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3 **Kin selection and sexual conflict: male relatedness and**  
4 **familiarity do not affect female fitness in seed beetles**

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## 26 **Summary**

27 Theory maintains that kin selection can mediate sexual conflict because  
28 within-group male relatedness should reduce male-male competition,  
29 thereby reducing collateral harm to females. We tested whether male  
30 relatedness and familiarity can lessen female harm in the seed beetle  
31 *Callosobruchus maculatus*. Neither male relatedness nor familiarity  
32 influenced female lifetime reproductive success or individual fitness.  
33 However, male relatedness, but not familiarity, marginally improved female  
34 survival. Because male relatedness improved female survival in late life  
35 when *C. maculatus* females are no longer producing offspring, our results  
36 do not provide support for the role of kin selection in mediating sexual  
37 conflict. The fact that male relatedness improves the post-reproductive part  
38 of female life cycle strongly suggests that the effect is non-adaptive. We  
39 discuss adaptive and non-adaptive mechanisms that could result in  
40 reduced female harm in this and previous studies and suggest that  
41 cognitive error is a likely explanation.

42

43 **Keywords:** sexual conflict, kin selection, mate harm, *Callosobruchus*

44 *maculatus*

45

## 46 1. INTRODUCTION

47 Males and females have different routes to successful reproduction [1], and  
48 this can lead to evolutionary conflict between the sexes [2-4]. One extreme  
49 form of this conflict is mate harm, when one sex (usually the male) physically  
50 injures the opposite sex (usually the female) [4, 5]. Male mate harm occurs in  
51 many animals and has been especially well studied in insects, including the  
52 bed bug *Cimex lectularius*, the seed beetle *Callosobruchus maculatus*, and  
53 the fruit fly *Drosophila melanogaster*. Male bed bugs stab females with their  
54 genitalia, inseminating females directly into the abdominal cavity [6, 7]. Male  
55 seed beetles have spines on their intromittent organs that pierce holes in the  
56 female's genital tract, reducing female longevity [8-10]. In the fruit fly, males  
57 may harass females during courtship [11-14] or physically harm them during  
58 the mating process [11, 15]. *Drosophila* and *C. maculatus* ejaculates also  
59 contain accessory gland proteins that modulate female reproductive behaviour  
60 [5, 16-18]. Mate harm can evolve for two reasons. One possibility is that the  
61 harm itself increases male fitness by causing females to allocate more  
62 resources into current reproduction and away from future reproductive  
63 attempts with other males [19]. A better-supported alternative in most cases is  
64 that mate harm is simply a deleterious side effect of male-male competition  
65 over fertilization [4, 20, 21].

66 Kin selection theory [22, 23] suggests that the level of genetic  
67 relatedness among competing males can moderate sexual conflict in viscous  
68 populations [24-27]. In populations in which close adult relatives are likely to  
69 interact, males would gain indirect fitness benefits by helping their close male  
70 kin to reproduce. Such cooperation might take the form of reducing mate harm

71 to facilitate sexual access to those females. As Chippindale *et al.* [28] point  
72 out, for this kind of kin selection to occur, three conditions must be met: 1)  
73 males must harm their mates in some way, thereby reducing female  
74 reproductive success; 2) there must be some mechanism in place for reliably  
75 recognizing kin; and 3) groups of related males must have a reasonable  
76 chance of encountering each other during the reproductive period.

77 A series of recent studies on *Drosophila melanogaster* has investigated  
78 this possible role of kin selection in moderating mate harm, with conflicting  
79 results [28-33]. In support of the kin selection hypothesis, Carazo *et al.* [28]  
80 found that females exposed to groups of three full-sib brothers did indeed  
81 have higher lifetime reproductive success and slower reproductive ageing  
82 than females exposed to trios of unrelated males. Groups of brothers were  
83 less aggressive towards each other, courted females less vigorously, and  
84 lived longer. Finally, Carazo *et al.* [28] found that when two brothers were  
85 housed with an unrelated male, that unrelated male sired more than one-third  
86 of the offspring, suggesting that groups of cooperating relatives are vulnerable  
87 to invasion by non-cooperative non-relatives.

88 One important point to consider in the Carazo *et al.* [28] study is that it  
89 confounded familiarity and relatedness. Brothers used in the study had also  
90 been reared together in the same vial, whereas the unrelated males had been  
91 raised in separate vials. Hollis *et al.* [30] conducted a follow-up study on a  
92 different population of *Drosophila* in which they controlled for familiarity by  
93 testing the effect of brothers raised together versus apart. Females exposed  
94 to brothers raised together had higher lifetime reproductive success, but this  
95 effect disappeared when females were raised with brothers raised apart.

96 Hollis *et al.* [30] concluded that familiarity and not relatedness *per se* was  
97 likely driving the patterns Carazo *et al.* [28] observed.

98         However, one weakness of Hollis *et al.*'s [30] study is that they did not  
99 include a treatment with unrelated males that had been reared together.  
100 Without this treatment it is impossible to conclude whether familiarity alone  
101 would be sufficient for cooperation. To address this issue, Chippindale *et al.*  
102 [29] performed a fully crossed experiment in which they exposed females to  
103 brothers that had been raised together, brothers that had been raised apart,  
104 and unrelated males that had been raised apart. Unlike Carazo *et al.* [28],  
105 they found no evidence that either familiarity or relatedness among males had  
106 any effect on female lifespan or reproductive success, a result corroborated in  
107 a separate study by Martin and Long [31]. Further, Chippindale *et al.* [29]  
108 found no evidence that unrelated or unfamiliar males sired a disproportionate  
109 number of offspring when introduced to pairs of brothers or males raised in  
110 the same environment. They conclude that cooperation among males does  
111 not appear to lower mate harm in this system, at least not in the populations  
112 they examined. Finally, a later study by Le Page *et al.* [32] suggested that  
113 both relatedness and familiarity are required for reduced female harm in *D.*  
114 *melanogaster* in the population used by Carazo *et al.* [28, 33].

115         These conflicting results suggest that, at least in *Drosophila*, it remains  
116 unclear what role kin selection plays in mediating male-male cooperation and  
117 mate harm. Moreover, if we are to understand whether inclusive fitness  
118 benefits mediate sexual conflict in the animal kingdom, we need to expand our  
119 research focus into other model systems. One excellent candidate is the seed  
120 beetle *C. maculatus*. As described above, male seed beetles inflict physical

121 harm on their mates [9, 10] and this species has been used routinely as a  
122 model system to study the economics and genetics of sexual conflict over  
123 lifespan and reproduction [34-36].

124 A recent study by Lymbery *et al.* [37] generally supported the  
125 importance of male relatedness in mediating male harm to females. They  
126 found that females housed with familiar brothers produced more offspring,  
127 suggesting that relatedness and familiarity among males act together to  
128 reduce male-induced harm to females. However, the beetles in the Lymbery  
129 *et al.* [37] study were provided with Baker's yeast, which is rather unusual for  
130 a species that inhabits human grain storages and is commonly kept in the  
131 laboratory as a capital breeder that is aphagous in the adult stage.  
132 Furthermore, while *C. maculatus* beetles can technically ingest yeast, yeast  
133 consumption *per se* does not necessarily have a positive effect on longevity,  
134 fecundity or offspring production [38] suggesting that access to yeast is not a  
135 part of a normal life cycle of this species. Therefore, we investigated the effect  
136 of male relatedness and familiarity in a large outbred and well-described  
137 population of *C. maculatus* (SI USA) that was not provided with yeast in the  
138 adult stage, which is in line with the recent evolutionary history of this species  
139 and this population. We conducted a fully crossed experiment with respect to  
140 male relatedness and familiarity, quantifying the lifetime reproductive success  
141 and lifespan of virgin females exposed to four different trios of males: 1)  
142 brothers raised together, 2) brothers raised apart, 3) unrelated males raised  
143 together, and 4) unrelated males raised apart. If kin selection is indeed  
144 mediating sexual conflict in this system, then brothers raised together should

145 exhibit less mate-harming behaviour than other groups, resulting in higher  
146 relative fitness of females.

147

## 148 **2. METHODS**

### 149 **(a) Study system**

150 Seed beetles are common pest of stored legumes indigenous to Asia and  
151 Africa. Females lay their eggs on the surface of dried beans. Once the larvae  
152 hatch, they burrow into the bean and eclose as reproductively mature adults  
153 approximately 23-27 days later. *C. maculatus* are facultatively aphagous,  
154 obtaining all the nutrients they require for survival and reproduction during the  
155 larval stage [39]. Adult feeding increases fecundity and longevity [39]. Early  
156 studies used a combination of yeast and sugar solutions, so it was difficult to  
157 disentangle the effect of the separate components in fitness-related traits.  
158 Ursprung *et al.* [38] found that sugar solution and water do increase fecundity  
159 and longevity, but there was no effect of yeast on these key life-history traits.  
160 Lymbery *et al.* [37] provided their study beetles with *ad lib* access to yeast, but  
161 there was no obvious benefit in terms of fecundity or longevity, although the  
162 direct comparison is not possible because their study did not include standard  
163 aphagous conditions.

164 The study population was derived from an outbred South Indian stock  
165 population ("SI USA") of *C. maculatus* originally obtained from C. W. Fox at  
166 the University of Kentucky, USA, and then subsequently moved to Uppsala  
167 University and finally to The American University of Paris three months prior to  
168 the first block of the experiment. The original SI USA stock population was  
169 collected from infested mung beans (*Vigna radiata*) in Tirunelveli, India in

170 1979 [40]. Both prior to and during the experiment, beetles were cultured  
171 exclusively on mung beans and kept at aphagy (no food or water) in climate  
172 chambers at 29°C, 50% relative humidity and a 12:12 h light:dark cycle. One  
173 great advantage of this system is that the laboratory conditions closely  
174 resemble natural conditions, because these beetles have associated with  
175 dried legumes for thousands of years and their life history is adapted to life in  
176 a storage environment [41, 42].

177

### 178 **(b) Establishing the four treatment groups**

179 The experiment was carried out in two blocks. The first block was completed  
180 in 2015. In 2018 the experiment was replicated and expanded to provide  
181 additional data on daily fecundity of females. During both blocks, base  
182 populations of beetles were kept in 1L jars with 150g of mung beans, and  
183 approximately 250 newly hatched beetles were transferred to new jars with  
184 fresh beans every 24 days on a continual basis. From this base population,  
185 we established four different treatment groups (with a goal of approximately N  
186 = 75 each in each block, 150 total): **1) related familiar (RF), 2) related**  
187 **unfamiliar (RU), 3) non-related familiar (NF), and 4) non-related unfamiliar**  
188 **(NU)**. In the RF treatment, three full-sib brothers were housed in a Petri dish  
189 together for 24 hours before being added to a dish with a (non-related)  
190 female. In the RU treatment, three brothers with no prior experience with each  
191 other were added all at once to a dish with a female. In the NF treatment,  
192 three non-related males were housed together for 24 hours before being  
193 added to a dish with a female. Finally, in the NU treatment, three non-related  
194 and unfamiliar males were added to a dish with a female.



195           To generate full sibling brothers for the RF and RU treatments, we  
196 transferred a random subset of beans with developing larvae into virgin  
197 chambers (aerated plastic culture plates with a separate well for each  
198 individual) and monitored the virgin chambers daily. Approximately one day  
199 after hatch, we randomly paired 180 males and females and placed them into  
200 180 60-mm Petri dishes with 75 beans each. We then removed the males and  
201 females after 48 hours and allowed the eggs to develop. Since females can  
202 lay up to 65 eggs per day (Berg, unpublished data), we wanted to provide  
203 enough beans that females would lay only one egg on each bean. Before the  
204 offspring hatched, we transferred the fertilized beans from the Petri dishes to  
205 virgin chambers, carefully marking which beans came from which parents,  
206 and monitoring hatch daily. Once these offspring hatched, we set up the four  
207 different treatment groups above.

208           For both “familiar” treatments, trios of males were introduced to each  
209 other on the same day that they hatched. For both “unfamiliar” treatments,  
210 males were housed in their separate virgin cells until one day post hatch and  
211 then introduced together with the female without any time to acclimatize to  
212 each other. In all treatments, females were randomly selected from the base  
213 population one day after hatch and were unrelated to the males. Males from  
214 the non-related treatments were randomly selected from the base population  
215 as well. All males and females used in this study were maintained as virgins  
216 prior to the pairing. The Petri dishes in which males and females were housed  
217 measured 100-mm and contained 150 beans. This number is sufficient to  
218 allow females to lay just one egg per bean, reducing any larval competition  
219 that might affect data on reproductive success. All sets of brothers used in this

220 study came from different parents, thus obviating the need to control for  
221 parental identity in the analyses.

222 Three days after the trios of males were introduced to females, we  
223 swapped out all the males for new males. This was done to reduce the  
224 variance caused by male condition or behaviour on female reproductive  
225 success or lifespan. In preparation for this, we set up new trios of freshly  
226 hatched related familiar and non-related familiar males one day before. For all  
227 the “related” dishes, we used brothers of the previous trio. Since fewer males  
228 were eclosing this late in the hatch cycle, we had to use slightly older males in  
229 some cases. We excluded few females that escaped/died from unnatural  
230 causes resulting in slight deviations from the initial sample size (N = 75 for  
231 RF, N = 75 for RU, N = 71 for NF, and N = 76 for NU in the first block; N = 75  
232 for all treatments in the second block).

233

### 234 **(c) *Lifespan and fitness assays***

235 During both blocks of the experiment, we conducted both lifespan and fitness  
236 assays for each female within each treatment group. For lifespan assays, we  
237 monitored the Petri dishes daily and recorded the date of death of each  
238 female. Once all adults were dead, we removed them from the dishes. We  
239 collected two kinds of fitness data. During the first block of the experiment, we  
240 measured total offspring production only. We did this by counting the number  
241 of eclosed young per female, a standard measure of lifetime reproductive  
242 success in this system. To facilitate the counting of offspring, we froze the  
243 dishes 37 days after the initial pairing, well after all the offspring had eclosed  
244 but before a subsequent generation could develop.

245           During the second block, we also measured daily offspring production  
246 for each female. To do this we moved the female and males to new Petri  
247 dishes with new beans every 24 hours until the female died (maximum of 9  
248 sets of Petri dishes per female). Approximately 37 days later, we froze the  
249 dishes and counted number of eclosed offspring per day per female.

250

#### 251 **(d) Statistical analyses**

252 Before analysis, we excluded all individuals that did not reproduce (NF = 2,  
253 NU = 4, RF = 4, RU = 10). We analysed the lifetime offspring production as  
254 well as age-specific reproduction using a generalized mixed effect model with  
255 a Poisson error structure implemented in the *lme4* package in R 3.3.3. ,  
256 treating Relatedness and Familiarity as crossed fixed factors. We tested for  
257 overdispersion using the *dispersion\_glm* function in the *blmeco* package,  
258 and if above 1.4, we controlled for overdispersion by adding a subject-level  
259 random effect. For total reproduction, we used Block as a random factor.

260           Age-specific reproduction and individual fitness was only analysed for  
261 Block 2, as this was the only block where age-specific fecundity data was  
262 collected. For age-specific reproduction, we included Relatednes and  
263 Familiarity as crossed fixed factors, as well as all interactions with Age and  
264 Age<sup>2</sup>. In addition, we also included Age at last reproduction (ALR) as a  
265 crossed covariate. Age and ALR were scaled and centered before analysis  
266 (mean = 0, s.d. = 1) and we used the *bobyqa* optimizer as well as increased  
267 the default number of iterations to 10.000 in order to obtain good model  
268 convergence. For all mixed-effect models, chi-square tests of fixed effects  
269 were performed using the *car* package.

270 Individual fitness ( $\lambda_{\text{ind}}$ ) was calculated from the life-table of age-specific  
271 reproduction [43, 44], with a development time of 23 days, by solving the  
272 Euler-Lotka equation for each individual using the *lambda* function in the  
273 *popbio* package.  $\lambda_{\text{ind}}$  was then analysed in a linear model using Relatedness  
274 and Familiarity as crossed fixed factors.

275 Survival was analysed in a Cox proportional hazard model using the  
276 *coxme* package, with Relatedness and Familiarity as crossed fixed factors,  
277 and Block as a random effect.

278

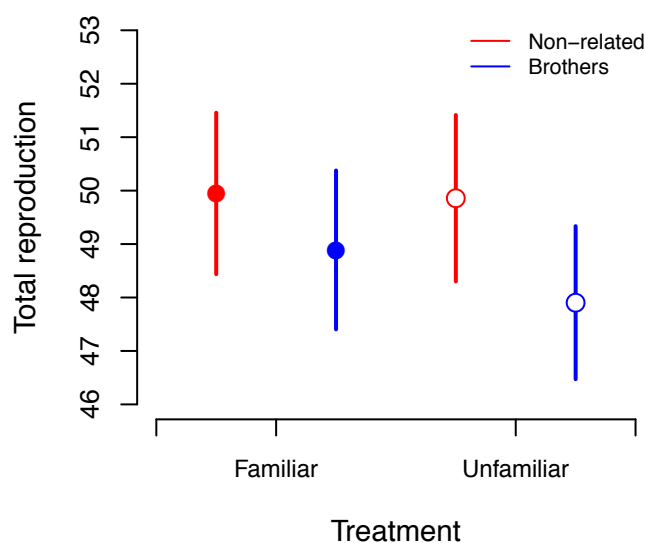
### 279 **3. RESULTS**

280 We measured lifetime reproductive success (total number of eclosed  
281 offspring) of individual females introduced to one of four different groups of  
282 male trios: brothers raised together (related familiar, or RF, N = 146), brothers  
283 raised separately (related unfamiliar, RU, N = 140), non-related males raised  
284 together (non-related familiar, NF, N = 144), or non-related males raised  
285 separately (non-related unfamiliar, NU, N = 147). There was no significant  
286 difference in female lifetime reproductive success between the four treatments  
287 (Relatedness:  $\chi^2 = 1.37$ , df = 1, p = 0.392; Familiarity:  $\chi^2 = 0.00$ , df = 1, p =  
288 0.999; Relatedness  $\times$  Familiarity:  $\chi^2 = 0.0015$ , df = 1, p = 0.969; Figure 1). If  
289 anything, mean number of eclosed young was slightly higher for the non-  
290 related treatments. If the non-reproducing females are included in the dataset,  
291 we actually find higher reproduction in the non-related treatment group  
292 (Relatedness:  $\chi^2 = 4.30$ , df = 1, p = 0.038; Familiarity:  $\chi^2 = 1.33$ , df = 1, p =  
293 0.248; Relatedness  $\times$  Familiarity:  $\chi^2 = 0.240$ , df = 1, p = 0.624, Supplementary  
294 figure 1). We did find different shapes of the age-specific fecundity, illustrated

295 by the significant interaction Relatedness  $\times$  Familiarity  $\times$  Age<sup>2</sup> (Table 1, Figure  
296 2). However, we found no effect on individual fitness  $\lambda_{ind}$  (Relatedness:  $F =$   
297 0.026,  $df = 1$ ,  $p = 0.872$ , Familiarity:  $F = 0.244$ ,  $df = 1$ ,  $p = 0.622$ , Relatedness  
298  $\times$  Familiarity:  $F = 0.072$ ,  $df = 1$ ,  $p = 0.789$ ).

299 When we measured the lifespan of the females introduced to the  
300 different treatment groups, we found that male relatedness, but not familiarity,  
301 improved female survival (Relatedness:  $z = -2.57$ ,  $df = 1$ ,  $p = 0.01$ ; Familiarity:  
302  $z = 0.34$ ,  $df = 1$ ,  $p = 0.73$ ; Relatedness  $\times$  Familiarity:  $z = 0.06$ ,  $df = 1$ ,  $p = 0.96$ ;  
303 Figure 3).

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305  
306



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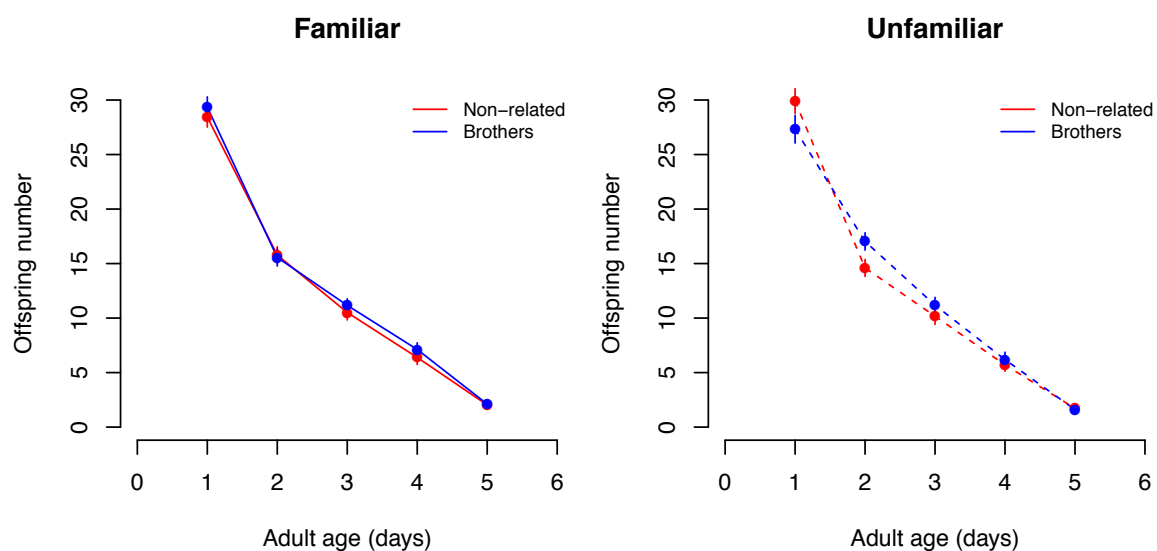
308 **Figure 1.** Lifetime reproductive success LRS (number of adult offspring) by  
309 treatment group: brothers (blue), non-related males (red), familiar individuals  
310 (solid symbols) and unfamiliar individuals (open symbols). Symbols represent  
311 mean  $\pm$  SE.

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316

317 **Table 1.** Age-specific reproduction, result from generalized linear model with  
 318 poisson error structure.  
 319

Parameter	$\chi^2$	df	p
Relatedness	0.2719	1	0.60204
Familiarity	0.1325	1	0.71588
ALR	52.8875	1	< 0.001
Age	1459.7816	1	< 0.001
Age <sup>2</sup>	623.6481	1	< 0.001
Relatedness × Familiarity	0.0653	1	0.79830
Relatedness × ALR	0.2163	1	0.64190
Familiarity × ALR	0.0061	1	0.93791
Relatedness × Age	1.3663	1	0.24246
Familiarity × Age	1.0830	1	0.29802
ALR × Age	214.4389	1	< 0.001
Relatedness × Age <sup>2</sup>	1.6252	1	0.20237
Familiarity × Age <sup>2</sup>	1.8441	1	0.17448
ALR × Age <sup>2</sup>	64.3620	1	< 0.001
Relatedness × Familiarity × ALR	1.6448	1	0.19967
Relatedness × Familiarity × Age	0.9137	1	0.33914
Relatedness × ALR × Age	0.0013	1	0.97119
Familiarity × ALR × Age	2.4446	1	0.11793
Relatedness × Familiarity × Age <sup>2</sup>	4.7754	1	0.02887
Relatedness × ALR × Age <sup>2</sup>	0.5634	1	0.45291
Familiarity × ALR × Age <sup>2</sup>	2.1438	1	0.14315
Relatedness × Familiarity × ALR × Age	1.8233	1	0.17692
Relatedness × Familiarity × ALR × Age <sup>2</sup>	3.3245	1	0.06825

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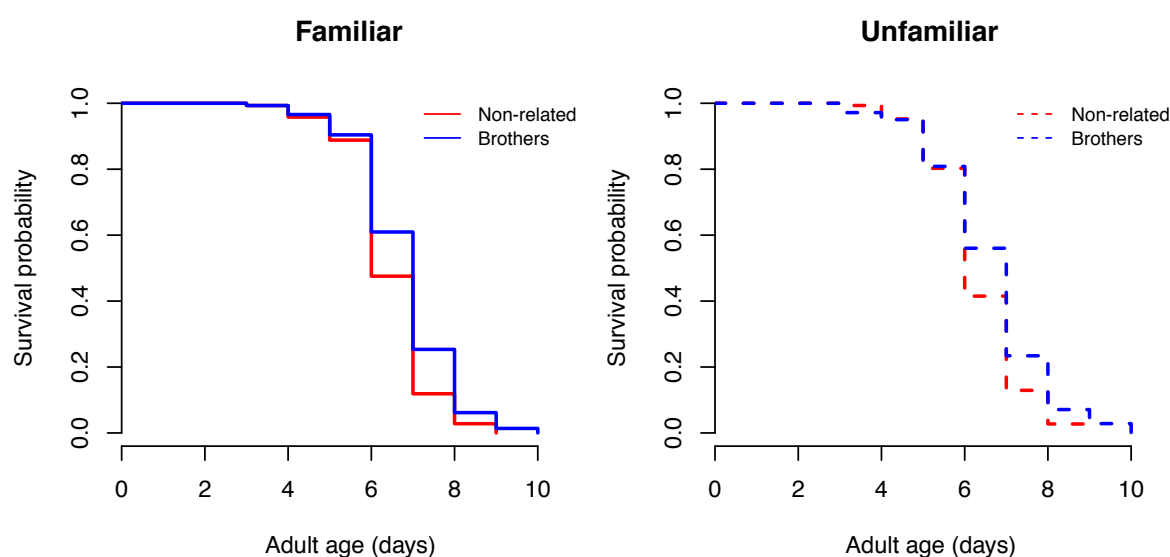


321

322 **Figure 2.** Age-specific reproduction for females mated with (A) familiar and  
 323 (B) non-familiar males. Brothers are shown as blue, and non-related males as  
 324 red.

325

326



327

328

329 **Figure 3** Survival probability for females mated to (A) familiar and (B)  
330 unfamiliar males. Blue represents brothers and red represents non-related  
331 males.

332

#### 333 4. DISCUSSION

334 Adaptive reduction in mate harm can only evolve if 1) there are reliable  
335 mechanisms for recognizing kin, and 2) populations are sufficiently viscous  
336 (i.e. genetically structured) for relatives to have a reasonable chance of  
337 encountering each other while they are reproductively active. The population  
338 genetic structure is one challenge facing the hypothesis that kin selection can  
339 mitigate the evolution of male harm via interlocus sexual conflict. For example,  
340 while kin recognition mechanisms may exist in *Drosophila* (e.g. through  
341 cuticular hydrocarbons [45, 46]), Chippindale *et al.* [29] point out that both  
342 natural and laboratory populations of *Drosophila* are unlikely to be sufficiently  
343 structured to promote kin-selected reduction in male-female conflict. Simply

344 put, adults emerge and fly off and are unlikely to remain in or disperse into  
345 genetically structured populations. Le Page *et al.* [32] countered this point by  
346 suggesting that genetic structure may occur in fruit flies during colonization of  
347 new patches by a small group of females, such that male relatedness-driven  
348 reduction of female harm in the established populations are a relic of “the  
349 foundation past”. Future work will test whether selection during the foundation  
350 of a new population in the natural environment is sufficiently strong to  
351 generate long-lasting effects on male reproductive behaviour.

352 In contrast, *C. maculatus* beetles may, in theory, meet the necessary  
353 pre-conditions without difficulty. Female seed beetles lay eggs in clusters, and  
354 will deposit all of their eggs in close proximity to each other provided there is  
355 sufficient supply of unoccupied beans. Upon emergence from the bean, male  
356 seed beetles aggressively court females and begin mating immediately, which  
357 increases the probability of encountering relatives. *C. maculatus* is a pest  
358 species that infests supplies of stored legumes – under those conditions, it is  
359 likely that many beetles will emerge and mate simultaneously providing  
360 sufficient variation in the relatedness of competitors. At the same time, we  
361 note that *C. maculatus* males are relatively indiscriminate in their mating  
362 behaviour, likely because of the high “missing opportunity” cost that is  
363 associated with living in high density populations, and commonly mount other  
364 males because of perception errors [47, 48], which would complicate selection  
365 for fine-tuned kin recognition mechanism that could lead, in theory, to reduced  
366 female harm.

367 In this study, similar to Chippindale *et al.*'s [29] *Drosophila* study, we  
368 found that neither male relatedness nor familiarity influenced female lifetime



369 reproductive success. However, in contrast to Chippindale *et al.* [29], male  
370 relatedness, but not familiarity, improved female survival. This is also in  
371 contrast to Lymbery *et al.* [37], who found that both familiarity and relatedness  
372 increase reproductive success, but not survival. In our study, since the effect  
373 on survival occurred only in late life, around day six of age when females  
374 already stopped producing eggs, it failed to increase female lifetime  
375 reproductive success. Therefore, our results do not provide support for the  
376 role of kin selection in mitigating the effects of male harm.

377         Seed beetles are facultatively aphagous – that is, eclosed adults do not  
378 require food or water to breed and survive [49]. While in many bruchid beetles  
379 adults commonly consume pollen, nectar or fungi [50], *Callosobruchus* beetles  
380 do not usually feed as adults. In the current study, we opted to keep the  
381 beetles under the aphagous conditions in which they have evolved for over  
382 500 generations since they were first brought into the laboratory in 1979.  
383 Thus, our schedule reflects not only the original conditions of the human grain  
384 storage, but also the recent evolutionary history of this large outbred  
385 population.

386         Nevertheless, it is interesting to consider how male relatedness could  
387 affect the fitness of female beetles in different environments. One may  
388 hypothesize that increased survival could result in increased fecundity when  
389 beetles have access to additional resources to continue reproduction in late  
390 life. Yet, in a recent study by Lymbery *et al.* [37], beetles were raised with the  
391 access to yeast but neither lived longer, nor produced more offspring  
392 compared to normal non-feeding conditions. This finding seems concordant  
393 with earlier reports that did not find positive effects of yeast consumption on

394 fitness in *C. maculatus* [38]. However, despite the lack of positive effects of  
395 access to yeast on life-history traits, females kept with related familiar males  
396 produced more offspring in Lymbery *et al.* [37] study. This suggests that the  
397 presence of yeast interacts with male behaviour in an unknown way to affect  
398 female reproductive performance. In our study, we found a three-way  
399 interaction between relatedness, familiarity and the shape of age-specific  
400 reproduction curve, stemming from the increased early-life reproduction and  
401 steeper age-specific decline of females mated to unrelated males than  
402 females mated to groups of brothers when both males were raised in a way  
403 that precluded familiarity. Increased reproductive performance in early-life  
404 could translate into increased individual fitness, but this was not the case. On  
405 the other hand, when we included females that failed to produce viable  
406 offspring in the analysis, we found that females kept with groups of unrelated  
407 males had higher reproductive success, because most of failed reproductive  
408 attempts were among females kept with groups of brothers. This finding is in  
409 line with the idea that multiple mating increases female fitness when it  
410 increases the genetic diversity of partners [51-54].

411         To summarize, there is little conclusive evidence to date for the role of  
412 kin selection in mediating sexual conflict, and, specifically, in reducing male-  
413 induced harm to females. More importantly, it is not always easy to see how  
414 the selection for such an effect can operate in the natural environment  
415 because it requires many opportunities for sib-sib interaction that may not be  
416 very common in wild populations of invertebrates [29]. Some organisms, such  
417 as bulb mites (*Rhizoglyphus robini*), maybe more prone to the evolution of kin-  
418 selected benefits of reduced male harm because of the metapopulation

419 structure characterized by rapid population growth and colonization of the new  
420 patches [55]. Indeed, recent work on bulb mites suggest that there is standing  
421 genetic variation for male harm that evolves rapidly under kin selection [55], in  
422 line with theoretical models [24, 27, 56]. Le Page *et al.* [32] discussed several  
423 possible explanations for the fact that reduction in male-induced harm to  
424 females is observed in some populations. One likely non-adaptive explanation  
425 is a perception error. Indeed, as suggested by Le Page *et al.* [32], males can  
426 use cuticular hydrocarbon profiles, or gut microbiota, as a measure of male-  
427 male competition, and they may underestimate the level of competition when  
428 surrounded exclusively by related males with similar odours, thereby investing  
429 less in sperm competition. Such a non-adaptive hypothesis fits squarely with  
430 the results of our study, because females housed with groups of related males  
431 did enjoy improved survival, suggesting reduced male harm, but this effect  
432 was entirely limited to post-reproductive part of their life cycle and had no  
433 effect on their individual fitness. We suggest that more work is needed to  
434 evaluate the importance of kin selection in the evolution of mating systems.

435

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