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3 **Polyandry and fitness in female horned flour beetles (*Gnatocerus***  
4 ***cornutus*).**

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25 **ABSTRACT**

26 Although polyandry is common, it is often unclear why females mate with multiple males,  
27 because although polyandry may provide females with direct or indirect fitness benefits, it can  
28 also be costly. Our understanding of polyandry is also restricted by the relative paucity of  
29 studies that disentangle the fitness effects of mating more than once with a single male and  
30 mating with multiple males. Here we investigated potential benefits and costs of polyandry  
31 in the horned beetle (*Gnatocerus cornutus*) while controlling for number of matings. We  
32 found that female lifespan was independent of mating frequency, indicating that mating itself  
33 is not very costly. However, females that mated more than once laid more eggs and had  
34 greater lifetime reproductive success than singly mated females. Because the magnitude of  
35 these effects was similar in monandrous and polyandrous females, this improved fertility was  
36 due to multiple mating itself, rather than mating with multiple males. However, polyandrous  
37 females produced more attractive sons, but they tended to have smaller mandibles and so  
38 may fare less well in male-male competition. These results indicate that polyandry is  
39 relatively cost free, at least in the laboratory, and has direct and indirect benefits to female  
40 fitness. However, because the attractive sons produced by polyandrous females may fight  
41 less well, the indirect benefits of polyandry will depend on the intensity of male-male  
42 competition and how free females are to exert mate choice. Where competition between  
43 males is intense, polyandry benefits via son attractiveness may be reduced and perhaps even  
44 carry costs to female fitness.

45

46 **KEYWORDS**

47 Coleoptera, Female choice, Fisherian process, Good genes, Insecta, Polyandry, Monandry,  
48 Sexual selection

49

## 50 INTRODUCTION

51 Polyandry, where females mate with multiple males, is common in many species of  
52 birds, mammals and insects (Arnqvist & Nilsson, 2000; Hosken & Stockley, 2003; Jennions &  
53 Petrie, 2000; Taylor, Price, & Wedell, 2014). However, we do not fully understand why  
54 females mate with more than one male, especially because mating can be costly. Mating  
55 requires time and energy (Huchard et al., 2012) and can increase the risk of parasitism (Kemp,  
56 2011), predation (Rowe, 1994), disease (Poiani & Wilks, 2000) and injury (Arnqvist, Nilsson, &  
57 Katvala, 2005). Polyandry can also elevate the intensity of sexual conflict (Holman & Kokko,  
58 2013; but see Pizzari & Wedell, 2013) and select against male parental care (Kokko & Jennions,  
59 2008; Queller, 1997). However, given that polyandrous mating is so common both across and  
60 within taxa (Taylor et al., 2014), its benefits must sometimes outweigh these considerable  
61 costs.

62 Polyandry may allow females to minimize the costs of male harassment if resisting  
63 courting males is more expensive than accepting these extra males as mates (Harano, 2015;  
64 Panova et al., 2010; Rivera & Andrés, 2002; Thornhill & Alcock 1983). Alternatively,  
65 polyandry could offer direct, material benefits transferred at or after mating that improve  
66 female lifespan or fecundity (reviewed in Arnqvist & Nilsson, 2000; Hosken & Stockley, 2003).  
67 For example, in decorated crickets males transfer a nutrient rich nuptial gift to females at  
68 mating and females that mate multiple times and receive many such gifts, live longer than  
69 females who mate less frequently (Burpee & Sakaluk, 1993). Polyandry could also provide  
70 indirect heritable, genetic benefits that improve the survival or reproductive success of  
71 offspring (Arnqvist & Nilsson, 2000; Hosken & Stockley, 2003; Jennions & Petrie, 2000;  
72 Simmons, 2001; Taylor et al., 2014; Zeh & Zeh, 2001). This is because polyandry creates the  
73 opportunity for post-copulatory sexual selection, potentially improving the fertilization success

74 of the sperm of genetically compatible (Zeh & Zeh, 2001), diverse (Taylor et al., 2014),  
75 unrelated (Hosken & Blanckenhorn, 1999; Tregenza & Wedell, 2002), viable or attractive  
76 (Slatyer, Mautz, Backwell, & Jennions, 2012) males. While there is evidence for indirect  
77 benefits of polyandry (Simmons, 2005; Slatyer et al., 2012), their role in the evolution of  
78 polyandry remains controversial (Reding, 2014; Uller & Olsson, 2008).

79 To understand why females mate polyandrously the costs and benefits (both direct  
80 and indirect) of polyandry on female fitness need to be estimated (Arnqvist & Nilsson, 2000;  
81 Jennions & Petrie, 2000). The problem is that very few studies have successfully decoupled  
82 the female fitness effects of mating more than once (e.g. multiple times with a single male)  
83 and mating with multiple males (Slatyer et al., 2012). Studies that have made this distinction  
84 have been conducted in a very few species and have often only measured a small number of  
85 fitness related traits, over a short period of time (Slatyer et al., 2012). Collecting these data is  
86 vital to improving our understanding of polyandry (Slatyer et al., 2012; Taylor et al., 2014) as  
87 well as its evolutionary and ecological consequences (Holman & Kokko, 2013).

88 Here, we investigated polyandry and its effects on female fitness in the polyandrous  
89 beetle *Gnatocerus cornutus* (Okada & Miyatake, 2009; Yamane, Okada, Nakayama, & Miyatake,  
90 2010). We use a long-running laboratory population that has been kept in large numbers  
91 (approx.  $N_e > 1000$ ), and has been able to exert free mate choice and express other behaviours  
92 in conditions that closely mimic their natural habitat. This regime has ensured the  
93 persistence of ample genetic diversity, as shown by rapid phenotypic responses to selection on  
94 mandible size (Harano, Okada, Nakayama, Miyatake, & Hosken, 2010). Additionally, traits  
95 including mass, development time and mandible size in these beetles overlap or are very close  
96 to trait estimates from independent laboratories (Demuth, Naidu, & Mydlarz, 2012; Holloway  
97 et al., 1987). This indicates that the beetles used in this study are a good representative of

98 natural populations of *G. cornutus*. In this species females exert mate choice and gain  
99 genetic benefits via the attractiveness of their sons (Okada, Katsuki, Sharma, House, & Hosken,  
100 2014). However, female choice is limited by male-male competition as unpreferred males,  
101 those with larger mandibles, gain more mates through their superior competitive abilities  
102 (Okada et al., 2014). We compared the fitness of singly mated females with those that mated  
103 multiply with a single male (monandry) or with multiple males (polyandry) using direct (e.g.  
104 lifespan, fecundity) and indirect (e.g. offspring lifespan, fertility and attractiveness) fitness  
105 measures. We then discuss the costs and benefits of polyandry in the female *G. cornutus*.

106

## 107 **MATERIALS AND METHODS**

### 108 *Stock Culture Maintenance*

109 *G. cornutus* larvae do not pupate at high densities and so following Okada and  
110 Miyatake (2010) final instar larvae were individually housed in wells of a 24-well tissue culture  
111 plate with 1g of food (Cellstar; Greiner Bio-One, Frickenhausen, Germany). After adult  
112 emergence, *G. cornutus* males can take up-to seven days to attain sexual maturity (Katsuki,  
113 Harano, Miyatake, Okada, & Hosken, 2012; Katsuki, Okada, & Okada, 2012) and so we allowed  
114 individuals from both sexes to mature for 14 days before using them in experiments. All  
115 experiments within this study follow this maintenance protocol unless stated otherwise.

116

### 117 *Mating Regimes*

118 Experimental animals were removed from the stock population at their final instar  
119 stage and then randomly allocated to one of three experimental treatments: monandry  
120 (multiple mated to one male), polyandry (multiply mated with multiple males) or single mating  
121 (one copulation with one male) (n = 50 / treatment). Two weeks after adult emergence,

122 experimental females were weighed to the nearest 0.01 mg on an electronic balance  
123 (Mettler-Toledo AG, Laboratory and Weighing Technologies, Greifensee, Switzerland) and then  
124 individually aspirated into plastic containers (17 mm diameter, 20 mm high), which contained a  
125 disc of filter paper for traction (17 mm diameter). After thirty minutes, a virgin  
126 stock-population male was added to each dish and the pair were observed until they finished  
127 mating. After copulating, pairs were separated and females placed in a well of the tissue  
128 culture plate with 1g of food. Mating began on day 14 post eclosion, such that singly mated  
129 females were mated once on day 14, while monandrous and polyandrous females were mated  
130 once every day between days 14 and 17 post-eclosion to either a single male (monandry) or to  
131 each of four different males (polyandry).

132

### 133 *Assaying Direct Effects on Female Fitness*

134 Fifty females from each treatment were divided into two groups ( $n = 25$  /group). One  
135 of these groups was used to measure lifetime reproductive success (LRS - the number of adult  
136 offspring that hatch from eggs), while the other group was used to assay fecundity (the  
137 number of eggs laid). Lifespan was examined in females from both of these groups.

138 Females used to measure LRS were transferred after mating into an egg-laying vial (70  
139 mm diameter, 25 mm high) containing excess food (20 g) for two months. LRS of each female  
140 was used as a proxy for female fitness (Katsuki, Harano, et al., 2012; Katsuki, Okada, et al.,  
141 2012; Tsuda & Yoshida, 1985) and measured as the total number of adult offspring that  
142 emerged from these vials. Females from all treatment groups (singly mated, monandry or  
143 polyandry) were transferred into egg-laying vials at the same age i.e. 18 days post-eclosion.  
144 This ensured that females had the same amount of time in which to lay eggs and therefore, all  
145 fertility measures are directly comparable across treatment groups. After two months,

146 females were moved into new survival assay vials (40 mm high, 15 mm diameter) containing  
147 an excess of the culture medium (4 g).

148 Females in the fecundity assay group were transferred after mating into an egg laying  
149 vial containing excess food (20 g) for two months. Their eggs were separated from this food by  
150 sieving and were counted twice a week, because eggs take 4.6 days to hatch (Tsuda & Yoshida,  
151 1984). After two months, females were also moved into survival assay vials. Their survival  
152 after mating in both groups monitored weekly until death.

153 Lifespan and fecundity (e.g. egg counts) measure the direct effects of polyandry on  
154 female fitness, while LRS may indicate indirect effects of polyandry if differences between  
155 females are due to variation in embryo survival or direct effects if differences are due to how  
156 many ova are fertilized. Our mating regime allows us to distinguish between these  
157 possibilities: direct benefits of multiple mating are likely to improve fertility in polyandrous and  
158 monandrous females to a similar extent relative to singly mated females, while indirect genetic  
159 benefits will improve fertility in polyandrous females relative to other treatment regimes. It  
160 is important to highlight that our mating regime means that monandrous multiple-mating  
161 females (mated four times to a single male), could potentially suffer from the effects of male  
162 sperm depletion more than polyandrous females, mated to four different, virgin males.  
163 However, past work has shown that sperm counts do not differ between a male's first and  
164 second mating, 24 hours later. This reduces the potential for sperm depletion effects on  
165 fertility in our monandrous females but does not preclude it completely.

166

#### 167 *Assaying Indirect Effects on Female Fitness*

168 From the females that were used to assay fecundity, twenty eggs were randomly  
169 selected per female (N=20 / treatment) and each of these newly laid eggs were reared in a

170 glass vial (15 mm diameter, 40 mm high) with an excess of the culture medium (4 g). We  
171 recorded development time from egg to adult by monitoring these larvae daily. On the day  
172 of adult emergence, offspring were weighed to the nearest 0.01 mg. Longevity and LRS were  
173 measured in daughters (mean = 8/dam), all of which were mated just once, but were  
174 otherwise treated in the same way as their mothers, while additional traits were assayed in  
175 sons. To assay son attractiveness and courtship behaviors, virgin females were randomly  
176 chosen from the stock population and individually aspirated into in plastic dishes (17 mm  
177 diameter, 20 mm high) lined with filter paper. After 30 minutes, one experimental male was  
178 added to each dish. The pair were continuously observed until the end of mating, when sons  
179 were removed to avoid re-mating. Following Okada et al. (2014), copulation latency (the  
180 time from male introduction to commencement of copulation) was measured as an indicator  
181 of son attractiveness. This is a widely used measure of male attractiveness (Okada, Blount,  
182 Sharma, Snook, & Hosken, 2011; Shackleton, Jennions, & Hunt, 2005; Taylor, Wedell, & Hosken,  
183 2007). We also measured courtship rate as a measure of courtship quality (e.g. Simmons &  
184 Holley, 2011). Copulation rate is measured as the number of bouts of tapping of the female'  
185 body a male performs per unit time. We measure this by counting how many times a male  
186 performs this courtship behavior from the point at which they are introduced to a female until  
187 the point at which copulation begins. Both copulation latency and courtship rate are highly  
188 repeatable measures (Okada et al., 2014). After mating trials, mandible length ( $\pm 0.01$  mm) of  
189 each male was measured using a dissecting microscope linked to a PC (VM-60, Olympus, Tokyo,  
190 Japan). Longevity was then assayed in these sons, as described for their mothers.

191

## 192 *Statistical Analysis*

193 The LRS, fecundity and longevity of mothers were analyzed using analysis of variance



194 (ANOVA) with mating treatment (monandry, polyandry or singly mated) and female body size  
195 as fixed effects. Sex-specific mean values of each trait measured in sons and daughters  
196 (development time, body size, longevity, and LRS) and for traits only assayed in sons  
197 (copulation latency, courtship rate, mandible size) were calculated for each mother. Each  
198 trait mean was then analyzed using an ANOVA with mating treatment as a fixed effect.  
199 Student's *t* tests were used to compare means, correcting the significance level for multiple  
200 comparisons by the sequential Bonferroni method (Rice, 1989). All analyses were performed  
201 using JMP 7.0 for windows (SAS Institute 2007).

202

### 203 *Ethical Note*

204 The stock population of *G. cornutus* used in this study originated from adults collected in  
205 Miyazaki City, Japan (31° 54'N, 131° 25' E) in June 1957. This population has been maintained  
206 on whole-meal flour enriched with yeast (Okada & Miyatake, 2010; Okada, Yamane, &  
207 Miyatake, 2010) at 25°C and 60% relative humidity under a 14 : 10 h light / dark cycle. These  
208 laboratory conditions closely resemble natural conditions of this stored product pest. All  
209 individuals in the experiment were handled with care and handling time was kept to an  
210 absolute minimum. Note that the use of these beetles conforms to the University of Exeter's  
211 Animal Ethics Policy.

212

## 213 **RESULTS**

### 214 *Direct Effects on Female Fitness*

215 We found significant effects of female mating treatment and female body size on  
216 female fecundity (treatment,  $F_{2, 71} = 16.91$ ,  $P < 0.001$ ; mass,  $F_{1, 71} = 21.59$ ,  $P < 0.001$ ) and LRS  
217 (treatment,  $F_{2, 71} = 15.523$ ,  $P < 0.0001$ ; body size,  $F_{1, 71} = 22.651$ ,  $P < 0.0001$ ). Multiple

218 comparisons showed that the number of eggs laid and LRS was significantly higher in  
219 monandrous and polyandrous females than in singly mated animals (Table 1). This  
220 comparable LRS in both monandrous and polyandrous females suggests that this elevated LRS  
221 is due to direct effects of mating. Crucially, this result also suggests that monandrous  
222 females (mated four times to one male) did not experience reduced fertility relative to  
223 polyandrous females (mated four times, to four virgin males), as a result of sperm depletion in  
224 their mate.

225 In the LRS and fecundity groups, female longevity was not affected by mating  
226 treatment or body size (Table 1; LRS, treatment,  $F_{2, 71} = 0.951$ ,  $P = 0.3912$ ; body size,  $F_{1, 71} =$   
227  $3.187$ ,  $P = 0.0785$ ; fecundity,  $F_{2, 71} = 0.361$ ,  $P = 0.6982$ ; body size,  $F_{1, 71} = 0.012$ ,  $P = 0.9131$ ).  
228 Moreover, within each treatment, there was no significant difference in longevity between  
229 both the LRS and fecundity assay groups (single mating,  $t = 1.007$ ,  $P = 0.3191$ ; monandry,  $t =$   
230  $-0.301$ ,  $P = 0.7649$ ; polyandry,  $t = -0.174$ ,  $P = 0.8628$ ).

231

### 232 *Indirect Effects on Female Fitness*

233 Sons of polyandrous females developed faster than sons from any other treatment  
234 groups and were more attractive (i.e. had shorter copulation latency) and had a greater  
235 courtship rate than other sons (Tables 2 & 3). However, sons of polyandrous females also  
236 had significantly shorter mandibles compared to sons produced from other mating treatments  
237 (Tables 2 & 3). Female mating treatment had no significant effect on sons' body size or  
238 longevity. Daughters of polyandrous females developed more quickly than other female  
239 offspring but daughters did not differ in any other trait assayed (Tables 2 & 3).

240

241

242 **DISCUSSION**

243           We demonstrate a clear, direct fitness advantage to multiple mating in *G. cornutus*.  
244 Irrespective of whether females mate with one or four males, mating multiply improved  
245 female fecundity and LRS. In insects in general it appears that mating just once does not  
246 always maximize female fertility (Arnqvist & Nilsson, 2000), and that it is often multiple mating,  
247 rather than polyandry, that has direct positive effects on female fecundity (South & Lewis,  
248 2011). These increases in female fecundity may be driven by accessory substances  
249 transferred with a male's ejaculate (Avila, Sirot, LaFlamme, Rubinstein, & Wolfner, 2011) or  
250 resources, such as amino acids or water, that trigger or elevate egg laying (Vahed, 1998).  
251 Alternatively, improved female fecundity may simply reflect that a single mating does not  
252 provide females with enough sperm to fertilize all of their eggs (Kraus, Neumann, Praagh, &  
253 Moritz, 2004; Slatyer et al., 2012). Consistent with this latter explanation, *G. cornutus*  
254 copulation is brief and relatively few sperm are transferred (Okada et al., 2010; Yamane et al.,  
255 2010). Irrespective of the underlying mechanism, multiple mating is often associated with  
256 reduced female lifespan (Arnqvist & Nilsson, 2000; Slatyer et al., 2012). This can either be  
257 due to the survival costs of mating itself (e.g. injury) (Blanckenhorn et al., 2002) or the direct  
258 costs of elevated egg production following multiple mating (Chapman, Takahisa, Smith, &  
259 Partridge, 1998). In *G. cornutus*, neither multiple mating nor the subsequent increases in  
260 fecundity reduced female survival: females that mated multiply, laid more eggs than once  
261 mated females, without experiencing significant reductions in lifespan. Overall, this suggests  
262 that the costs of both mating and of laying eggs are low in this species (at least in the  
263 laboratory) and that mating offers females direct fitness benefits.

264           In addition to these direct benefits of mating multiply, polyandry had clear indirect  
265 benefits for female fitness. Firstly, polyandry was associated with rapid development to

266 adulthood, something seen in other taxa (Hosken, Garner, Tregenza, Wedell, & Ward, 2003).  
267 The fitness consequences of rapid growth are unknown in flour beetles (Okada et al., 2014),  
268 but we did not detect any costs to fast development later in life (e.g. reduced lifespan or LRS)  
269 and rapidly developing genotypes are probably generally advantageous in grain pests (Jayas,  
270 White, & Muir, 1994). Thus rapid development may improve offspring fitness in natural  
271 populations, although this needs to be tested. Less ambiguously, polyandry clearly improved  
272 the attractiveness of sons: males whose mothers mated polyandrously invested more  
273 intensely in courtship behaviors and were more attractive to females. Investment in  
274 courtship is under strong pre-copulatory sexual selection via female choice in this species  
275 (Okada et al., 2014) and so it appears that polyandrous mating in *G. cornutus* can indirectly  
276 elevate fitness. It is unclear if this is driven by cryptic female choice for attractive males or  
277 because these males produce highly competitive sperm (Jennions & Petrie, 2000) but there is  
278 some evidence for the latter idea. Attractive males produced by polyandrous mothers  
279 tended to have smaller mandibles. Mandible size is positively genetically associated with  
280 competitive ability in males (Okada & Miyatake, 2009; Yamane et al., 2010) and mating success  
281 (Harano et al., 2010) and so is a major determinant of male fitness. However, males with  
282 large mandibles transfer fewer sperm per ejaculate and have smaller testes (Yamane et al.,  
283 2010). This may mean males with large mandibles are disadvantaged in sperm competition –  
284 if sperm competition proceeds via the raffle principle whereby the more sperm a male  
285 transfers, the greater the probability of fertilization success (Parker, 1990).

286           These results highlight the complex interplay between male-male competition,  
287 female choice and sexual conflict over mating in this species. Males with large mandibles  
288 gain high reproductive success by out-competing other males and by coercing females into  
289 mating but transfer few sperm. Males that invest heavily in courtship are highly attractive to

290 females, despite having smaller mandibles, and transfer more sperm, which may improve their  
291 paternity during sperm competition. Clearly there are different routes to reproductive  
292 success in *G. cornutus*. Crucially, the benefits of either of these male strategies (attractive  
293 versus competitive phenotypes) probably depend on population density. This is because the  
294 probability of males gaining reproductive success by outcompeting their rivals (e.g. via combat)  
295 are reduced when rivals are rare (Kokko & Rankin, 2006). This means that at high densities,  
296 the benefits of having large mandibles are likely to increase because male reproductive success  
297 increasingly depends on the outcome of male-male competition. At low population densities,  
298 males that are very attractive may gain reproductive success by readily attracting females  
299 without having to frequently fight off competitors. This means that as population density  
300 increases, the indirect fitness benefits of polyandry (i.e. producing attractive but less  
301 competitive sons) may decrease and it is easy to imagine that this increased offspring  
302 attractiveness could even carry fitness costs in male biased, high density populations.

303           Environmental factors often influence female remating decisions (Bleu, Bessa-Gomes,  
304 & Laloï, 2012; Pai & Yan, 2002) and social situation (e.g. sex ratio), mate encounter rates and  
305 demography can all affect the costs and benefits of polyandry (Bleu et al., 2012; Holman &  
306 Kokko, 2013; Kokko & Mappes, 2013). Clearly, to understand the evolution of polyandry, the  
307 costs and benefits of multiple mating need to be considered in an ecological context. Indeed,  
308 while recent meta-analyses that have shown a weak, positive effect of polyandry on offspring  
309 viability (Slatyer et al., 2012), indirect genetic benefits to polyandrous mating are controversial  
310 (Reding, 2014; Uller & Olsson, 2008). Perhaps this controversy persists because the  
311 magnitude of the indirect benefits of polyandry depend enormously on population ecology  
312 and demography, factors that are often overlooked in laboratory research.

313

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459 **Table 1.** Means  $\pm$  SE of fitness measures for mothers.

Trait	Mating Treatment		
	Singly mated	Monandry	Polyandry
Lifetime reproductive success (LRS)	158.276 $\pm$ 5.53 <sup>a</sup>	189.36 $\pm$ 5.94 <sup>b</sup>	191.84 $\pm$ 4.32 <sup>b</sup>
Fecundity	142.20 $\pm$ 5.05 <sup>c</sup>	170.40 $\pm$ 4.30 <sup>d</sup>	174.76 $\pm$ 6.21 <sup>d</sup>
Longevity – fecundity group (weeks)	33.60 $\pm$ 1.00	31.56 $\pm$ 1.59	30.64 $\pm$ 2.03
Longevity – LRS group (weeks)	32.08 $\pm$ 1.13	32.08 $\pm$ 0.69	30.64 $\pm$ 2.03

460 Different letters indicate a significant difference at  $P < 0.05$  by Student's t test with the  
 461 sequential Bonferroni method (Rice, 1989).

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465 **Table 2.** Means  $\pm$  SE of offspring traits of each treatment.

Trait	Mating Treatment		
	Singly mated	Monandry	Polyandry
<i>Male offspring</i>			
Development time (days)	46.07 $\pm$ 0.33 <sup>a</sup>	46.71 $\pm$ 0.31 <sup>a</sup>	44.95 $\pm$ 0.29 <sup>b</sup>
Body mass (mg)	2.72 $\pm$ 0.006	2.73 $\pm$ 0.006	2.72 $\pm$ 0.007
Longevity (weeks)	29.78 $\pm$ 0.51	29.98 $\pm$ 0.56	30.08 $\pm$ 0.49
Copulation latency (seconds)	1181.38 $\pm$ 38.01 <sup>a</sup>	1148.39 $\pm$ 40.66 <sup>a</sup>	1004.07 $\pm$ 47.38 <sup>b</sup>
Courtship rate (per second)	0.06 $\pm$ 0.003 <sup>a</sup>	0.06 $\pm$ 0.003 <sup>a</sup>	0.09 $\pm$ 0.004 <sup>b</sup>
Mandible length (mm)	0.40 $\pm$ 0.007 <sup>a</sup>	0.40 $\pm$ 0.006 <sup>a</sup>	0.38 $\pm$ 0.005 <sup>b</sup>
<i>Female offspring</i>			
Development time (days)	44.49 $\pm$ 0.40 <sup>a</sup>	44.62 $\pm$ 0.29 <sup>a</sup>	43.34 $\pm$ 0.25 <sup>b</sup>
Body mass (mg)	2.70 $\pm$ 0.007	2.70 $\pm$ 0.004	2.70 $\pm$ 0.005
Longevity (weeks)	34.49 $\pm$ 0.46	33.48 $\pm$ 0.56	34.99 $\pm$ 0.46
LRS	124.23 $\pm$ 2.79	124.88 $\pm$ 2.69	123.55 $\pm$ 2.58

466 Different letters indicate a significant difference at  $P < 0.05$  by Student's t test with the  
 467 sequential Bonferroni method (Rice, 1989). Average values for offspring of 20  
 468 mothers/treatment.

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472 **Table 3.** Analysis of variance in traits measured in sons and daughters

Traits	Effect	df	Mean square	F	P
<i>Male offspring</i>					
Development Time	Treatment	2	15.80	8.24	<0.01
	Error	57	1.92		
Body size	Treatment	2	<0.01	0.51	0.60
	Error	57	<0.01		
Longevity	Treatment	2	0.49	0.09	0.91
	Error	57	5.39		
Copulation latency (attractiveness)	Treatment	2	177849	4.99	0.01
	Error	57	35621		
Courtship rate	Treatment	2	<0.01	21.04	<0.01
	Error	57	<0.01		
Mandible size	Treatment	2	<0.01	5.24	0.01
	Error	57	<0.01		
<i>Female offspring</i>					
Development Time	Treatment	2	9.93	4.88	0.01
	Error	57	2.03		
Body size	Treatment	2	<0.01	0.20	0.82
	Error	57	0.001		
Longevity	Treatment	2	11.89	2.43	0.10
	Error	57	4.89		
LRS	Treatment	2	8.90	0.06	0.94
	Error	57	144.77		

