fitted with normally distributed errors that examined if the total mass of the brood of larvae  
382 was influenced by proxies for female and male quality. We used female pre-breeding mass  
383 and the mass of the food-deprived male after food deprivation as proxies for female and  
384 male quality, respectively. This is because body mass is the best indicator we have for  
385 variation in an individual's underlying genetic or phenotypic quality. However, there was no  
386 evidence that the mass of the brood of larvae at dispersal was influenced by either the  
387 female's pre-breeding mass (GLM; estimate  $\pm$  SE =  $-1.29 \pm 3.88$ ,  $t = -0.33$ ,  $p = 0.74$ ) or the  
388 mass of the food-deprived male to which she had been mated (GLM; estimate  $\pm$  SE =  $-1.71 \pm$   
389  $3.17$ ,  $t = -0.54$ ,  $p = 0.59$ ).

390

391 *Allocation to future reproduction*

392 We found no evidence that differential allocation by females in the initial breeding attempt  
393 had consequences for allocation to future reproduction. Firstly, our univariate analyses  
394 found no difference in mass change during the first breeding attempt between females that  
395 had mated with a food-deprived or a control male (Table 2). Secondly, there were no  
396 differences in clutch size, egg size, hatching success, the number of dispersing larvae or  
397 mean larval mass at dispersal in the second breeding attempt between females that initially  
398 mated with a food-deprived or a control male (Table 2). We found no evidence that direct  
399 effects of male condition on larvae in the first breeding attempt had consequences for  
400 future reproduction. There was no difference in female mass change during the first  
401 breeding attempt when females cared for broods of larvae sired by a food-deprived or a  
402 control male (Table 2). Similarly, there were no differences in clutch size, egg size, hatching  
403 success, the number of dispersing larvae or mean larval mass at dispersal in the second  
404 breeding attempt between females that had reared brood of larvae sired by food-deprived  
405 or control males in the initial breeding attempt (Table 2). In the second breeding attempt,  
406 females laid smaller eggs and produced heavier larvae when breeding on a larger carcass  
407 (Table 2). Variation in carcass size had no effect on clutch size, hatching success, the number  
408 of dispersing larvae or female mass change during the second breeding attempt (Table 2).

409 Finally, we found a significant, positive relationship between brood mass in the first  
410 breeding attempt and brood mass in the second breeding attempt (i.e. proxies for allocation  
411 to current and future reproduction, respectively) (Pearson's correlation:  $r = 0.35$ ,  $t = 3.32$ ,  $p$   
412  $= 0.001$ ). Thus, the relationship between current and future reproduction seems to be  
413 driven by variation in resource acquisition or heterogeneity in genetic or phenotypic quality  
414 among females rather than variation in resource allocation. Furthermore, our bivariate  
415 analysis found no evidence that differential allocation in the initial breeding attempt

416 influenced this relationship as there was no effect of the condition of a female's initial  
417 mating partner on the association between brood mass in the first and second breeding  
418 attempts (Pillai's trace = 0.059,  $F_{2, 75} = 2.36$ ,  $p = 0.10$ ; Figure 4). Similarly, the condition of  
419 the male that sired the larvae in the initial breeding attempt had no effect on the  
420 relationship between brood mass in the first and second breeding attempts (Pillai's trace =  
421 0.015,  $F_{2, 75} = 0.57$ ,  $p = 0.56$ ; Figure 4).

422

## 423 **Discussion**

424 We find evidence for differential allocation by the female but no evidence for direct effects  
425 of male condition on offspring in *N. vespilloides*. Females that had mated with a food-  
426 deprived male (i.e. a male in poor condition) had fewer larvae at dispersal than females that  
427 had mated with a control male (i.e. a male in good condition), whilst there was no  
428 difference in either the number or mean mass of larvae sired by food-deprived or control  
429 males. This finding provides clear evidence for differential allocation given that our cross-  
430 fostering experimental design controlled for any direct effects of the condition of the  
431 larvae's sire. We also found evidence that differential allocation influenced the relationship  
432 between traits within a breeding attempt as there was a positive relationship between the  
433 number of larvae and mean larval mass when females mated with a food-deprived male.  
434 Thus, our results show that differential allocation influenced relationships between  
435 reproductive traits, but not necessarily by influencing trade-offs between them.

436 We found evidence for positive differential allocation as females had more larvae at  
437 dispersal when mating with a male in good condition than when mating with a male in poor  
438 condition. Our study was motivated by a recent theoretical model predicting that females  
439 mating with a good quality male should produce a larger number of offspring (Ratikainen et

440 al. 2018). This is because the male's quality influences the offspring's reproductive value and  
441 therefore the optimal per offspring investment. Our findings support this prediction. Thus,  
442 our results suggest that females use the condition of their mating partner as an indicator of  
443 the reproductive value of their larvae, adjusting the number of larvae accordingly. However,  
444 the model also predicts that females mating with a good quality male should produce  
445 smaller offspring as a result of the trade-off between the number and size of offspring. We  
446 found no support for this prediction in our study as there was no difference in mean larval  
447 mass when females mated with a good condition or poor condition male. Furthermore, the  
448 model predicts that a female's total investment in reproduction is higher when mating with  
449 a good quality male. Again, our results did not match this prediction as we found no  
450 evidence that the condition of a female's mating partner influenced the trade-off between  
451 current and future reproduction. A potential explanation for why our results do not fully  
452 match these theoretical predictions is that females received direct benefits such as nuptial  
453 gifts from mating that varied with respect to male condition. However, there is no evidence  
454 that males transfer nuptial gifts or that females receive direct benefits from mating in our  
455 system (House et al. 2008). Thus, although our study provides clear evidence for differential  
456 allocation, more work is required to understand why our results only match some of the  
457 predictions of Ratikainen et al. (2018).

458         The finding that females had fewer larvae at dispersal when mating with a food-  
459 deprived male shows that females adjusted their reproductive allocation after hatching.  
460 Post-hatching filial cannibalism is the most likely mechanism of differential allocation by  
461 females in *N. vespilloides*. It is difficult to observe culling behaviour directly in this species,  
462 but there is good evidence that females adjust brood size after hatching by cannibalising  
463 some larvae when too many eggs hatch (Bartlett 1987; Bartlett & Ashworth 1988; Müller et

464 al. 1990). Food provisioning is a plausible alternative mechanism of differential allocation.  
465 For example, females could potentially reduce food provisioning to larvae when mating with  
466 a food-deprived male. However, it is unlikely that this mechanism can explain our results  
467 given that food provisioning has a stronger effect on larval body mass at dispersal than on  
468 larval survival (Andrews et al. 2016). Thus, had differential occurred through changes in food  
469 provisioning, this would have been detected as a change in mean larval mass rather than in  
470 the number of dispersing larvae. As discussed below, information about the potential  
471 mechanistic basis of differential allocation can be a useful consideration when deciding on  
472 the most appropriate experimental design.

473         There are several potential explanations for why females mating with a food-  
474 deprived male would have fewer larvae. Firstly, females may have reared fewer larvae in  
475 order to allocate more resources to individual larvae and thereby compensate for any direct  
476 effects of poor male condition on larval performance. This explanation seems unlikely given  
477 that, as noted above, we found no evidence of negative direct effects of male condition on  
478 larvae. Furthermore, we would have expected females mating with a food-deprived male to  
479 produce heavier larvae through the trade-off between number and size of larvae. Instead,  
480 we found that the relationship between the number of larvae and larval mass was positive,  
481 rather than negative, for females mating with food-deprived males. However, we cannot  
482 exclude the possibility that reducing the number of larvae benefitted larvae in other ways,  
483 for example by enhancing their immunity, lifespan, or reproductive success as adults.  
484 Secondly, females may reduce the number of larvae after mating with a food-deprived male  
485 to decrease allocation to the current brood and thereby increase allocation to future  
486 reproduction. We found no support for this argument as females mating with food-deprived  
487 males gained a similar amount of weight during breeding (a proxy for investment to future

488 reproduction; Creighton et al. 2009; Billman et al. 2014) and had similar reproductive  
489 success in a second breeding attempt as females mating with control males. Furthermore,  
490 the positive relationship between brood mass in the first and second breeding attempts  
491 suggests that reduced allocation to the current brood did not lead to increased allocation to  
492 future broods. Thus, further work is required to identify any adaptive benefits of differential  
493 allocation in response to mating with a male in poor condition in our system.

494         We found a negative relationship (i.e., a trade-off) between the number and size of  
495 larvae at dispersal when females mated with a control male, but a positive relationship  
496 when females mated with a food-deprived male. This positive relationship is surprising given  
497 that the carcass is a limited resource and represents the sole source of food for developing  
498 larvae. The observation that some females had small numbers of very small larvae when  
499 mating with a food-deprived male (Figure 3) provides a potential explanation for this  
500 observation – as these broods may represent females that abandoned their broods or  
501 greatly reduced their effort in the breeding attempt, after mating with a male in poor  
502 condition. We did not record instances of brood abandonment in our study. However, in our  
503 system, larvae that are abandoned by their parents can survive to dispersal (Eggert et al.  
504 1998; Smiseth et al. 2003; Pilakouta et al. 2015), although they suffer reduced survival and  
505 growth due to the lack of parental food provisioning (Eggert et al. 1998). Thus, brood  
506 abandonment offers a plausible mechanism for the presence of small numbers of very small  
507 larvae that could generate a positive relationship between the number and size of larvae  
508 and obscure the expected trade-off.

509         Regardless of the mechanism, one explanation for why some females produced  
510 small numbers of small larvae is the presence of cryptic heterogeneity in quality amongst  
511 females or their mating partners. Such heterogeneity could arise from many sources,

512 including variation in an individual's genetic quality or in the amount of resources they have  
513 acquired during development (Wilson & Nussey 2010; Bergeron et al. 2012). However, our  
514 post-hoc analyses found no relationship between either male or female quality and brood  
515 mass when using body mass prior to breeding as a proxy for individual quality. Although  
516 body mass prior to breeding is a reasonable proxy for individual quality, we cannot rule out  
517 other cryptic sources of heterogeneity in individual quality as the underlying reason why  
518 some females produced small numbers of small larvae after mating with a poor condition  
519 male.

520         Our cross-fostering approach has several advantages over the traditional approach  
521 used to separate between differential allocation and direct effects of male condition or  
522 quality based on the manipulation of male attractiveness. For example, studies on birds  
523 have manipulated male attractiveness by fitting males with coloured leg rings (e.g., Burley et  
524 al. 1982). The first advantage of our approach is that it requires no assumptions or  
525 knowledge about which male traits females find attractive. Manipulating male  
526 attractiveness has limitations given that (1) females often use multiple cues to assess male  
527 attractiveness (Candolin 2003), (2) females may respond directly to male condition or  
528 quality rather than to male attractiveness (Michl et al. 2005), and (3) manipulating  
529 attractiveness may influence male behaviour or physiology indirectly through social  
530 feedbacks from conspecifics (Rubenstein & Hauber 2008; Royle & Pike 2010). A second  
531 advantage of our approach is that it allowed us to simultaneously test for both differential  
532 allocation and direct effects of male condition on offspring. This is an advantage because  
533 differential allocation and direct effects of male condition are not mutually exclusive (e.g.  
534 Watson & Simmons 2012), and may even have opposing effects on offspring that cancel  
535 each other out. For example, if poor male condition has a negative effect on offspring,



536 differential allocation by females may compensate for any direct effects of male condition,  
537 leading to no overall effect on offspring performance. Such a scenario would only be  
538 detected using experimental designs that allow us to separate between the two processes.

539         It is important to recognise the limitations of our cross-fostering approach. The most  
540 important limitation is that cross-fostering can only separate differential allocation from  
541 direct effects of male condition in traits expressed after hatching. For example, in our study,  
542 we found evidence of reduced hatching success of eggs when females mated with a food-  
543 deprived male. However, our design does not allow us to determine if this was due to  
544 differential allocation by the female (e.g., through adjustments of egg components), or a  
545 direct effect of male condition such as a reduction in the number or quality of sperm  
546 transferred by food-deprived males. Crucially, this limitation did not influence our results on  
547 post-hatching reproductive allocation given that we provided all females with a  
548 standardised brood of newly hatched larvae, thereby eliminating any confounding effects  
549 due to potential prenatal effects on the number of larvae in the brood. Thus, the putative  
550 mechanisms of differential allocation by females will determine which experimental  
551 approach is more appropriate for examining evidence for differential allocation. We suggest  
552 that a cross-fostering approach may be better suited for species in which differential  
553 allocation is likely to occur through mechanisms operating after hatching, such as  
554 provisioning of resources to offspring as in many birds (e.g., Limbourg et al. 2012) or culling  
555 of offspring as in our study species (Bartlett 1987; Bartlett & Ashworth 1988; Müller et al.  
556 1990). In contrast, manipulation of male attractiveness is a better approach for studies  
557 where differential allocation is likely to occur via prenatal mechanisms, such as the number,  
558 size, and/or components of eggs.

559 In conclusion, we found evidence for differential allocation as female *N. vespilloides*  
560 had fewer larvae after mating with a male in poor nutritional condition. Crucially, our cross-  
561 fostering approach allowed us to address a limitation of prior work by experimentally  
562 excluding any direct effects of male condition. Furthermore, whilst prior work has focused  
563 on the trade-off between current and future reproduction, our results show that differential  
564 allocation can also expose complex patterns of reproductive allocation within a breeding  
565 attempt. There was a trade-off between larval size and number when females mated with a  
566 male in good condition, whilst there was a positive relationship between the number and  
567 size of larvae when females mated with a male in poor condition. Thus, our study adds to  
568 our understanding of differential allocation in three main ways. Firstly, we demonstrate  
569 that cross-fostering can provide clear evidence for differential allocation by separating  
570 decisions by the female from direct effects of male condition. Secondly, we show that  
571 differential allocation can be associated with complex patterns of reproductive allocation  
572 within and between breeding attempts. Finally, we suggest that differential allocation in  
573 response to male condition can expose otherwise cryptic heterogeneity among individuals  
574 with respect to genetic and/or phenotypic quality.

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## 576 **Figure legends**

- 577 Figure 1 – Schematic diagram of our cross-fostering experimental design (not drawn to  
578 scale) used to separate differential allocation by the female in response to her mating

579 partner from direct effects of the offspring's sire. This design detects differential allocation  
580 as effects due to the condition of the mating partner and direct effects of male condition as  
581 effects due to the condition of the sire. Filled symbols represent control males in good  
582 condition, females mating with control males in good condition, and larvae sired by control  
583 males in condition. Open symbols represent food-deprived males in poor condition, females  
584 mating with food deprived males in poor condition and larvae sired by food-deprived males  
585 in poor condition.

586

587 Figure 2 – Effects of differential allocation by females (evident as effects due to the  
588 condition of a female's mating partner) and direct effects of male condition (evident as  
589 effects due to the condition of the larvae's sire) on the number of larvae (A) and mean larval  
590 mass (B). Filled symbols represent means  $\pm$  SE for larvae sired by a control male in good  
591 condition while open symbols represent means  $\pm$  SE for larvae sired by a food-deprived  
592 male in poor condition. Grey circles represent data on individual broods with the size of the  
593 circle representing the frequency of observations.

594

595 Figure 3 – Effects of differential allocation by females (evident as effects due to the  
596 condition of a female's mating partner) and direct effects of male condition (evident as  
597 effects due to the condition of the larvae's sire) on the trade-off between the number of  
598 larvae and mean larval mass (g). Filled symbols and solid lines represent larvae sired by a  
599 control male in good condition while open symbols and dashed lines represent larvae sired  
600 by a food-deprived male in poor condition. The grey shaded area around the line indicates  
601 the 95% confidence intervals.

602



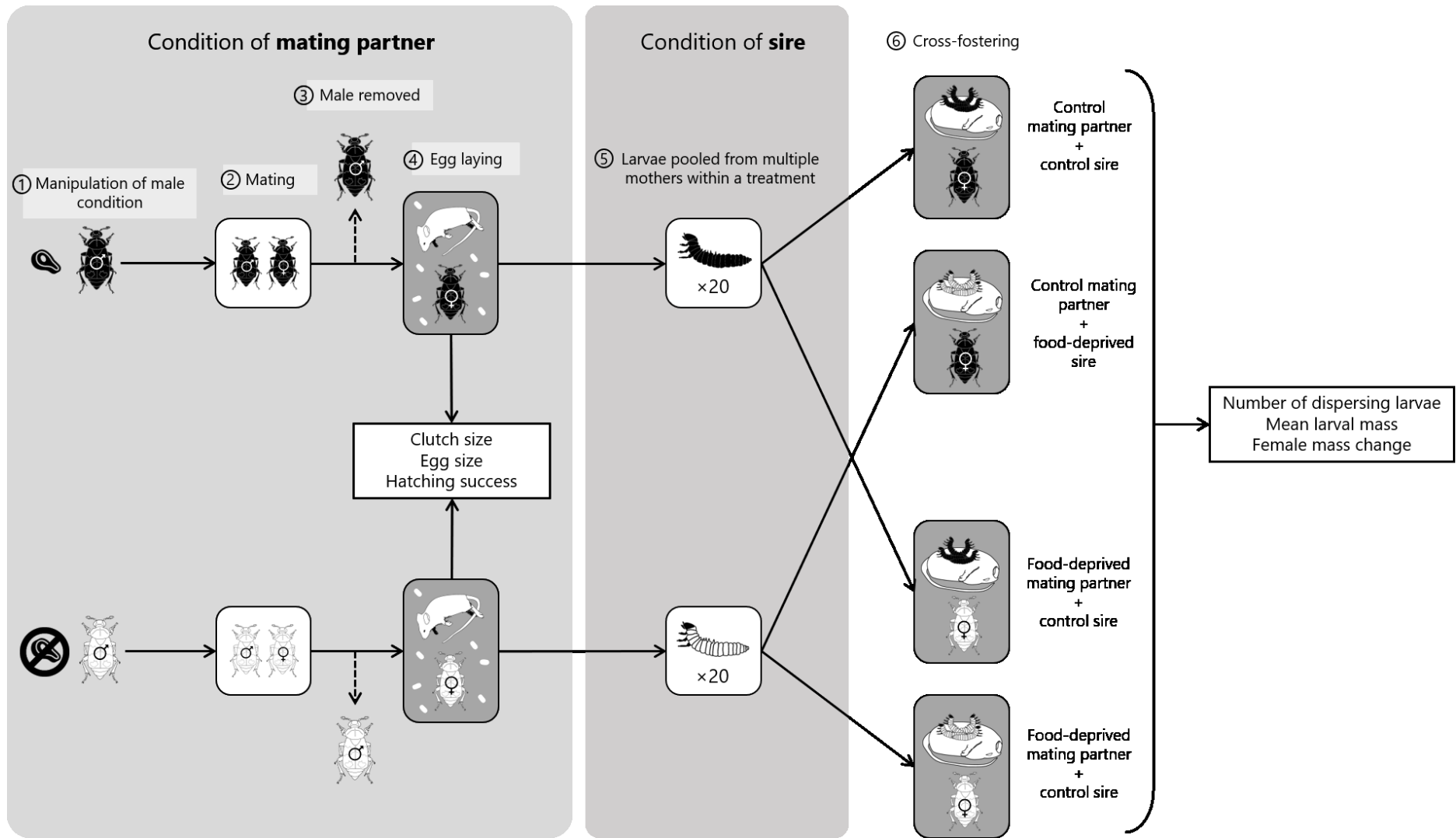
603 Figure 4 – Effects of differential allocation by females (evident as effects due to the  
604 condition of a female’s mating partner) and direct effects of male condition (evident as  
605 effects due to the condition of the larvae’s sire) on the trade-off between brood mass in the  
606 first breeding attempt (g) and brood mass in the second breeding attempt (g). Filled symbols  
607 and solid lines represent larvae sired by a control male in good condition, while open  
608 symbols and dashed lines represent larvae sired by a food-deprived male in poor condition.  
609 The grey shaded area around the line indicates the 95% confidence intervals.

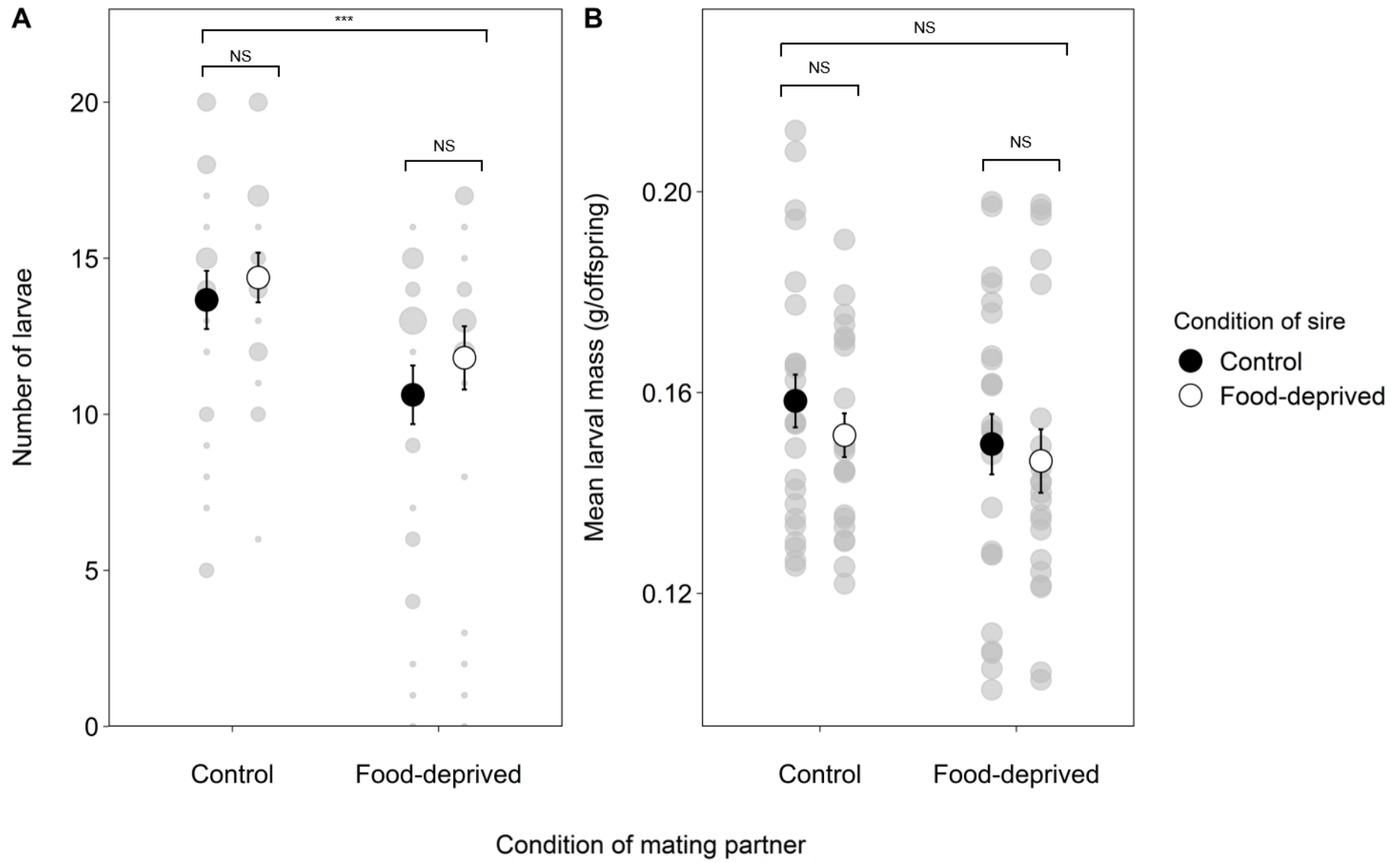
Table 1 – Evidence for differential allocation by the female (evident as effects due to the condition of the female’s mating partner) and direct effects of male condition (evident as effects due to the condition of the larvae’s sire) on reproductive traits. After measuring pre-hatching traits, we provided all females with a standardised number of larvae. We used control males in good condition as the reference level for comparison to food-deprived males in poor condition. We provide parameter estimates ( $\pm$  SE), test statistics (z or t) and p-values from univariate analyses. Significant p-values are indicated in bold.

Initial breeding attempt		Differential allocation (condition of female’s mating partner)			Direct effect of male condition (condition of larvae’s sire)			Carcass size		
		Trait	Estimate ( $\pm$ SE)	Test statistic	p-value	Estimate ( $\pm$ SE)	Test statistic	p-value	Estimate ( $\pm$ SE)	Test statistic
Pre-hatching	Clutch size	-0.098 $\pm$ 0.052	z = -1.86	0.062	-	-	-	-0.031 $\pm$ 0.033	z = -0.94	0.34
	Egg size (mm <sup>3</sup> )	-0.13 $\pm$ 0.14	t = -0.96	0.33	-	-	-	-0.15 $\pm$ 0.090	t = -1.73	0.087
	Hatching success (%)	-1.06 $\pm$ 0.48	t = -2.18	<b>0.031</b>	-	-	-	-0.12 $\pm$ 0.28	t = -0.44	0.65
Post-hatching	Number of dispersing larvae	-3.07 $\pm$ 0.87	t = -3.49	<b>&lt;0.001</b>	0.55 $\pm$ 0.88	t = 0.63	0.53	2.46 $\pm$ 0.56	t = 4.37	<b>&lt;0.001</b>
	Mean larval mass (g)	-0.0062 $\pm$ 0.0057	t = -1.09	0.27	0.00053 $\pm$ 0.0057	t = 0.091	0.92	0.011 $\pm$ 0.0036	t = 2.96	<b>0.0040</b>
	Female mass change (g)	0.0082 $\pm$ 0.0058	t = 1.40	0.16	0.0021 $\pm$ 0.0059	t = 0.36	0.72	-0.0042 $\pm$ 0.0038	t = -1.11	0.27

Table 2 – Evidence for differential allocation by the female (evident as effects due to the condition of the female’s first mating partner) and direct effects of male condition (evident as effects due to the condition of the larvae’s sire) on reproductive traits in a second breeding attempt. We used control males in good condition as the reference level for comparison to food-deprived males in poor condition. We provide parameter estimates ( $\pm$  SE), test statistics (z or t) and p-values from univariate analyses. Significant p-values are indicated in bold.

Second breeding attempt Trait	Differential allocation (condition of female’s first mating partner)			Direct effect of male condition (condition of sire in first breeding attempt)			Carcass size		
	Estimate ( $\pm$ SE)	Test statistic	p-value	Estimate ( $\pm$ SE)	Test statistic	p-value	Estimate ( $\pm$ SE)	Test statistic	p-value
Clutch size	0.024 $\pm$ 0.061	z = 0.39	0.69	0.10 $\pm$ 0.061	z = 1.70	0.088	0.011 $\pm$ 0.040	z = 0.28	0.77
Egg size (mm <sup>3</sup> )	0.014 $\pm$ 0.12	t = 0.12	0.91	0.039 $\pm$ 0.12	t = 0.32	0.74	0.16 $\pm$ 0.080	t = 2.03	<b>0.041</b>
Hatching success (%)	-0.42 $\pm$ 0.46	t = -0.92	0.36	-0.37 $\pm$ 0.45	t = -0.82	0.41	-0.25 $\pm$ 0.33	t = -0.77	0.41
Number of dispersing larvae	-0.81 $\pm$ 1.63	t = -0.49	0.62	1.83 $\pm$ 1.62	t = 1.13	0.26	0.81 $\pm$ 1.05	t = 0.77	0.44
Mean larval mass (g)	0.0048 $\pm$ 0.0095	t = 0.58	0.61	-0.015 $\pm$ 0.0095	t = -1.60	0.11	0.020 $\pm$ 0.0062	t = 3.19	<b>0.0023</b>
Female mass change (g)	-0.0027 $\pm$ 0.0062	t = -0.44	0.66	0.0020 $\pm$ 0.0062	t = 0.032	0.97	-0.0053 $\pm$ 0.0039	t = -1.37	0.17





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