Switching tactics: Phenotypic plasticity in the alternative mate-finding tactics of burying beetles

TESS E. PIENING MULREY^a, ANNE-KATRIN EGGERT^a, & SCOTT K. SAKALUK^a

^a School of Biological Sciences, Illinois State University, Normal, Illinois

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*Correspondence and present address: S.K. Sakaluk, Behavior, Ecology, Evolution & Systematics Section, 4120 Biological Sciences, Illinois State University, Normal, IL 61720-4120, U.S.A.

E-mail address: skakal@ilstu.edu (S. K. Sakaluk)

30 ABSTRACT

31 Male *Nicrophorus* burying beetles utilize two alternative mate-finding tactics: searching and signalling.
32 The searching tactic involves flying in search of a carcass on which to mate, while the signalling tactic
33 involves emitting pheromone in the absence of a carcass to attract females. All males employ both
34 tactics, but the amount of time spent employing each tactic differs between individuals. Because all
35 males are phenotypically plastic in the time spent employing alternative behaviours, the expression is
36 likely to be condition dependent on some environmental cue, such as the availability of reproductive
37 resources (i.e. females and carcasses). We tested how the expression of alternative tactics is influenced
38 by previous mating experience and the perceived availability of females versus carcasses by using a
39 repeated-measures design in which mate-finding behaviour of males was observed before and after
40 exposure to: 1) multiple females but no carcasses, 2) multiple females and multiple carcasses, 3)
41 multiple carcasses but no females, or 4) no females or carcasses (control). Males in the multiple-female
42 group (but lacking a carcass) allocated significantly more time to signalling, as did males in the control
43 group lacking both mating and carcass experience. Thus, males that did not encounter carcasses during
44 the treatment period increased pheromone emission thereafter. In the two groups that were given
45 carcasses, however, investment in signalling was not altered. With respect to time allocated to searching,
46 control, multiple-female, and multiple-carcass groups all significantly decreased time spent searching,
47 whereas males in the female-and-carcass group did not alter time allocated to searching. Our results
48 demonstrate that environmental cues do influence which alternative tactic male burying beetles employ,
49 and that the chosen tactic is influenced by the availability of both receptive females and carcasses on
50 which to breed.

51 Keywords: alternative mating tactics, burying beetles, conditional strategy, phenotypic plasticity,
52 pheromone, *Nicrophorus orbicollis*, signalling

53 Alternative mating behaviours, prevalent across a wide range of taxa, are characterized by
54 multiple distinct behavioural phenotypes of the sexually selected sex, typically males (Waltz & Wolf,
55 1984; Shuster, 2010). Most alternative reproductive behaviours comprise multiple tactics expressed in a
56 condition-dependent manner (Dominey, 1984; Taborsky, Oliveira, & Brockmann, 2008). These
57 conditional strategies are characterized by alternative phenotypes that are influenced by an
58 environmental cue or some aspect of the male's condition or status (Gross, 1996; Lee, 2004; Shuster &
59 Wade, 2003; Tomkins & Hazel, 2007). Conditional strategies occur when there is a certain threshold or
60 switchpoint at which males switch from expressing one tactic to the other; this switchpoint could be an
61 intrinsic quality of the individual such as size or an extrinsic environmental cue (Shuster & Wade, 2003;
62 Tomkins & Hazel, 2007).

63 While some conditional strategies are characterized by an irreversible developmental switch, as
64 documented in salmonid fishes (Aubin-Horth & Dodson, 2004; Piché, Hutchings, & Blanchard, 2008),
65 many others exist in which shifts between tactics are rapid and reversible. This specific type of
66 conditional strategy is termed a "behavioural strategy" and is characterized by phenotypically plastic
67 males that can switch between tactics based on an extrinsic cue such as their relative status in the
68 population (as in the status-dependent selection model of Gross, 1996) or another environmental cue
69 such as population density or resource availability (Brockmann, 2001; Shuster & Wade, 2003).

70 Such phenotypically plastic behavioural strategies are most likely to evolve when males have
71 few mating opportunities, but when the availability of mating opportunities can be predicted by the
72 environment (Shuster & Wade, 2003). In this case, males will perceive the environment as "fine-
73 grained", as it may change rapidly and multiple times throughout an individual's life (Levins, 1968;
74 Brockmann, 2001; Shuster & Wade, 2003). In the context of male mating behaviours, the environmental
75 grain may be determined by the spatiotemporal distribution of females (Shuster & Wade, 2003).

76 Environments in which the circumstances surrounding a successful reproductive attempt are highly
77 variable may also result in phenotypically plastic mating behaviours (Shuster & Wade, 2003).

78 Alternative tactics comprising behavioural strategies are known throughout diverse taxa.
79 Commonly, the alternative phenotypes are expressed in response to the immediate environmental
80 conditions, as when individuals adjust their tactic according to their status relative to competitors.
81 However, instances may exist in which the current environment is not indicative of the reproductive
82 landscape as a whole and provides little information on which tactic use is optimal. Such may be the
83 case with alternative mate-finding tactics, where all males find themselves in a similar immediate
84 environment devoid of reproductive resources at the time they have to decide which tactic to employ. In
85 this case, males may use information gained from past experiences with respect to the availability of
86 reproductive resources in making their decision on which tactic to employ.

87 Male *Nicrophorus* burying beetles represent an ideal model with which to test whether
88 individuals use experience in decisions about tactic use. To successfully reproduce, burying beetles
89 require a small vertebrate carcass, a rare and ephemeral resource. The two alternative mate-finding
90 tactics employed by male burying beetles are to actively fly in search of a carcass on which to meet a
91 female and raise a brood, or to emit pheromone in the absence of a carcass (henceforth termed
92 “signalling”; Müller & Eggert, 1987). If a searching male is able to successfully locate a carcass, he may
93 be presented with a number of different situations, depending on the presence of other beetles. If another
94 male is already present, the two fight over possession of the resource (Müller et al., 1990; Sakaluk &
95 Müller, 2008). If a female is present on the carcass, the pair prepare and bury the carcass, mating
96 repeatedly in the process. When no females are present on the carcass when the male arrives, he
97 superficially buries the carcass and emits pheromone to aid in attracting a female (Pukowski, 1933;
98 Eggert, 1992; Wilson & Fudge, 1984), which might inadvertently attract conspecific males or congeners

99 of either sex who may attempt to usurp the resource (Müller & Eggert, 1987; Eggert & Sakaluk, 2000;
100 Haberer, Schmitt, Shreier, & Müller, 2011). While this tactic is risky, it can have a high payoff; in *N.*
101 *vespilloides*, the average paternity on a single carcass for a successful searching male is likely to be
102 84%, with paternity up to 92% if there are no satellite males present (Eggert, 1992).

103 The signalling tactic is unlikely to have as great a return per brood, but likely has lower costs in
104 terms of risky agonistic encounters and energetic demands. Females are attracted to the scent of the
105 pheromone emitted by signalling males, and they cannot determine whether a male holds a carcass until
106 they come into physical contact (Eggert & Müller, 1989a; Eggert & Müller, 1989b). Upon meeting a
107 signalling male, the female allows a single copulation before flying away (Eggert & Müller, 1989b),
108 likely to ensure an adequate supply of viable sperm in case she locates a carcass on which another male
109 is not present (Eggert, 1992). Field studies have found that between 19 and 39% of females raise broods
110 without a mate, presumably using sperm stored from mating with signalling males (Eggert, 1992; Scott
111 & Traniello, 1990; Müller et al., 2007). It is estimated that signalling males can achieve 5% paternity of
112 a single clutch if they are the last male to mate with a female (Eggert, 1992). However, their actual
113 success depends on how long it takes for a female to subsequently locate a carcass, how many additional
114 males she mates with, and whether another male is present on the carcass. It follows that a signalling
115 male must mate with many females to achieve similar reproductive returns as a searcher. In spite of the
116 difference between single-clutch paternities between the two tactics, a field study of *N. vespilloides*
117 estimated that up to 45% of all larvae were sired by signalling males (Eggert, 1992; Müller et al., 2007).

118 All male burying beetles employ both tactics, but the proportion of time spent employing each
119 tactic differs between individuals. While there is genetic variation underlying these behaviours, it does
120 not account for all of the variation, and there is even substantial variation in behaviour within an
121 individual (Eggert, 1992). Thus, these behavioural phenotypes are best viewed as alternative tactics

122 within a conditional strategy. As a conditional strategy, there should be intrinsic or extrinsic cues that
123 influence tactic expression. Previous studies have found that small males signal more frequently than
124 large males, and that the influence of this intrinsic cue is not contingent on interactions with other
125 beetles (Beeler et al., 1999; Walling et al, 2009). However, it is likely that there are also extrinsic
126 environmental cues that influence a male's relative investment in the alternative tactics.

127 Because the reproductive success of male burying beetles is dependent on the spatiotemporal
128 distribution of females and carcasses, experience with these resources may provide a valuable cue,
129 allowing males to determine which tactic is likely to have greater returns in their specific environment.
130 We tested this hypothesis by observing the behaviour of naïve male *N. orbicollis* before and after
131 exposure to different reproductive experiences. We predicted that if males are able to gain information
132 about the distribution of resources from their experience, they should adjust the relative amount of time
133 invested in each tactic accordingly. Specifically, males that are allowed to mate with multiple females in
134 the absence of a carcass may perceive a greater availability of potential mates; we predicted that these
135 males would increase investment in signalling and decrease investment in searching. Males that
136 encounter multiple carcasses should perceive a greater abundance of carcasses, which may yield higher
137 returns for searching. We predicted that males encountering carcasses would increase investment in
138 searching and decrease investment in signalling. However, if a male encounters a carcass in nature but is
139 unable to attract a female, the carcass is of no reproductive value. Thus, we exposed males to carcasses
140 with females either present or absent, to determine whether the presence of a female affects a male's
141 perception of reproductive opportunities and differential investment in the alternative tactics.

142

143 METHODS

144 *Experimental animals*

145 *Nicrophorus orbicollis* occurs throughout eastern North America, primarily inhabiting hardwood
146 forests. Experimental individuals were second- and third- generation lab-reared descendants of beetles
147 caught in baited pit-fall traps in autumn 2013 at the Parklands Nature Preserve near Lexington, Illinois
148 (40.6657N, 88.897W). Field-caught individuals were brought into the lab and maintained in plastic deli
149 containers (0.5 L) with moist paper towels as a substrate, either individually or with another individual
150 of the same sex. The beetles were fed small pieces of ground beef twice per week, at which time the
151 paper towels were replaced with clean ones.

152 Field-caught beetles were paired randomly for breeding. Each pair was placed in a breeding
153 chamber with a defrosted previously frozen 20-g mouse carcass. When at least one member of the pair
154 began inspecting the carcass, the beetles and the carcass were transferred to another container with moist
155 peat. The pairs were then placed in a darkroom and allowed to mate, bury and prepare the carcass, and
156 raise larvae. Once larvae dispersed from the carcass, they were removed from the breeding container. Up
157 to five sibling larvae were placed into containers filled with about 4 cm of packed clean moist peat to
158 pupate. These containers were kept in a dark incubator at 16°C until adults eclosed. The newly emerged
159 beetles were sexed and placed in a new container with about 2 cm of moist peat, either individually or
160 with one same-sex sibling, and maintained in an incubator on a 16L:8D photoperiod at 21°C. They were
161 fed two decapitated mealworms twice weekly, and the peat in their containers was replaced every other
162 week.

163 Upon maturity, F₁ adults were randomly mated with unrelated individuals of the opposite sex to
164 create an F₂ population as described above. Larvae and newly emerged adults were treated as described
165 above for the F₁ generation, except that all larvae were housed individually until eclosion. As adults,
166 male beetles were housed individually to avoid any variation in social environment, whereas females
167 were housed with one or two same-sex siblings. These same procedures were also used to create an F₃

168 population. All F₂ and F₃ adults were maintained in incubators on a 16L:8D photoperiod at 21°C, with
169 males and females in separate incubators. These adult beetles were maintained in the same manner as
170 described for the F₁ adults.

171 *Experimental design*

172 All beetles used in this study were sexually mature F₂ and F₃ adults, ranging in age from 29 to 59
173 days post-eclosion on the first day their behaviour was observed. The experiment was done in three
174 rounds of 60 males, as this was determined to be the largest manageable number of beetles to observe at
175 one time. Sexually mature male beetles ($N=180$) were first weighed to the nearest milligram on a Mettler
176 PE360 Delta Range digital scale balance, and then placed individually into observation chambers.
177 Observation chambers were lidded rectangular acrylic glass boxes (20cm x 10cm x 10cm), with a small
178 hole drilled about 1 cm from the top on each end to allow for gas exchange and to facilitate feeding.
179 Observation chambers were filled with about 2 cm moist peat substrate and kept in a darkroom on the
180 same 16L:8D photoperiod to which the beetles were acclimated. Red LED lights remained on in the
181 darkroom 24 hours. After being placed in the observation chambers, each beetle was fed half a
182 mealworm and allowed to acclimate to the chamber and the darkroom for 15-18 hours before
183 observations began.

184 Observations started 2 hours after ‘lights off’ and continued for 6 hours, until ‘lights on’ because
185 pilot studies had shown that beetles were most active during this time. Beetle behaviour was scan-
186 sampled at 10-minute intervals during this time. Males were recorded as either signalling, searching, or
187 inactive during each scan. Males were scored as signalling if they were in the characteristic “head-stand”
188 posture, with their abdomen extended upwards and their final abdominal segment fully extruded. Males

189 were considered to be searching if they were flying, digging, walking, or running. If a male was
190 stationary, but not signalling, he was scored as inactive.

191 After an observation period ended, each beetle was fed half a mealworm, which was inserted
192 through one of the holes drilled in the side of the chamber to minimize disturbances associated with
193 removing the lid. Observations were carried out on four consecutive days to establish each male's
194 baseline signalling and searching frequencies. At the end of day four, each male was randomly assigned
195 to: 1) one of two carcass treatments (provided with a carcass(es) or no carcass) and 2) one of two female
196 treatments (provided with a female(s) or no female). This design yielded four treatment groups: 1)
197 control (neither carcass nor female); 2) multiple-female; 3) multiple-carcass; and 4) female-and-carcass.
198 An equal number of siblings from each family were assigned to each treatment to avoid potential
199 confounding effects of genetic variation on signalling and searching frequencies (see Eggert, 1992).

200 After the pre-treatment observations were complete, males were transferred individually to
201 small deli containers containing about 2 cm of moist peat substrate. Males in the multiple-carcass and
202 female-and-carcass treatment were provided with a thawed 20-g mouse carcass; a single female was also
203 added to the containers with males in the female-and-carcass treatment. All containers were transferred
204 to incubators set to the same 16L:8D photoperiod for the duration of the seven-day treatment period.
205 The control and multiple-female groups were kept in a separate incubator from the carcass treatments to
206 prevent these males from receiving any olfactory cues from the carcasses.

207 Males in the two treatments with carcasses were allowed to remain on a carcass for three days,
208 and the males in the female-and-carcass group were allowed to mate with the female and bury and
209 prepare the carcass with her. After these three days, all males in these treatments were transferred into
210 new deli containers with about 2 cm of moist peat, and given one day to recover before repeating the

211 process again. Thus, in the seven-day treatment period, males in the multiple-carcass treatment were
212 given two carcasses in succession and males in the female-and-carcass group were given two carcasses
213 and two females in succession.

214 In the multiple-female treatment, males were given access to three females for 4-5 hours per
215 day. These females were added to the male's container 3-4 hours after 'lights-off', and removed after
216 'lights-on'. When adding or removing females, we tried to minimize disturbance to the male. However,
217 removal of females did require some disturbance while searching through the peat although we had
218 marked females with white paint on the pronotum to facilitate their discrimination from the male.
219 During the seven-day treatment period, three novel females were added to a male's container every day.

220 Control males were maintained individually in deli containers for seven days, without access to
221 either females or carcasses. To control for any effect that disturbances may have had on the other
222 treatment groups, the control male's substrate was stirred with forceps daily at 'lights-on'. Males in the
223 control and multiple-female treatments were fed two decapitated mealworms every three days. As males
224 in the two carcass treatments had the opportunity to feed on carcasses, they were not fed any mealworms
225 during the treatment period.

226 Following the seven days of treatments, males were transferred back into clean observation
227 chambers with about 2 cm of fresh moist peat, fed half a mealworm, and placed in the darkroom to
228 acclimate for 15-18 hours before observations began. Each male was randomly assigned a new
229 identification number by a person other than the observer to prevent any potential observer bias during
230 observations (i.e., post-treatment observations were made blind to treatment). Male behaviour was
231 observed on four consecutive days post-treatment, in the same manner and under the same conditions as
232 during the pre-treatment period.

233 Following the four days of observations, males were placed individually in labelled film
234 canisters and frozen. Using a Nikon SMZ800 zoom stereoscope affixed with an Infinity 1-2 camera and
235 NI-150 high intensity illuminator, the length and width of each male's pronotum was measured to the
236 nearest .01 μm in NIS Elements D 3.10. Measurements were taken by capturing a still photograph of the
237 pronotum. The pronotum width was measured at the widest part of the pronotum. Pronotum length was
238 measured along the suture line. Each measurement was taken three times for each male, using a different
239 still photograph for each set of measurements.

240 *Statistical analyses*

241 SAS 9.3 was used for all statistical analyses. To calculate baseline rates of signalling and
242 searching, observations were totalled over the four pre-treatment days. Signalling and searching
243 observations were also totalled over the post-treatment days for each male. We employed a repeated-
244 measures, mixed-model ANCOVA, with time (pre- and post-treatment) as the repeated factor, family as
245 a random effect, carcass experience (carcass or no carcass) and mating experience (female or no
246 female) as main effects, and size and age as covariates. We used pronotum length as our only size
247 measurement, as we found pronotum length, pronotum width, and mass to be highly correlated.
248 Parameter estimates were obtained using restricted maximum likelihood (REML) with a compound
249 symmetry covariance structure, and degrees of freedom were estimated using the Satterthwaite
250 approximation (Littell et al., 2006). The effect of family was determined by comparing the -2 log
251 likelihood values between models that included or excluded family as a random effect.

252 Repeatabilities for the signalling and searching behaviours were calculated using mean squares
253 from general linear models as in Lessels and Boag (1987), and standard errors were calculated using the

254 protocol from Becker (1992). The pre-treatment and post-treatment repeatability of each behaviour was
 255 calculated across all four observation days within each time period.

256 RESULTS

257 Male beetles altered their investment in alternative mate-finding tactics as a function of their
 258 previous experience. We found a significant time-by-carcass interaction for the amount of time spent
 259 signalling, but no time*female interaction; the three-way interaction between time, carcass treatment and
 260 female treatment was not significant, which indicates that the effect of carcass on male signalling was
 261 not contingent on their previous mating experience (Table 1; Fig. 1). Males in the multiple-female

Table 1. Repeated-measures mixed-model ANCOVA of the effect of previous experience with carcasses and females on subsequent signalling and searching effort in males (male pronotum length and age included as covariates).

Effect	Signalling			Searching		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Carcass	0.07	1, 140	0.7958	0.45	1, 140	0.5024
Female	0.47	1, 139	0.4958	0.02	1, 138	0.8750
Time	12.46	1, 174	0.0005	15.75	1, 175	0.0001
Time*Carcass	7.86	1, 174	0.0056	2.85	1, 175	0.0932
Time*Female	1.73	1, 174	0.1901	2.21	1, 175	0.1389
Time*Carcass*Female	0.31	2, 155	0.7338	0.83	2, 155	0.4398
Pronotum Length	55.98	1, 172	<0.0001	21.28	1, 169	<0.0001
Age	10.14	1, 174	0.0017	0.54	1, 155	0.4625

262 group (but without a carcass) allocated significantly more time to signalling ($F_{1, 174}=7.7$, $P=0.0061$), as
 263 did males in the control group lacking both mating and carcass experience ($F_{1, 175}=12.77$, $P=.0005$; Fig.

264 1). Thus, males that did not encounter carcasses during the treatment period increased pheromone
265 emission thereafter. In the two groups that were given carcasses, however, investment in signalling was
266 not altered (carcass: $F_{1, 175}=1.56$, $P=0.2127$; female and carcass: $F_{1, 174}=0.3$, $P=0.5870$; Fig. 1).

267 There was a significant difference across time periods in time spent searching, with males
268 spending less time searching post-treatment. The time-by-carcass interaction for time spent searching
269 was nearly significant ($P=0.09$; Table 1; Fig. 1), but no other higher-order interaction was significant.
270 Pairwise comparisons revealed that males in the control, multiple-female, and carcass groups
271 significantly decreased the amount of time spent searching after the treatment period ($F_{1, 176}=9.57$,
272 $P=0.0023$, $F_{1, 174}=6.67$, $P=0.0106$, and $F_{1, 176}=5.45$, $P=0.0208$). Males in the female-and-carcass group,
273 the only ones that were given access to both resources required for successful reproduction, did not alter

Figure 1. Least squares means ($\pm$ SE) of the total number of observations spent signaling (solid line) and searching (dashed line) pre- and post-treatment for the four treatment groups.

274 their investment in searching ($F_{1, 174}=0.01$, $P=0.9374$; Fig. 1).

275 Fig.1 illustrates how males adjusted their investment in both alternative tactics simultaneously
 276 after treatment. Males in the multiple-female treatment group both increased signalling and decreased
 277 searching following treatment, as did males in the control group. The males that were provided access to
 278 carcasses but not females, decreased their investment in searching, but did not alter their time spent
 279 signalling post-treatment. Males that were provided with females and carcasses, simulating two
 280 subsequent successful reproductive attempts, did not alter their investment in either behaviour post-
 281 treatment. There was no effect of either carcass or female experience on the time males spent inactive,
 282 and nor did the amount of time males spent inactive differ across pre- or post-treatment observation
 283 periods (results not shown).

284 We included family in our model as a random effect, as previous studies have shown genetic
 285 variation in the time invested in alternative mate-finding tactics (Eggert, 1992). We found a significant
 286 effect of family on both signalling ($X^2=32.6$,
 287 $P<0.0001$) and searching ($X^2=8.0$, $P=0.0046$).
 288 Individual signalling and searching behaviour
 289 were repeatable across all trials, both pre- and
 290 post-treatment (Table 2).

Table 2. Signalling and searching were repeatable, both before and after the treatment period.

	Pre-treatment		Post-treatment	
	<i>R</i>	<i>S.E.</i>	<i>R</i>	<i>S.E.</i>
Signalling	0.583	0.2346	0.562	0.241
Searching	0.455	0.2636	0.787	0.1465

291 Pronotum length, our measure of male
 292 body size, had a significant effect on both signalling and searching (Table 1). For signalling, there was
 293 an inverse relationship with male size, such that smaller males invested more time signalling (parameter
 294 estimate ($\pm$ SE) = -0.02498 ± 0.0033). The opposite relationship was found between male size and
 295 investment in searching, as larger males spent more time searching (parameter estimate =

0.01136±0.002541). Male age also had a significant effect on male signalling (Table 1), with older males spending significantly more time signalling than younger males (parameter estimate = 0.7957±0.2499). There was, however, no effect of male age on searching (parameter estimate = -0.1382±0.1877).

300

301 DISCUSSION

302 The results of our experiment demonstrate that male burying beetles adjust their use of
303 alternative mate-finding tactics based on their previous experience with reproductive resources.
304 Experimental males that did not encounter any carcasses during the treatment period significantly
305 increased their investment in signalling while significantly decreasing investment in searching. Males in
306 the groups that were given access to carcasses during the treatment period did not alter their investment
307 in signalling, but males that only encountered carcasses in the absence of females decreased their
308 investment in searching following the treatment period. Because the pre- and post-treatment
309 observations were done under identical conditions and the beetles were not observed during the
310 treatment period, these behavioural adjustments should be considered a response to prior experience
311 rather than to the immediate conditions.

312 The alternative mate-finding tactics of male burying beetles are part of a conditional behavioural
313 strategy. A male's own body size is one condition that affects his use of the alternative tactics, but
314 environmental indicators of the profitability of each tactic are also expected to factor into the use of
315 alternative tactics. These types of phenotypically plastic strategies are expected to evolve when the
316 reproductive landscape is perceived as "fine-grained" (Levins, 1968), but males are able to predict the
317 availability of mating opportunities based on some environmental cue (Shuster & Wade, 2003). We

318 hypothesized that the spatiotemporal distribution of reproductive resources (i.e. females and carcasses)
319 would serve as such a cue, indicating which tactic would yield higher payoff. We found support for this
320 hypothesis, as mate-finding tactic expression was influenced by experience with both females and
321 carcasses. We note, however, that all males maintained both tactics to some degree post-treatment,
322 although the relative investment in each was influenced by experience. It is likely that using a
323 combination of both tactics is generally beneficial because rapid changes in the availability of carcasses
324 may not be predictable based on prior experience.

325 Males that were allowed to mate with multiple females during the trial period subsequently
326 increased their investment in signalling and decreased time spent searching. While we had predicted
327 such an adjustment, we found that the control group, which did not have any interactions with females
328 or carcasses, similarly increased signalling time and decreased searching time. This result suggests that a
329 high perceived availability of females is not likely to be the cue stimulating an increased investment in
330 the signalling tactic. It is more likely that increased investment in signalling and decreased investment in
331 searching is triggered by temporal variation in the availability of carcasses. When carcasses are
332 perceived to be unavailable in the near future, the payoff for searching should be low, and signalling
333 may yield higher returns, regardless of the availability of females in the current environment.
334 Alternatively, the increased pheromone emission of control males may have been due to an age effect, as
335 we found that the time allocated to signalling increased significantly with male age. In *N. vespilloides*, in
336 contrast, the quantity of pheromone emitted did not change with male age, but the ratio of the two
337 components of the sex pheromone varied with male age such that older males were more successful in
338 attracting females than younger males (Chemnitz et al., 2015).

339 Although the availability of females does not appear to play a role in tactic expression in the
340 absence of carcasses, female presence does influence tactic expression when carcasses are available.

341 While neither of the treatment groups that received carcasses altered their investment in signalling, those
342 beetles that received only carcasses but no females decreased their searching time. Males that received
343 both carcasses and females, however, did not show any change in searching behaviour. This suggests
344 that the presence of females and carcasses is required for males to maintain their investment in the
345 search tactic. The reduced time spent searching in the group that received only carcasses could be due
346 simply to a reduction in overall activity, as well-fed burying beetles have been shown to be less active
347 (Cordes, Kaschuba, & Müller, 1988). However, if nutritional status did play a role, males that were on
348 carcasses with females during the treatment period should have similarly reduced their search activity,
349 but they did not.

350 We had predicted that males given both females and carcasses would subsequently increase their
351 time spent searching and decrease signalling, as they would experience the reproductive situation of
352 successful searchers. These males should perceive high availability of both reproductive resources, and
353 thus we expected the benefits of searching to be greater than those of signalling. However, males that
354 were allowed to mate with females on carcasses did not alter their investment in either tactic, instead
355 continuing to search and signal at the same frequencies as they did before encountering the resources.
356 We attribute this apparent lack of response to the fact that these males experienced the ideal
357 reproductive situation: a carcass, a female, and no competitors. These males achieved ideal reproductive
358 success at the tactic frequencies employed prior to the treatments, and therefore would be under no
359 pressure to alter their strategy. Moreover, if the current level of searching is sufficient to maximize
360 carcass location, concurrent maintenance of the current allocation to signalling would mean that males
361 do not forego additional fitness returns through insemination of ancillary females with whom they do
362 cooperate in rearing a brood.

363 In contrast to the results of the present study, Walling et al. (2009) found no effect of experience
364 on signalling behaviour in *N. vespilloides*. While the differences between the two studies could
365 conceivably reflect interspecific differences in the capacity for behavioural plasticity between *N.*
366 *orbicollis* and *N. vespilloides*, we think they are more likely due to differences in experimental design.
367 In order to simulate environmental differences that might affect the profitability of the alternative
368 tactics, we varied the males' exposure to carcasses and females for a week. In Walling et al.'s (2009)
369 study, in contrast, the treatment that simulated different experiences for the males consisted of brief
370 interactions with either a conspecific male or a female in the absence of any carcasses. For the mating
371 experience treatment, Walling et al. (2009) allowed males a single mating before measuring their post-
372 experience signalling rate. We suggest that this single mating experience is likely insufficient to alter a
373 male's perceptions of the spatiotemporal distribution of females. We chose the design of our study based
374 on the assumption that providing males with multiple females over several days would more accurately
375 mimic the experience of successful use of the signalling tactic in the field. The lack of a significant
376 effect of previous experience in Walling et al.'s (2009) study could also be due to the manner in which
377 the use of tactics was measured: we observed males for six-hour periods on four days pre-treatment and
378 another four days post-experience, whereas Walling et al.'s (2009) pre-and post-experience observations
379 were made during a single hour on each of two consecutive days. We believe our methods may be more
380 reflective of the natural situation, insofar as we observed males throughout their entire daily activity
381 period and not just a small portion of it. Additionally, we were able to confirm the repeatability in male
382 behaviour before and after treatments, ensuring that any changes were not simply due to stochastic
383 variation.

384 Consistent with earlier studies in *N. orbicollis* and *N. vespilloides* (Beeler et al., 1999; Walling et
385 al., 2009; Chemnitz et al., 2015), we found an effect of absolute male size on signalling frequency, with

386 small males signalling more than large males. We also report the novel finding of a significant positive
387 relationship between absolute male size and time spent searching. While these size effects may be
388 attributable to differences in competitive ability on a carcass as suggested by Beeler et al. (1999), we
389 propose that they might also be affected by a discrepancy in the energetic costs of the two tactics. If the
390 energetic demands of searching are greater than those of signalling, larger males with greater fat
391 reserves should be able to invest more into searching at a lower cost than small males (but higher lipid
392 reserves could also result in greater costs of flight due to increased wing loading; see Marden & Chai,
393 1991). While no specific data are available for our study species, flight is generally considered to be a
394 very energetically expensive activity for insects (Reinhold, 1999; Kammer & Heinrich, 1978), while
395 pheromone production is considered relatively inexpensive. Thus, energy reserves may play an
396 important role in a male's decision about the use of alternative tactics.

397 Although our study demonstrates that mating experience and the availability of reproductive
398 resources serve as environmental cues influencing the tactic switchpoint of a conditional strategy, we do
399 not yet know the amount of genetic variation for the switchpoint, if any. By varying the number of
400 reproductive resources available to males, or the frequency with which they are encountered, it may be
401 possible to determine whether males differ in the exact value of the environmental cue that is required to
402 alter investment in alternative tactics, as predicted by the environmental threshold model (Tomkins &
403 Hazel, 2007). Additionally, it might be useful to investigate the effects that other social experiences,
404 such as the presence of a competitor and non-mating interactions, have on relative investment in
405 alternative mate-finding tactics. While Walling et al. (2009) clearly intended to simulate male
406 interactions with conspecific competitors, it is unlikely that the brief encounters during 30 minutes of
407 confinement with another male were sufficient to trigger adjustments in tactic use. Moreover, it is
408 unclear what exactly the conditions were that caused males to fight each other. In our experience, male

burying beetles do not engage in aggressive interactions unless a carcass or mating partner is present, and the description of the simulated “agonistic experience” in Walling et al. (2009) makes no mention that either reproductive resource was present with the two males.

Because the alternative mate-finding tactics of male burying beetles are dependent on environmental conditions rather than being strictly genetically determined, they constitute a conditional strategy. Alternative strategies are considered to be conditional whenever the expression of tactics is contingent on non-genetic factors, but there is considerable variation among taxa in the manner in which such factors impact tactic expression. Tactic use is frequently correlated with phenotype. This can occur when a certain phenotype is fixed after a certain stage of development and constrains the subsequent use of tactics for the individual. Well-known examples include horned beetles, acarid mites, and salmon, in which an individual’s phenotype (and therefore their tactic) is determined by some threshold cue during development (Emlen, 1994; Emlen, 1997; Aubin-Horth & Dodson, 2004; Radwan, 2009). Correlations with phenotype can be looser in other systems where tactic use remains flexible, such as in horseshoe crabs in which the tactic a male employs is determined by his absolute body condition (Brockmann, 2002). In burying beetles, a male’s body size clearly affects his relative use of alternative tactics. Our study confirmed earlier findings in *N. orbicollis* (Beeler et al. 1999) and *N. vespilloides* (Walling et al. 2009) that larger males tend to spend less time signalling than do smaller males, and it also showed that larger males spend more time searching than do smaller ones. As males in our study were kept singly, these findings could not have been affected by social interactions as suggested by Walling et al. (2009)

When conditional strategies are not strictly correlated with phenotype, individuals may use different tactics under different environmental or social conditions. This is commonly seen when the tactic employed is dependent on the individual’s status relative to competitors. When larger competitors are present, males may switch to a less profitable tactic, such as satellite behaviour or female mimicry

432 (in rove beetles: Forsyth & Alcock, 1989; in cuttlefish: Norman, Finn, & Tregenza, 1999; in shrimp:
433 Correa, Baeze, Hinojosa, & Thiel, 2003). Similarly, in the grasshopper, *Ligurotettix coquillettii*, a male's
434 signalling behaviour is dependent on the density and proximity of conspecific males; a male is more
435 likely to signal when alone in a bush than when sharing a bush with conspecific males (Shelley &
436 Greenfield, 1985). Male signalling can also be influenced by the amount of resources available for
437 reproduction. In the burying beetle, *N. vespilloides*, males paired with females on large carcasses that
438 can support the young of more than one female, engage in polygynous signalling, whereas those paired
439 on small carcasses do not (Eggert & Sakaluk, 1995). In all of these examples, individuals are making
440 decisions based on the current conditions and immediate social context.

441 In burying beetles, such behavioural adjustments based on the outcome of fights around
442 carcasses are well documented. One recent example is a study by Carter, Head, Moore, & Royle (2015)
443 that investigated the signalling behaviour of competing *N. vespilloides* males on carcasses on which no
444 female was present. These males emit pheromone in an apparent attempt to increase the attractiveness of
445 the carcass to conspecific females, whose presence they require for successful reproduction. The males
446 in Carter et al.'s (2015) study adjusted the location of their signalling behaviour based on their
447 interactions with such competitors, with larger males being more likely to signal directly on the carcass.
448 Although referred to in the study as alternative tactics, these different choices of signalling location are
449 not equivalent to the alternative mate-finding tactics in our study, and are directly and immediately
450 affected by the presence of competing conspecifics. In addition to differences in signalling location,
451 males and females that lose fights over carcasses exhibit a whole host of alternative behaviours around
452 carcasses that have been described as "satellite" behaviour for males and "intraspecific brood
453 parasitism" for females (Bartlett, 1988; Eggert and Müller, 1997; Müller et al., 1990). Subordinate
454 individuals largely remain inactive in the soil near the carcass while exploiting opportunities to mate and

455 oviposit around the carcass; their behaviour differs clearly from that of the winners of such interactions,
456 and they do not stay to provide parental care (Eggert & Müller, 1997). Even winners behave differently
457 in the presence of competitors than when they are uncontested: female *N. vespilloides* postpone
458 oviposition on a carcass for close to a day when a smaller female is present, which enables them to
459 selectively destroy their competitor's offspring (Eggert & Müller, 2011).

460 While behavioural adjustments in response to immediate conditions are common, adjustments
461 can also occur based on earlier experience, as seen in our study. Prior mating experience can influence
462 mate choice decisions in spiders (Wilder & Rypstra, 2008; Molina & Christenson, 2008) and
463 lepidopterans (Fowler-Finn & Rodriguez, 2001). In terms of conditional strategies, however, the only
464 other example we know of where alternative mating tactics are influenced by experience is in the field
465 cricket *Teleogryllus oceanicus*, in which the acoustic environment experienced by juvenile males has
466 been shown to have a significant effect on subsequent choice of alternative mating tactics as adults
467 (Bailey, Gray, & Zuk, 2010). To the best of our knowledge, our study is the first to provide evidence of
468 males adjusting their investment in alternative tactics based on experience with reproductive resources
469 as adults.

470 Aside from investment in alternative mating tactics, burying beetles also use past experiences to
471 adjust their behaviour in other contexts. Both social history (frequency of previous interactions with
472 conspecifics) and reproductive history (the latency to production of a first brood) influence the duration
473 of parental care in male *N. orbicollis* (Scott, 1998). Based on the frequency of encounters with other
474 conspecifics, *N. orbicollis* appears to assess the competitive environment, adaptively adjusting the extent
475 of brood reduction behaviours to best match the current population density and intensity of competition
476 (Creighton, 2005). Behaviour in agonistic encounters can also be affected by the outcome of earlier
477 interactions (Otronen, 1990; Lee, Head, Carter, & Royle, 2014). In conjunction with our own findings,

478 these studies demonstrate that burying beetles possess an impressive capacity for adaptive adjustments
479 of behaviour based on their immediate and past social context and local environment.

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489 REFERENCES

- 490 Aubin-Horth, N. & Dodson, J.J. (2004). Influence of individual body size and variable thresholds on the
491 incidence of a sneaker male reproductive tactic in Atlantic salmon. *Evolution*, **58**, 136-144.
- 492 Bailey, N.W., Gray, B., & Zuk, M. (2010). Acoustic experience shapes alternative mating tactics and
493 reproductive investment in male field crickets. *Current Biology*, **20**, 845-849.
- 494 Bartlett, J. (1988). Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera:
495 Silphidae). *Behavioral Ecology & Sociobiology*, **23**, 297-303.
- 496 Becker, W. A. (1992). *Manual of Quantitative Genetics*. Academic Enterprises, Pullman.
- 497 Beeler, A.E., Rauter, C.M. & Moore, A.J. (1999). Pheromonally mediated mate attraction by males of
498 the burying beetle *Nicrophorus orbicollis*: alternative calling tactics conditional on both intrinsic
499 and extrinsic factors. *Behavioral Ecology*, **10**, 578-584.
- 500 Brockmann, H.J. (2001). The evolution of alternative strategies and tactics. *Advances in the Study of*
501 *Behaviour*, **30**, 1-51.
- 502 Brockmann, H.J. (2002). An experimental approach to alternating mating tactics in male horseshoe
503 crabs (*Limulus Polyphemus*). *Behavioral Ecology*, **13**, 232-238
- 504 Carter, M.J., Head, M.L., Moore, A.J., & Royle, N.J. (2015). Behavioral plasticity and GxE of
505 reproductive tactics in *Nicrophorus vespilloides* burying beetles. *Evolution*, **69**, 969-978.
- 506 Chemnitz, J., Jentschke, P.C., Ayasse, M., & Steiger, S. (2015). Beyond species recognition: somatic
507 state affects long distance sex pheromone communication. *Proceedings of the Royal Society B*,
508 **282**, 20150832.

- 509 Cordes, R., Kaschuba, A., & Müller, J.K. (1988). Comparison of strategies of diurnal activity of
510 *Necrophorus vespilloides* (Sil., Col.) and *Pterostichus oblongopunctatus* (Car., Col.).
511 *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **81**, 339-340.
- 512 Correa, C., Baeze, J.A., Hinojosa, I.A., & Thiel, M. (2003). Male dominance hierarchy and mating
513 tactics in the rock shrimp *Rhynchocinetes typus* (Decapoda: Caridae). *Journal of Crustacean*
514 *Biology*, **23**, 33-45.
- 515 Creighton, J.C. (2005). Population density, body size, and phenotypic plasticity of brood size in a
516 burying beetle. *Behavioral Ecology*, **16**, 1031-1036.
- 517 Dominey, W.J. (1984). Alternative mating tactics and evolutionarily stable strategies. *American*
518 *Zoologist*, **24**, 385-396.
- 519 Eggert, A.-K. (1992). Alternative male mate-finding tactics in burying beetles. *Behavioral Ecology*, **3**,
520 243-254.
- 521 Eggert, A.-K. & Müller, J.K. (1989a). Mating success of pheromone-emitting *Necrophorus* males: do
522 attracted females discriminate against resource owners? *Behaviour*, **110**, 248-258.
- 523 Eggert, A.-K. & Müller, J.K. (1989b). Pheromone-mediated attraction in burying beetles. *Ecological*
524 *Entomology*, **14**, 235-237.
- 525 Eggert, A.-K. & Müller, J.K. (2011). Timing of oviposition enables dominant female burying beetles to
526 destroy brood-parasitic young. *Animal Behaviour*, **82**, 1227-1233.
- 527 Eggert, A.-K. & Sakaluk, S.K. 1995. Female-coerced monogamy in burying beetles. *Behavioral Ecology*
528 *and Sociobiology*, **37**, 147-153.

- 529 Eggert, A.-K. & Sakaluk, S.K. (2000). Benefits of communal breeding in burying beetles: a field
530 experiment. *Ecological Entomology*, **25**, 262-266.
- 531 Emlen, D.J. (1994). Environmental control of horn length dimorphism in the beetle *Onthophagus*
532 *acuminatus* (Coleoptera: Scarabaeidae). *Proceedings of the Royal Society B*, **256**, 131-136.
- 533 Emlen, D.J. (1997). Alternative reproductive tactics and male-dimorphism in the horned beetle
534 *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Behavioral Ecology & Sociobiology*, **41**,
535 335-341.
- 536 Forsyth, A. & Alcock, J. (1989). Female mimicry and resource defense polygyny by males of a tropical
537 rove beetle, *Leistotrophus versicolor* (Coleoptera: Staphylinidae). *Behavioral Ecology &*
538 *Sociobiology*, **26**, 325-330.
- 539 Fowler-Finn, K.D. & Rodriguez, R. (2011). Experience-mediated plasticity in mate preferences: mating
540 assurance in a variable environment. *Evolution*, **66**, 459-468.
- 541 Gross, M.T. (1996). Alternative reproductive strategies and tactics: diversity within the sexes. *Trends in*
542 *Ecology & Evolution*, **11**, 92-98.
- 543 Haberer, W., Schmitt, T., Schreier, P. & Müller, J.K. (2011). Intended and unintended receivers of the
544 male pheromones of the burying beetles *Nicrophorus humator* and *Nicrophorus vespilloides*.
545 *Entomologia Experimentalis et Applicata*, **140**, 122-126.
- 546 Kammer, A.E., & Heinrich, B. (1978). Insect flight metabolism. *Advances in Insect Physiology*, **13**, 133-
547 228.
- 548 Lee, J.S.F. (2004). Alternative reproductive tactics and status-dependent selection. *Behavioral Ecology*,
549 **16**, 566-570.

- 550 Lee, V.E., Head, M.L., Carter, M.J., & Royle, N.J. (2014). Effects of age and experience on contest
551 behaviour in the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology*, **25**, 172-179.
- 552 Lessels, C.M. & Boag, P.T. (1987). Unrepeatable repeatabilities: A common mistake. *The Auk*, **104**,
553 116-121.
- 554 Levins, R. (1968). *Evolution in Changing Environments: Some Theoretical Explanations*. Princeton, NJ:
555 Princeton University Press.
- 556 Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D. & Schabenberger, O. (2006). *SAS® for*
557 *mixed models, Second Edition*. Cary, NC: SAS Institute Inc.
- 558 Marden, J.H. & Chai, P. (1991). Aerial predation and butterfly design: how palatability, mimicry, and
559 the need for evasive flight constrain mass allocation. *American Naturalist*, **138**, 15-36.
- 560 Molina, Y. & Christenson, T. (2008). Effects of mating experience on subsequent reproductive
561 behaviour and sperm release in the spider *Nesticodes rufipes*. *Animal Behaviour*, **76**, 1327-1334.
- 562 Müller, J.K., Braunisch, V., Hwang, W., & Eggert, A.-K. (2007). Alternative tactics in individuals
563 reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*.
564 *Behavioral Ecology*, **18**, 196-203.
- 565 Müller, J.K. & Eggert, A.-K. (1987). Effects of carrion-independent pheromone emission by male
566 burying beetles (Silphidae: *Necrophorus*). *Ethology*, **76**, 297-304.
- 567 Müller, J.K., Eggert, A.-K., & Dressel, J. (1990). Intraspecific brood parasitism in the burying beetle,
568 *Nicrophorus vespilloides*. *Animal Behaviour*, **40**, 491-499.

- 569 Norman, M.D., Finn, J. & Tregenza, T. (1999). Female impersonation as an alternative reproductive
570 strategy in giant cuttlefish. *Proceedings of the Royal Society B*, **266**, 1347-1349.
- 571 Otronen, M. (1990). The effect of prior experience on the outcome of fights in the burying beetle,
572 *Nicrophorus humator*. *Animal Behaviour*, **40**, 980-982.
- 573 Piché, J., Hutchings, J.A., & Blanchard, W. (2008). Genetic variation in threshold reaction norms for
574 alternative reproductive tactics in male Atlantic salmon, *Salmo salar*. *Proceedings of the Royal*
575 *Society B*, **275**, 1571-1575.
- 576 Pukowski, E. (1933). Okologische Untersuchungen an *Necrophorus* F. *Zeitschrift für Morphologie und*
577 *Oekologie der Tiere*, **27**, 518-586.
- 578 Radwan, J. (2009). Alternative mating tactics in acarid mites. *Advances in the Study of Behavior*, **39**,
579 185-208.
- 580 Reinhold, K. (1999). Energetically costly behaviour and the evolution of resting metabolic rate in
581 insects. *Functional Ecology*, **13**, 217-224.
- 582 Sakaluk, S.K. & Müller, J.K. (2008). Risk of sperm competition mediates copulation duration, but not
583 paternity, of male burying beetles. *Journal of Insect Behaviour*, **21**, 153-163.
- 584 Scott, M.P. (1998). Facultative adjustment of the duration of parental care by burying beetles. *Journal of*
585 *Insect Behavior*, **11**, 597-603.
- 586 Scott, M.P. & Traniello, J.F.A. (1990). Behavioural and ecological correlates of male and female
587 parental care and reproductive success in burying beetles (*Nicrophorus* spp.). *Animal Behaviour*,
588 **39**, 274-283.

- 589 Shelley, T.E. & Greenfield, M.D. (1985). Alternative mating strategies in a desert grasshopper: a
590 transitional analysis. *Animal Behaviour*, **33**, 1211-1222.
- 591 Shuster, S.M. (2010). Alternative Mating Strategies. In D.F. Westneat & C.W. Fox (Eds.), *Evolutionary*
592 *Behavioral Ecology* (pp. 434-450). New York: Oxford University Press.
- 593 Shuster, S.M. & Wade, M.J. (2003). *Mating Systems and Strategies*. Princeton, NJ: Princeton University
594 Press.
- 595 Taborsky, M., Oliveira, R.F., & Brockmann, H.J. (2008). The evolution of alternative reproductive
596 tactics: concepts and questions. In R.F. Oliveira, M. Taborsky & H.J. Brockmann (Eds.),
597 *Alternative Reproductive Tactics* (pp. 1-21). Cambridge: Cambridge University Press.
- 598 Tomkins, J.L. & Hazel, W. (2007). The status of the conditional evolutionary stable strategy. *Trends in*
599 *Ecology & Evolution*, **22**, 522-528.
- 600 Walling, C.A., Stamper, C.E., Salisbury, C.L. & Moore, A.J. (2009). Experience does not alter
601 alternative mating tactics in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology*,
602 **20**, 153-159.
- 603 Waltz, E.C. & Wolf, L.L. (1984). By Jove!! Why do alternative mating tactics assume so many different
604 forms? *American Zoologist*, **24**, 333-343.
- 605 Wilder, S.M. & Rypstra, A.L. (2008). Prior encounters with the opposite sex affect male and female
606 mating behaviour in a wolf spider (Araneae, Lycosidae). *Behavioral Ecology*, **62**, 1813-1820.
- 607 Wilson, D.S. & Fudge, J. (1984). Burying beetles: intraspecific interactions and reproductive
608 success in the field. *Ecological Entomology*, **9**, 195-203.