

1 **Male burying beetles extend, not reduce, parental care duration when**
2 **reproductive competition is high.**

3

4 Running title: Parental care as paternity protection.

5

6 Paul E. Hopwood ¹, Allen J. Moore ^{1,2}, Tom Tregenza ¹ and Nick J. Royle ^{1*}.

7

8 1: Centre for Ecology and Conservation, Biosciences, College of Life &

9 Environmental Sciences, University of Exeter, Cornwall Campus, Penryn,

10 TR10 9EZ, UK.

11 2: Department of Genetics, University of Georgia, Athens, Georgia, 30602,

12 USA

13

14 *author for correspondence: N.J.Royle@exeter.ac.uk

15

16

17 **Summary**

18 Male parents spend less time caring than females in many species with
19 biparental care. The traditional explanation for this pattern is that males have
20 lower confidence of parentage, so they desert earlier in favor of pursuing
21 other mating opportunities. However, one recent alternative hypothesis is that
22 prolonged male parental care might also evolve if staying to care actively
23 improves paternity. If this is the case, an increase in reproductive competition
24 should be associated with increased paternal care. To test this prediction we
25 manipulated the level of reproductive competition experienced by burying
26 beetles, *Nicrophorus vespilloides* (Herbst, 1783). We found that caregiving
27 males stayed for longer and mated more frequently with their partner when
28 reproductive competition was greater. Reproductive productivity did not
29 increase when males extended care. Our findings provide support for the
30 increased paternity hypothesis. Extended duration of parental care may be a
31 male tactic both protecting investment (in the current brood) and maximising
32 paternity (in subsequent brood(s) via female stored sperm) even if this fails to
33 maximise current reproductive productivity and creates conflict of interest with
34 their mate via costs associated with increased mating frequency.

35

36 **Keywords:** Parental care; sexual conflict; paternity assurance; paternal care;
37 phenotypic plasticity.

38 **Introduction**

39 In most species with biparental care females spend more time caring than
40 males (Kokko & Jennions 2012). The reason why male parents desert before
41 females and why they vary in the length of time they stay with their caring
42 partner is often unclear (Kokko & Jennions 2012). Regardless of intersexual
43 differences in prenatal reproductive costs (such as egg versus sperm
44 production) offspring fitness benefits associated with increased postnatal
45 parental effort should be shared by both parents, all else being equal.
46 Conflicts of interest between parents occur because parental care is costly: it
47 takes time that could be used in trying to find more mating opportunities, and
48 energy invested in current offspring may trade-off with future reproductive
49 productivity (Clutton-Brock & Vincent 1991; Royle *et al.* 2012). As a result of
50 these costs, each individual parent stands to gain in the future if they can
51 minimize their own current effort at the expense of their partner (Trivers 1972;
52 Parker *et al.* 2002; Royle *et al.* 2002; Harrison *et al.* 2009).

53

54 This paradigm has been used to explain variation in the level of male care:
55 maternity is typically more assured than paternity in species with biparental
56 care (Alonzo & Klug 2012). Consequently selection is expected to disfavor
57 males that care for offspring unrelated to themselves (Whittingham *et al.*
58 1992; Houston 1995; Kokko & Jennions 2008; Alonzo & Klug 2012) and/or
59 favor paternity protection behaviors such as mate guarding or high mating
60 frequency that counter the threat from sperm competition (Birkhead 1979,
61 1982). A general, positive relationship between paternity assurance and
62 paternal effort has been found across species (Griffin *et al.* 2013) and, within

63 species, cues indicating declining paternity assurance may select for
64 facultative adjustment in paternal care (Sheldon 2002; Kokko & Jennions.
65 2008). Empirical evidence exists that shows males decreasing parental effort
66 when they obtain fewer matings (e.g., Burke *et al.* 1989; Hartley *et al.* 1995)
67 or when risks of cuckoldry increase (e.g., Hunt & Simmons 2002; Benowitz *et*
68 *al.* 2013) but overall support for the relationship between paternity assurance
69 and paternal effort is mixed (Alonzo, 2010).

70

71 An underlying assumption is that male care is driven by improved parental
72 productivity and/or indirect benefits through increased fitness of offspring but
73 an alternative hypothesis is that males stay if this increases the proportion of
74 their paternity in current and/or future broods, rather than providing care *per*
75 *se* (Kvarnemo 2006; Kahn *et al.* 2013). This 'increased paternity' hypothesis
76 casts male parental care in a different light: male care need not be beneficial
77 to offspring and it may create additional conflicts of interest between mates.
78 Under this scenario, male care in the current brood should be extended when
79 reproductive competition increases, as this will increase his paternity in the
80 current brood and/or in future broods (Kvarnemo 2006; Kahn *et al.* 2013). In
81 contrast, where selection disfavors males that provide care for offspring less
82 likely to be their own, an increase in reproductive competition is expected to
83 lead to a decrease in parental effort in the current brood.

84

85 Some empirical observations appear to support the increased paternity
86 hypothesis (e.g., in some fish, females prefer to spawn in the nests of males
87 already caring for eggs (Ridley & Rechten 1981; Forsgren *et al.* 1996)) but to

88 our knowledge the key prediction of the hypothesis, outlined above, has not
89 been explicitly tested. Here we provide a test using *Nicrophorus vespilloides*
90 burying beetles as a model system. Male and female burying beetles provide
91 complex prenatal and postnatal parental care, either alone or together and, for
92 uniparental care, male and female parental behavior has been shown to
93 increase offspring fitness (Scott 1989; Eggert *et al.* 1998; Smiseth *et al.* 2003;
94 Smiseth *et al.* 2007). A small vertebrate carcass is the necessary resource for
95 rearing a single brood of offspring (Pukowski 1933), and burying beetles often
96 engage in direct intrasexual contests for these scarce breeding resources.
97 Beetles that lose a contest to a dominant individual often remain and adopt a
98 satellite (male) or brood parasitic (female) role but the presence of these
99 subordinate individuals introduces uncertainty over the genetic parentage of a
100 brood for either or both parents. The extent of this uncertainty depends on the
101 sex ratio (and number) of these subordinate competitors, which varies among
102 reproductive events (Müller *et al.* 1990; Müller *et al.* 2007). Eggs of a brood
103 parasitic female (or females) reduce the dominant female's parentage but also
104 reduce the dominant male's proportion of paternity (because the carcass can
105 only support a finite brood) unless he mates with them and sires the resulting
106 offspring. In contrast, satellite males represent a threat to the paternity of the
107 dominant male by sneaking matings with the dominant female (Scott 1998;
108 Müller *et al.* 2007). These imbalances are reflected in the exclusively
109 intrasexual fights that establish dominance at a carcass (Otronen 1988; Lee *et*
110 *al.* 2013).

111

112 Studies on burying beetles have acknowledged the importance of intrasexual
113 competition in determining parentage during a breeding bout (e.g., Müller &
114 Eggert 1989; Müller *et al.* 2007) but have not tested the effect that variation in
115 this competitive social environment has on parental behavior and reproductive
116 output together. We provide such a test here in controlled laboratory
117 conditions designed to allow natural expression of beetle parental and social
118 behavior. We manipulated the sex ratio of reproductive competitors in the
119 burying beetle *N. vespilloides* to test the critical prediction of the increased
120 paternity hypothesis: that male care should be extended when this is likely to
121 increase paternity (Kvarnemo 2006). If male decisions to stay are based on
122 returns via increased paternal care provision, males should stay longer when
123 in a monogamous pair than when there is competition and greater duration of
124 care should be positively correlated with parental productivity in terms of
125 reproductive output. In contrast if, as predicted by Kvarnemo (2006), male
126 care decisions are based on increasing paternity of current and/or future
127 broods (e.g., securing a greater proportion of parentage via mate guarding
128 and/or repeated mating), the opposite pattern should be seen: males will stay
129 longer when there is intrasexual competition at the carcass with no, or
130 negative, effects on reproductive output.

131

132 **Methods**

133 Over 200 wild beetles were caught in funnel-type bottle-traps baited with small
134 pieces of putrescent salmon and hung in trees in a Cornish woodland, UK
135 (SW 772 376) during the autumn of 2011. Laboratory experimental stock was
136 generated from the outbred F4 offspring of these wild beetles. Beetles were

137 housed individually in clear plastic boxes, maintained at a temperature of 16 –
138 20°C with an 16:8hr light:dark cycle and fed decapitated mealworms *ad*
139 *libitum* from eclosion until introduced to their experimental environments, as
140 described by Head *et al.* (2012).

141

142 Laboratory stock beetles ($n = 246$) were weighed, measured and randomly
143 assigned to one of four treatments. Each beetle within each treatment
144 replicate was marked to enable identification of individuals. Marking was
145 achieved by lightly scratching a small area (~1mm Ø) on the dorsal surface of
146 elytra with a hobby-drill fine sanding-bit, in one of the four distinctive orange
147 patches of all beetles (i.e., either rear right; rear left; front right or front left),
148 and then applying a dot of black Indian ink. The orange quarter marked was
149 randomized for each of the four (or two) individuals within each treatment
150 group and replicate.

151

152 The whole duration of each breeding bout was filmed in the laboratory (from
153 the introduction of beetles to a carcass until larvae dispersed) using arenas
154 designed for this purpose (Hopwood *et al.* 2013). Each arena consisted of a
155 400mm length of black PVC-U 110mm Ø pipe placed upright in a 30L
156 plasterer's bucket containing approximately 25mm of moist compost. Three
157 40mm Ø exit holes were drilled in the inner pipe 5mm above the compost
158 surface level (these inner pipes we refer to as 'Nicrocosms' (Hopwood *et al.*
159 2013) while 'arena' refers to Nicrocosm and bucket combined). This design
160 allows beetles to escape from other beetles into the outer arena and also
161 permits caregiving beetles to desert broods at will. A closed-circuit, infra-red

162 surveillance camera (N08CX night vision CCTV camera) was positioned
163 inside the lumen of each Nicrocosm using motion detecting software
164 (AVerMedia NV6240 Express, DVR version 7.7.0.0007; www.avermedia-
165 dvrs.com) to capture beetle activity around a mouse carcass that was
166 positioned inside (see supplementary information in Hopwood *et al.* 2013 for
167 technical details). Experimental female beetles were mated with non-
168 experimental stock males 24 hours before being used, as almost all (93%)
169 sexually mature females caught in the wild have been found, in a previous
170 study, to be pre-mated (Müller & Eggert 1989). This is important because it
171 means both that wild females can breed without a male being present but also
172 that even as part of a 'monogamous' pair wild males face a threat to paternity
173 through stored sperm. Experimental beetles were assigned at random to
174 treatment groups when they were between 14 and 21 days old (post-eclosion)
175 but individuals were kept separate from one another until introduced to the
176 arena. Mouse size was standardised (mean \pm SD = 21.16 \pm 0.60g) across
177 treatments to enable comparisons of reproductive output and experimental
178 beetles' pronotal width was used as a proxy for body size.

179

180 Treatment groups were:

181 *Female bias (mfff)*: Three females and one male ($n = 17$) had access to
182 a single mouse carcass. The virgin male was placed in the arena with a
183 mouse carcass, and three randomly chosen pre-mated females, during the
184 activity period in the afternoon (when wild beetles fly in search of carcasses).

185 *Male bias (mmmf)*: One pre-mated female was introduced to three
186 virgin males and a mouse carcass during the afternoon activity period ($n =$
187 18).

188 *No sex-bias (mmff)*: Two pre-mated females and two virgin males were
189 placed in an arena containing a mouse carcass ($n = 17$).

190 *No current extra-pair competition (mf)*: A virgin male and a single pre-
191 mated female were placed in an arena with a mouse carcass ($n = 19$).

192

193 Beetle activity on and around the carcass (i.e., inside the Nicrocosm) was
194 recorded on video from the time experimental beetles were introduced to
195 carcasses until larval dispersal. Postnatal care duration was measured as the
196 proportion of time that a beetle remained with the brood between the first
197 larvae seen and larval dispersal from the carcass (individual parental
198 desertion defined as an unbroken six hour absence from the carcass with no
199 parental behavior witnessed). Number of matings was used as a measure of
200 paternity assurance behavior but in contrast to previous studies (e.g., House
201 *et al.* 2008; Head *et al.* 2014) matings in this experiment were recorded on
202 video in situ (i.e., the beetles were not removed from the carcass) for the
203 whole duration of reproductive bouts. Time taken to bury the carcass was
204 used as an indication of prenatal parental performance. This was measured
205 from the first contact with the carcass (defined as physical contact with
206 carcass combined with exploratory behavior, i.e., stopping and waving
207 antennae or burrowing under carcass) by the caregiving female individual, to
208 the time that only the distal half of the mouse tail remained above ground.
209 Postnatal parental performance was measured in terms of brood size and

210 larval mass at larval dispersal. Dominant (caregiving) beetles were designated
211 as the male and female that repelled initial same-sex incursions on to the
212 carcass; that subsequently engaged in prenatal preparation of the carcass;
213 that predominantly remained inside the Nicrocosm; and that became the
214 postnatal parental care providers (except in the case of two dominant males
215 which deserted prior to larval hatching but fulfilled the other criteria). In
216 treatments with no same-sex competition the uncontested caregiving beetle is
217 designated a dominant.

218

219 *Analysis*

220 Analyses were performed using 'R' version 2.14.1. Duration of paternal care
221 (the proportion of total postnatal larval developmental time spent performing
222 parental duties) was analysed using a generalised linear model with a quasi-
223 binomial error structure (to account conservatively with over-dispersion) with
224 social treatment (four categories) as the independent variable. Male versus
225 female desertion (counts of whether the male or female was the first to desert
226 among treatments) was analysed with a Fisher exact test. The effect of
227 treatment on brood size (the number of offspring that dispersed from the
228 carcass) was analysed using ANCOVA including larval mass as a covariate to
229 control for the correlation between offspring number and size. Mating
230 frequency was natural log transformed to normalise its distribution and
231 analysed using linear regression with treatment as the single independent
232 variable. The relationship between mating frequency and duration of burial
233 (natural log transformed) was analysed using a linear model. Out of 71 total
234 breeding bout replicates two failed to produce larvae and missing data-points

235 across treatments left a sample size of 65 for postnatal care analysis and
236 slight variation in total sample size across analyses. In all multivariate
237 analyses a minimal adequate model was determined through stepwise model
238 simplification starting with a full model including all interactions and
239 sequentially removing non-significant terms from the model starting with
240 highest order interactions (Crawley 2007). Post-hoc multiple comparisons
241 were obtained using Tukey's honest significant differences where necessary.

242

243 **Results:**

244 Dominant male parents adjusted their desertion decisions according to the
245 social competitive environment experienced at a carcass: they deserted
246 significantly earlier from monogamous pairs compared to carcasses where
247 there was intrasexual competition (GLM with quasi-binomial error structure:
248 treatment, $F_{3,61} = 5.863$, $p = 0.001$, Fig. 1a). The sex of the dominant carer
249 that deserted first was not influenced by the social competitive environment:
250 only three males cared for longer than their female partner (i.e., <5% of pairs)
251 and every other dominant female remained with their brood until the larvae
252 dispersed regardless of the nature or sex ratio of competition (Fisher exact
253 test: $n = 69$ pairs, $p = 0.319$).

254

255 Figure 1, a & b here.

256

257 *Social environment effects on reproductive productivity*

258 Variation in the social competitive environment affected offspring number, with
259 fewer larvae dispersing from carcasses in the treatment with most male

260 competitors (mmmf) (Fig. 1b). We analysed this with treatment as factor and
261 larval size as covariate, thereby controlling for a strong trade-off between
262 offspring number and size on a limited resource (ANCOVA, treatment, $F_{3,62} =$
263 3.309, $p = 0.026$; larval size, $F_{1,62} = 79.4$, $p < 0.0001$). The interaction term
264 between treatment and larval size was not significant ($F_{3,59} = 1.551$, $p =$
265 0.211) and hence removed from the model.

266

267 *Social environment effects on mating frequency*

268 The social environment treatment affected the number of times that the focal
269 male mated with the focal female: dominant males with male competitors
270 present (i.e., the male bias (mmmf) and no-sex bias (mmff) treatments) mated
271 more frequently with the dominant female than did males in monogamous
272 pairs (LM, treatment, $F_{3,62} = 4.667$, $p = 0.005$, Fig. 2a). The corollary of this
273 was that dominant females in treatments with male-male competition
274 experienced approximately twice the overall mating frequency (i.e., including
275 matings with subordinate males) than did those in pairs without additional
276 competitors (mf = 20.12 ± 6.08 ; mfff = 26.94 ± 5.62 ; mmff = 43.69 ± 9.70 ;
277 mmmf = 42.65 ± 4.72 times per hour, mean \pm SD; LM, treatment, $F_{3,65} =$
278 6.114, $p < 0.001$, see also Fig. 2a). In the two treatments with male
279 competitors mating frequency of the dominant male was significantly greater
280 than that of subordinates (Wilcoxon signed rank test, dominant status, $V =$
281 525, $n = 37$, $p < 0.0001$, Fig. 2a).

282

283 *Effects of mating frequency on carcass burial speed:*

284 Increased pre-burial mating frequency prolonged carcass burial (linear
285 regression: $\log(\text{carcass burial})$, $F_{1,65} = 31.302$, $p < 0.0001$, $R^2 = 0.31$, Fig. 2b).

286

287 Figure 2, a & b here.

288

289 **Discussion**

290 We found males that faced competition not just from males, but also from
291 females, extended the duration of time they remained with their partners and
292 the brood compared to males in monogamous pairs (Fig. 1a). Males in
293 treatments with satellite male competition mated more frequently with the
294 dominant female than did those in treatments with no satellite males
295 present (Fig. 2a). By staying longer and increasing his mating frequency a
296 caregiving male maximizes his current paternity against the success of
297 subordinate satellite males (Müller & Eggert 1989; House *et al.* 2008). He also
298 has the opportunity, by mating with subdominant (brood parasitic) females, to
299 improve his current paternity proportion and potentially secure paternity in
300 their future broods. Kvarnemo's (2006) model explicitly suggests that male
301 care may evolve when there is a net benefit resulting from the combined sum
302 of three factors: a) gained or lost opportunities for mating, b) increased
303 paternity, and c) improved offspring fitness. We found that potential benefits
304 for males via resource guarding and paternity protection may come at a cost
305 for females through higher mating frequency (see Head *et al.* 2014). We
306 discuss the likely role of these factors in the evolution of male care below:

307

308 *a) Opportunities for mating*

309 In many species, opportunities for mating are reduced for caregiving males
310 but this is not always the case (e.g., in nest-building fish species where males
311 caring for eggs are preferred by spawning females (Ridley & Rechten 1981;
312 Forsgren *et al.* 1996; Alonzo 2008)). In burying beetles reproductive success
313 has been viewed as resource-limited rather than mate-limited (Scott 1998).
314 This is because although males can call (release pheromones) for females
315 and mate without finding a carcass matings are only translated into
316 reproductive success when a female subsequently finds a carcass (Müller *et*
317 *al.* 2007). However, when competition occurs at a carcass reproductive
318 success can be mate-limited to an extent because subordinates do not have
319 the same opportunities to mate. When a pre-mated female locates a carcass
320 she may breed alone but a male finding a carcass must first call a female
321 (Eggert & Müller 1989). This is important because it means the dominant male
322 at a carcass has a potential route to future reproductive success even while
323 engaged in caregiving. He can replenish the sperm stored by his female
324 partner and any subordinate females that visit. Therefore, by prolonging
325 paternal care the apparent cost of his lost mating opportunities can be
326 ameliorated by the likelihood that another carcass is located by the current
327 (proven) female breeding partner(s).

328

329 *b) Increased paternity*

330 Kvarnemo (2006) points out that the benefits from increased paternity may be
331 divided into current and future benefits. Males of some nest-building goby
332 species line the inside of their nest (where females lay eggs) with sperm-
333 infused mucus helping to improve the current paternity of the caring male

334 against rival sneaker males' sperm (Scaggiante *et al.* 2005; Svensson &
335 Kvarnemo 2005, 2007). A study of savannah sparrows, *Passerculus*
336 *sandwichensis*, provides an example of a future paternity benefit of current
337 paternal caregiving: male parents that were attentive to a first brood were
338 rewarded by their mates with higher paternity (through higher female fidelity)
339 in their second broods (Freeman-Gallant 1996). Male burying beetles can
340 maximize their current paternity (to above 90%) when it is challenged by male
341 rivals who are present (satellites) or absent (stored sperm in pre-mated
342 females) by repeatedly mating with females (Müller & Eggert 1989). Sperm
343 precedence in *N. vespilloides* is an increasing function of repeated mating but
344 a male may have to mate seven times even to gain 50% paternity when a
345 female has been pre-mated (Müller & Eggert 1989; House *et al.* 2008). Thus,
346 dominant *N. vespilloides* males sharing a carcass with a female in the wild
347 can reduce the proportion of larvae sired by other males that employ
348 alternative male tactics (i.e., males having called and mated with females
349 without first finding a carcass or satellite males; Müller *et al.* 2007). When a
350 male encounters more than one female at a carcass (e.g., our 'mfff' and
351 'mmff' treatments) subordinate females lay eggs near the carcass and unless
352 mated by him they will use stored sperm from rival and/or absent male(s). In
353 this situation the caregiving male, by staying and mating with all females
354 present has an opportunity to improve his current and future reproductive
355 success. This is because any females with whom he has mated—especially
356 those that find a carcass and breed alone in the future—have the potential to
357 produce future broods using his sperm.

358

359 Female stored sperm has a limited lifespan, starting to become unviable after
360 three weeks (Eggert 1992) so males that delay their desertion from the
361 current breeding bout may increase their future success by ensuring the
362 departing dominant female has freshly replenished sperm stores. In this study
363 males continued to mate beyond the time when eggs were laid, and even
364 larvae hatched, and those facing threats to their paternity extended their care
365 period. Males across all treatments were observed mating after larvae
366 hatched (mf = 1 ± 0.41 ; mfff = 3.88 ± 1.83 ; mmff = 4.75 ± 1.82 ; mmmf = $3.47 \pm$
367 1.23 matings, mean \pm SD). These matings could have little or no benefit to
368 either sex with respect to the current brood but this prolonged residence with
369 repeated mating could influence a male's future paternity when females
370 inseminated by him (during the current breeding bout) find new breeding
371 opportunities. This may be an important component of his future reproductive
372 success because the incidence of females breeding without males can be
373 high in nature. A study in which 300 mouse carcasses were placed in the wild
374 found 39% (100 of 258) that were buried by beetles had only *N. vespilloides*
375 females present (Eggert 1992).

376

377 In our study, dominant males mated with sufficient frequency (i.e., more than
378 seven times) to achieve a high proportion of paternity in treatments with a
379 single (potentially pre-mated) dominant female. The frequency with which the
380 dominant male and female mated increased in the presence of satellite males,
381 as would be predicted if males are defending their paternity (Fig. 2a). The
382 mean prenatal mating frequency we recorded between dominant individuals,
383 from their first contact with each other to burial of the carcass was 15.1 ± 12.7

384 matings per hour (mean \pm SD) (over a mean period of 25 ± 18 hours (mean \pm
385 SD)) The dominant male invariably copulated with the dominant female
386 immediately after a satellite male was encountered, regardless of whether or
387 not a successful satellite mating had occurred (PEH pers. obs). As a result
388 dominant females were mated almost twice as frequently (in total) in groups
389 with multiple males compared to groups with a single male. A recent study
390 showed that female *N. vespilloides* suffer costs of repeated mating that affect
391 the provision of maternal care, leading to reduced offspring performance
392 (Head *et al.* 2014). Thus, optimal mating rate for females—for whom there
393 appears to be no fitness advantage beyond two matings (House *et al.* 2008,
394 2009)—conflicts with that of males who may secure an important component
395 of future paternity by mating frequently during the current reproductive bout,
396 especially if his female partner subsequently breeds alone, using stored
397 sperm (Eggert 1992). Another cost associated with increased mating
398 frequency is the overall time taken for carcass burial (Fig. 2b). It is not clear
399 whether this occurred because males were distracted from helping with
400 carcass burial or because dominant females suffered interference from
401 resistance to the intrusions of dominant males eager to upregulate their
402 mating frequency. Nevertheless, a potential cost of increased mating rate,
403 driven by males in response to threats to paternity from other males, is to
404 increase the risk of usurpation by a larger same-sex conspecific competitor or
405 total loss of the breeding resource to other competitors or scavengers through
406 increasing the time to successfully bury the carcass (Scott 1990; Müller *et al.*
407 1998; Trumbo 2007).

408

409 Brood size was smallest in the treatment with the most male competition
410 despite these dominant males' prolonged postnatal brood attendance
411 compared to males without threats to their paternity assurance. Cues
412 indicating a low proportion of brood parentage for a caregiving female (i.e.,
413 the female-biased treatment) might have been predicted to induce early
414 female desertion, leaving the male to care uniparentally. For example, in
415 penduline tits, *Remiz pendulinus*, either the male or the female is impelled to
416 provide uniparental care for a brood by the early desertion of their partner
417 (van Dijk *et al.* 2012). However, we found no evidence that variation in
418 parentage assurance between sexes determined which sex deserted first; in
419 only three cases (out of 69) males deserted before females.

420

421 One alternative explanation for the pattern of male residence with the brood is
422 resource competition in the face of high competitor density. In our design the
423 caregiving female has already established and maintained dominance status
424 against female competitors present (usually by virtue of a size advantage).
425 We think that if resource guarding against risk of brood takeover was the
426 primary factor explaining variation in male duration of care, males in both
427 treatments without male competitors (i.e., mf and mfff) should be expected to
428 desert early because as long as the lone male has mated sufficiently with any
429 subordinate females present the current brood will be comprised of his
430 offspring whether or not the dominant female is subsequently usurped by
431 these female rivals. In field studies of a wild population of *N. vespilloides* we
432 have recorded a very low incidence of intruding beetles arriving after carcass-
433 burial. In approximately 220 witnessed *N. vespilloides* reproductive events to-

434 date, only three were recorded with an intruding beetle arriving after carcass
435 burial was complete (PEH unpublished data). Nonetheless, studies of other
436 species support the idea that increased likelihood of current brood success
437 via resource guarding may select for extended male attendance in biparental
438 care at least where intruders sometimes arrive in later stages of reproductive
439 bouts (e.g., *N. orbicollis*: (Scott 1990; Trumbo 1991); *N. defodiens*: (Eggert &
440 Sakaluk 2000); *N. pustulatus*: (Trumbo 2007)).

441

442 *c) Offspring benefits*

443 Benefits to fathers through increased paternity need not be exclusive of
444 offspring benefits. One puzzle is that although biparental care is the most
445 common parental association in burying beetles (and both partners share all
446 post-natal parental duties) no clear benefit to offspring has been found for
447 biparental care over uniparental care from either sex (Müller *et al.* 1998;
448 Smiseth *et al.* 2005). An additional benefit to offspring via a male's
449 contribution to parental care is not a prerequisite of Kvarnemo's (2006)
450 hypothesis, which proposes that male care can evolve as long as there is a
451 net benefit to males via increased paternity and or mating opportunities. We
452 found no relationship between male duration of care and brood size but
453 rather, there appeared to be an inverse relationship between variation in
454 brood size and the level of intra-sexual competition males experienced (Fig.
455 1b). Artificial selection for increased repeated mating rate has also been
456 shown to affect the quality of female parental care provision leading to
457 reduced offspring performance (Head *et al.* 2014). Here we show that the
458 mating frequency experienced by caregiving females has a social

459 environmental component: mating increased in response to threats to male
460 paternity assurance.

461

462 **Conclusions**

463 Male *N. vespilloides* parents responded to experimentally manipulated
464 variation in the local competitive social environment: When cues indicated
465 reduced paternity assurance, caregiving males increased the duration of time
466 they stayed with their partner during the period of parental care and they
467 responded to threats to paternity by increasing mating frequency. Threats to
468 paternity come from direct competition from additional males and from sperm
469 competition with absent males with whom the female had previously mated,
470 but also from brood parasitic females who have the potential to lay eggs
471 fertilized with the stored sperm of absent males. Males in monogamous
472 partnerships (i.e., with no extra-pair competition) exhibited the shortest
473 duration of care, despite having the greatest confidence in paternity.
474 Collectively, results support the hypothesis that males may prolong the time
475 they spend with their partner and the brood when by doing so they can benefit
476 from increased paternity (Kvarnemo 2006). However, variation in the
477 competitive social environment experienced by individuals appears to affect
478 the intensity of sexual conflict between parents. The evolution of patterns of
479 parental investment between sexes in this and other species with biparental
480 or male uniparental care reflects a balance between sexual selection (e.g.,
481 direct benefit to males via increasing mating success versus costs to females
482 of increased mating) and natural selection (e.g., direct benefits of male

483 parental care contribution versus indirect costs in terms of reduced offspring
484 fitness and brood safety).

485

486 **Acknowledgements:**

487 Thanks to Curtis Creighton, Ian Hartley, Lotta Kvarnemo, Alastair Wilson and
488 an anonymous reviewer who read, and provided insightful comments on, the
489 manuscript. This work was funded by a Ph.D. studentship from the Natural
490 Environment Research Council (NE/1528326/1) and a grant from NERC to
491 NJR and AJM (NE/1025468/1).

492

493 **References:**

- 494 Alonzo, S. H. 2008. Female mate choice copying affects sexual selection in
495 wild populations of the ocellated wrasse. *Anim. Behav.* 75:1715-1723.
- 496 Alonzo, S. H. 2010. Social and coevolutionary feedbacks between mating and
497 parental investment. *Trends. Ecol. Evol.* 25:99-108.
- 498 Alonzo, S. H., & Klug, H. 2012. Paternity, maternity, and parental care. Pp.
499 189-205 *in* Royle, N. J., Smiseth, P. T. and Kölliker, M. eds. The
500 evolution of parental care. Oxford University Press, Oxford.
- 501 Benowitz, K. M., Head, M. L., Williams, C. A., Moore, A. J. & Royle, N. J.
502 2013. Male age mediates reproductive investment and response to
503 paternity assurance. *Proc. R. Soc. B* 280:20131667.
- 504 Birkhead, T. R. 1979. Mate guarding in the magpie *Pica pica*. *Anim. Behav.*
505 27:866-874.
- 506 Birkhead, T. R. 1982. Timing and duration of mate guarding in magpies, *Pica*
507 *pica*. *Anim. Behav.* 30:277-283.

508 Burke, T., Davies, N. B., Bruford, M. W. & Hatchwell, B. J. 1989. Parental care
509 and mating behaviour of polyandrous dunnocks *Prunella modularis*
510 related to paternity by DNA fingerprinting. *Nature* 338:249-251.

511 Clutton-Brock, T. H., & Vincent, A. C. J. 1991. Sexual selection and the
512 potential reproductive rates of males and females. *Nature* 351:58-60.

513 Crawley, M. J. 2007. *The R book*. Wiley and Sons, Chichester.

514 Eggert, A. K. 1992. Alternative male mate-finding tactics in burying beetles.
515 *Behav. Ecol.* 3:243-254.

516 Eggert, A. K., & Müller, J. K. 1989. Mating success of pheromone-emitting
517 *Necrophorus* males: do attracted females discriminate against resource
518 owners? *Behaviour* 110:248-257.

519 Eggert, A. K., Reinking, M. & Müller, J. K. 1998. Parental care improves
520 offspring survival and growth in burying beetles. *Anim. Behav.* 55:97-
521 107.

522 Eggert, A. K., & Sakaluk, S. K. 2000. Benefits of communal breeding in
523 burying beetles: a field experiment. *Ecol. Entomol.* 25:262-266.

524 Forsgren, E., A. Karlsson, & Kvarnemo, C. 1996. Female sand gobies gain
525 direct benefits by choosing males with eggs in their nests. *Behav. Ecol.*
526 *Sociobiol.* 39:91-96.

527 Freeman-Gallant, C. R. 1996. DNA fingerprinting reveals female preference
528 for male parental care in savannah sparrows. *Proc. R. Soc. B* 263:157-
529 160.

530 Griffin, A. S., Alonzo, S. H. & Cornwallis, C. K. 2013. Why do cuckolded males
531 provide paternal care? *PLoS biology* 11:e1001520.

532 Harrison, F., Barta, Z., Cuthill, I. & Székely, T. 2009. How is sexual conflict
533 over parental care resolved? A meta-analysis. *J. Evol. Biol.* 22:1800-
534 1812.

535 Hartley, I. R., Davies, N. B., Hatchwell, B. J., Desrochers, A., Nebel, D. &
536 Burke, T. 1995. The polygynandrous mating system of the alpine
537 accentor, *Prunella collaris*. II. Multiple paternity and parental effort.
538 *Anim. Behav.* 49:789-803.

539 Head, M. L., Berry, L. K., Royle, N. J. & Moore, A. J. 2012. Paternal Care:
540 Direct and indirect genetic effects of fathers on offspring performance.
541 *Evolution* 66:3570-3581.

542 Head, M. L., Hinde, C. A., Moore, A. J. & Royle, N. J. 2014. Correlated
543 evolution in parental care in females but not males in response to
544 selection on paternity assurance behaviour. *Ecol. Lett.* 17:803-810.

545 Hopwood, P. E., Moore, A. J. & Royle, N. J. 2013. Nutrition during sexual
546 maturation affects competitive ability but not reproductive productivity
547 in burying beetles. *Funct. Ecol.* 27:1350-1357.

548 House, C. M., Evans, G., Smiseth, P. T., Stamper, C. E., Walling, C. A. &
549 Moore, A. J. 2008. The evolution of repeated mating in the burying
550 beetle, *Nicrophorus vespilloides*. *Evolution* 62:2004-2014.

551 House, C. M., Walling, C. A., Stamper, C. E. & Moore, A. J. 2009. Females
552 benefit from multiple mating but not multiple mates in the burying
553 beetle *Nicrophorus vespilloides*. *J. Evol. Biol.* 22:1961–1966.

554 Houston, A. I. 1995. Parental effort and paternity. *Anim. Behav.* 50:1635-
555 1644.

- 556 Hunt, J., & Simmons, L. W. 2002. Confidence of paternity and paternal care:
557 covariation revealed through the experimental manipulation of the
558 mating system in the beetle *Onthophagus taurus*. J. Evol. Biol. 15:784-
559 795.
- 560 Kahn, A. T., Schwanz, L. E. & Kokko, H. 2013. Paternity protection can
561 provide a kick-start for the evolution of male-only parental care.
562 Evolution 67:2207-2217.
- 563 Kokko, H., & Jennions, M. 2008. Parental investment, sexual selection and
564 sex ratios. J. Evol. Biol. 21:919-948.
- 565 Kokko, H., & Jennions, M. 2012. Sex differences in parental care. Pp. 101-
566 116 in N. Royle, Smiseth, P. and Kölliker, M. eds. The evolution of
567 parental care. Oxford University Press, Oxford.
- 568 Kvarnemo, C. 2006. Evolution and maintenance of male care: is increased
569 paternity a neglected benefit of care? Behav. Ecol. 17:144-148.
- 570 Lee, V. E., Head, M. L., Carter, M. J. & Royle, N. J. 2013. Effects of age and
571 experience on contest behaviour in the burying beetle, *Nicrophorus*
572 *vespilloides*. Behav. Ecol. 25:172-179.
- 573 Müller, J. K., Braunisch, V., Hwang, W. & Eggert, A. K. 2007. Alternative
574 tactics and individual reproductive success in natural associations of
575 the burying beetle, *Nicrophorus vespilloides*. Behav. Ecol. 18:196-203.
- 576 Müller, J. K., & Eggert, A. K. 1989. Paternity assurance by "helpful" males:
577 adaptations to sperm competition in burying beetles. Behav. Ecol.
578 Sociobiol. 24:245-249.

- 579 Müller, J. K., Eggert, A. K. & Dressel, J. 1990. Intraspecific brood parasitism in
580 the burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae).
581 Anim. Behav. 40:491-499.
- 582 Müller, J. K., Eggert, A. K. & Sakaluk, S. K. 1998. Carcass maintenance and
583 biparental brood care in burying beetles: are males redundant? Ecol.
584 Entomol. 23:195-200.
- 585 Otronen, M. 1988. The effect of body size on the outcome of fights in burying
586 beetles (*Nicrophorus*). Ann. Zool. Fenn. 25:191-201.
- 587 Parker, G. A., Royle, N. J. & Hartley, I. R. 2002. Intrafamilial conflict and
588 parental investment: a synthesis. Phil. Trans. R. Soc. B 357:295-307.
- 589 Pukowski, E. 1933. Ökologische untersuchungen an *Nicrophorus* F.
590 Zoomorphology 27:518-586.
- 591 Ridley, M., & Rechten, C. 1981. Female sticklebacks prefer to spawn with
592 males whose nests contain eggs. Behaviour 76:152-161.
- 593 Royle, N. J., Hartley, I. R. & Parker, G. A. 2002. Sexual conflict reduces
594 offspring fitness in zebra finches. Nature 416:733-736.
- 595 Royle, N. J., Smiseth, P. T. & Kölliker, M. 2012. The evolution of parental
596 care. Oxford University Press, Oxford.
- 597 Scaggiante, M., Rasotto, M. B., Romualdi, C. & Pilastro, A. 2005. Territorial
598 male gobies respond aggressively to sneakers but do not adjust their
599 sperm expenditure. Behav. Ecol. 16:1001-1007.
- 600 Scott, M. P. 1989. Male parental care and reproductive success in the burying
601 beetle, *Nicrophorus orbicollis*. J. Insect Behav. 2:133-137.
- 602 Scott, M. P. 1990. Brood guarding and the evolution of male parental care in
603 burying beetles. Behav. Ecol. Sociobiol. 26:31-39.

604 Scott, M. P. 1998. The ecology and behavior of burying beetles. *Annu. Rev.*
605 *Entomol.* 43:595-618.

606 Sheldon, B.C. 2002. Relating paternity to paternal care. *Phil. Trans. R. Soc. B*
607 357, 341–350

608 Smiseth, P. T., Darwell, C. T. & Moore, A. J. 2003. Partial begging: an
609 empirical model for the early evolution of offspring signalling. *Proc. R.*
610 *Soc. B* 270:1773-1777.

611 Smiseth, P. T., Dawson, C., Varley, E. & Moore, A. J. 2005. How do caring
612 parents respond to mate loss? Differential response by males and
613 females. *Anim. Behav.* 69:551-559.

614 Smiseth, P. T., Lennox, L. & Moore, A. J. 2007. Interaction between parental
615 care and sibling competition: parents enhance offspring growth and
616 exacerbate sibling competition. *Evolution* 61:2331-2339.

617 Svensson, O., & Kvarnemo, C. 2005. The importance of sperm competition
618 risk and nest appearance for male behavior and female choice in the
619 sand goby, *Pomatoschistus minutus*. *Behav. Ecol.* 16:1042-1048.

620 Svensson, O., & Kvarnemo, C. 2007. Parasitic spawning in sand gobies: an
621 experimental assessment of nest-opening size, sneaker male cues,
622 paternity, and filial cannibalism. *Behav. Ecol.* 18:410-419.

623 Trivers, R. L. 1972. Parental investment and sexual selection. Pp. 136-179 *in*
624 B. G. Cambell, ed. *Sexual selection and the descent of man*. Aldine,
625 Chicago.

626 Trumbo, S. T. 1991. Reproductive benefits and the duration of paternal care
627 in a biparental burying beetle, *Necrophorus orbicollis*. *Behaviour*
628 117:82-105.

629 Trumbo, S. T. 2007. Defending young biparentally: female risk-taking with and
630 without a male in the burying beetle, *Nicrophorus pustulatus*. *Behav.*
631 *Ecol. Sociobiol.* 61:1717-1723.

632 van Dijk, R. E., Székely, T., Komdeur, J., Pogány, Á., Fawcett, T. W. &
633 Weissing, F. J. 2012. Individual variation and the resolution of conflict
634 over parental care in penduline tits. *Proc. R. Soc. B* 279:1927-1936.

635 Whittingham, L. A., Taylor, P. D. & Robertson, R. J. 1992. Confidence of
636 paternity and male parental care. *Am. Nat.* 139:1115-1125.

637 caption for Figure 1, a & b:

638 **Figure 1:** a) Mean proportion of the total postnatal larval developmental period (from first
639 larval arrival at carcass to larval dispersal from carcass) that the dominant male remained in
640 attendance performing parental activities. x-axis labels indicate the constituents of four
641 different social competitive treatments (i.e., 'mf' = one male and one female; 'mfff' = one male
642 and three females; 'mmff' = two males and two females; 'mmmf' = three males and one
643 female); b) effect of treatment on brood size at larval dispersal. Lower case letters above
644 error bars indicate significant differences among treatment groups.

645

646 caption for Figure 2, a & b:

647 **Figure 2:** a) Dominant male matings with dominant female (filled circles), and subordinate
648 male matings with dominant female (open triangles), lower case letters indicate significant
649 differences between treatments; b) Positive relationship between hours taken to bury carcass
650 (y axis) and frequency of mating between pairs of caregiving beetles (x axis). Figure shows
651 least squares regression on untransformed data for illustration purposes.

652



