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## Inbreeding produces trade-offs between maternal fecundity and offspring survival in a monandrous spider

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2 **monandrous spider**

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20           Offspring born to related parents often have lower fitness than those born to non-  
21 related parents, a phenomenon termed inbreeding depression. While many species have been  
22 shown to rely on pre- and/or post-copulatory mate choice to avoid inbreeding, such research  
23 has focussed largely on polyandrous rather than monandrous species. The absence of post-  
24 copulatory mate choice in monandrous species suggests that pre-copulatory mate choice  
25 should play a more important role in inbreeding avoidance. We used a monandrous wolf  
26 spider, *Pardosa astrigera*, as a model system to investigate whether (1) male spiders respond  
27 differently to sibling and non-sibling females; (2) female spiders respond differently to  
28 sibling versus non-sibling males; and (3) inbreeding affects females and their offspring. Male  
29 courtship behavior was similar for sibling and non-sibling females; although females were  
30 less likely to mate with siblings, over half did mate successfully with their sibs. Sibling-  
31 mated females produced fewer offspring from the first eggsac and fewer total offspring, but  
32 inbred offspring survived longer in a range of environments than their outbred counterparts.  
33 This suggests that the fitness costs of reduced fecundity in sibling-mated females may be  
34 offset by higher offspring survivorship. Our results highlight the importance of considering  
35 both parent and offspring fitness when addressing the costs of inbreeding, and are the first to  
36 document the impact of inbreeding on sexual behaviour and reproductive fitness in a  
37 monandrous spider.

38           **Keywords:** Courtship, fecundity, fitness, inbreeding avoidance, mate discrimination,  
39 mating, monandrous, spider

40 Inbred individuals are often less fit than outbred individuals, a phenomenon generally  
41 resulting from increased homozygosity at loci carrying rare deleterious recessive alleles or  
42 exhibiting over-dominance (Charlesworth & Charlesworth, 1987; Lynch, 1991). The fitness  
43 costs of inbreeding have been documented in an array of taxa, and exert a strong selective  
44 pressure on both mating and reproductive strategies (Bateson, 1982; Escobar et al., 2011;  
45 Muller & Muller, 2016; Szulkin, Stoper, Pemberton, & Reid, 2013). The impact of  
46 inbreeding on offspring can be altered by the surrounding environment. Varying  
47 environmental conditions, for example, can cause stress and often exacerbate the effects of  
48 inbreeding (Armbruster & Reed, 2005). These stressors can include suboptimal diets (Fox &  
49 Reed, 2011; Freitag, Bos, Stucki, & Sundstrom, 2014) and variation in temperature (Fox &  
50 Reed, 2011; Kristensen, Barker, Pedersen, & Loeschcke, 2008), and are widely recognized to  
51 exacerbate the fitness costs of inbreeding.

52 An array of mechanisms have evolved for avoiding inbreeding and/or reducing its  
53 fitness costs (Firman & Simmons, 2008; Pusey & Wolf, 1996; Ruch, Heinrich, Bilde, &  
54 Schneider, 2009). Prior to breeding, sex-biased dispersal from natal habitats decreases  
55 inbreeding risk in some species (Keane, 1990; Pusey & Wolf, 1996; Smith, Su, Berger-Tal, &  
56 Lubin, 2016), while other species prefer to mate with unrelated partners (Fischer, Karl,  
57 Heuskin, Janowitz, & Dotterl, 2015; Thomas & Simmons, 2011; Whitehorn, Tinsley, &  
58 Goulson, 2009). The recognition and avoidance of related individuals requires chemical or  
59 other cues that are indicative of relatedness (Firman & Simmons, 2008; Pusey & Wolf, 1996;  
60 Ruch et al., 2009). In insects, for instance, both mate recognition and pre-mating preference  
61 are affected by cuticular hydrocarbons (CHCs; Geiselhardt, Otte, & Hilker, 2009; Thomas &

62 Simmons, 2011) and other compounds (Chuine, Sauzet, Debias, & Desouhant, 2015;  
63 Herzner, Schmitt, Heckel, Schreier, & Strohm, 2006). The CHC profiles of several  
64 chrysomelid beetle species, for example, affect mate choice and facilitate outbred mating  
65 (Geiselhardt et al., 2009). Even if inbreeding does occur, its impact in polyandrous species  
66 can be reduced via post-copulatory mechanisms in which differential fertilization success  
67 depend on patterns of relatedness rather than intrinsic male quality (Bretman, Wedell, &  
68 Tregenza, 2004; Firman & Simmons, 2008; Fitzpatrick & Evans, 2014).

69         Research exploring inbreeding avoidance has primarily addressed polyandrous  
70 species, organisms capable of employing both pre- and post-copulatory mate choice strategies  
71 (Cornell & Tregenza, 2007; Firman & Simmons, 2008; Tregenza & Wedell, 2002; Welke &  
72 Schneider, 2009). This focus reflects the genetic benefits likely necessary for polyandry to  
73 evolve in species where females derive little or no material benefit from males (reviewed in  
74 Simmons, Beveridge, Wedell, & Tregenza, 2006). In contrast, inbreeding in monandrous  
75 species has received far less attention. Because monandrous females only mate once within a  
76 single reproductive episode, inbreeding avoidance must occur via pre-copulatory mechanisms  
77 (Hosken, Stockley, Tregenza, & Wedell, 2009). In situations where inbreeding is costly,  
78 monandrous species may thus possess especially effective pre-copulatory barriers. The  
79 strength of these barriers may, however, vary by sex: because male fitness is relatively  
80 unaffected by inbreeding, they should be more tolerant of sibling matings than females  
81 (Duthie, Lee, & Reid, 2016).

82         The wolf spider *Pardosa astrigera* Koch is widely distributed in East Asia. Male  
83 courtship consists of two distinct behaviours, body shaking and foreleg raising (Wu, Jiao, &

84 Chen, 2008). Olfaction plays a key role in male courtship. Males initiate courtship in  
85 response to pheromones associated with female dragline silk, and males can distinguish silk  
86 cues from individuals differing in sex and mating status (Xiao et al., 2015). While female *P.*  
87 *astrigera* are monandrous, the polygynous males can copulate with as many as five virgin  
88 females at 24h intervals (Jiao et al., 2011; Wu et al., 2008). While inbreeding depression has  
89 not previously been addressed in this species, prior research into its courtship and mating  
90 behavior make it an ideal model system for addressing such questions.

91 We report work investigating inbreeding avoidance through courtship behavior and  
92 the impact of inbreeding on reproductive output and offspring survival in the monandrous  
93 wolf spider *P. astrigera*. We compared male courtship behaviors in response to dragline silk  
94 of sibling and non-sibling females to test for male pre-copulatory kin discrimination. We also  
95 conducted non-choice mating experiments to compare the likelihood of sibling and non-  
96 sibling mating. In addition, we measured post-mating female reproductive output (both  
97 number and size of offspring) to determine the cost of inbreeding on female fitness. Finally,  
98 we compared the survival of inbred versus outbred offspring across a range of temperatures.  
99 We predicted that strong pre-copulatory barriers exist to sibling mating, that these barriers are  
100 stronger in females than in males, that inbreeding reduces both maternal and offspring fitness,  
101 and that higher temperatures increase the impact of inbreeding on the offspring.

## 102 **Methods**

103 Subadult *P. astrigera* of the overwintering generation were collected in April 2012  
104 from Ma'anshan Forest Park, Wuhan, Hubei Province, China. Spiders were housed  
105 individually in opaque Plexiglass enclosures (5.0 × 5.0 × 7.5 cm, l×w×h) at 25 ± 0.5 °C with

106 60 ± 10% relative humidity ('RH') and on a 14:10 light:dark ('l:d') cycle. Spiders were  
107 supplied with water *ad libitum* and fed every 3 days with a mixture diet of *Drosophila*  
108 *melanogaster* and mosquitoes (*Culicidae*). Individuals were checked daily for subadult  
109 molting in order to determine the exact date of adulthood. We used randomly-selected adult  
110 spiders to create the ten male:female pairs used to generate ten families. Mated females were  
111 maintained as above. We randomly selected and reared 30 spiderlings from each eggsac; each  
112 spiderling was reared individually in a glass tube (1.5 cm diameter). Spiderlings were  
113 supplied with water *ad libitum* and fed every two days with a mixture of *D. melanogaster* and  
114 mosquitoes. Once the spiders matured, similarly-sized females in their third day of adulthood  
115 were selected for silk collection and/or behavioural trials. All spiders were virgin and used  
116 only once; all adult spiders, except for those females whose lifespan was measured (details  
117 below) were released following their involvement in the experiment.

### 118 **Experiment 1: Male response to sibling/nonsibling female silk**

119 Silk was collected by placing each female in a 9-cm diameter glass petri dish lined  
120 with filter paper (15 cm diameter; Double Ring brand, Hangzhou, Zhejiang, China) for 12 h.  
121 All females were starved for 12 h beforehand to reduce faecal contamination. All silk was  
122 used within 18-24 h after its collection, a period of time over which silk-borne spider cues do  
123 not degrade under natural conditions (Baruffaldi, Costa, Rodriguez, & Gonzalez, 2010;  
124 Costa, Curbelo, & Perez-Miles, 2015).

125 We randomly selected similarly-sized virgin males ( $N = 65$ , 5-7 individuals per  
126 family) aged 5-10 days post-maturation and assigned each to one of the two female silk  
127 stimulus treatments. Male body size did not differ significantly between treatments ( $t_{61} =$



128 0.75,  $P = 0.45$ ). Thirty-three males were exposed to silk from a female in the same family  
129 (sibling), and 32 males were exposed to silk from a female from a different family (non-  
130 sibling); silk from a given female was only used for one male.

131 Behavioural trials were carried out in a cylindrical glass container open at both ends  
132 (10.5-cm diameter, 12-cm length). After setting the cylindrical glass container on the silk-  
133 covered filter paper, a single male was gently introduced onto the stimulus filter paper with a  
134 glass tube from above and its courtship behaviour videotaped (HDR-CX580E Sony video  
135 camera) for five min. We chose this cut-off period because preliminary experiments revealed  
136 that male spiders exposed to silk either began courtship rapidly (within two minutes) or never  
137 engaged in courtship behavior (Roberts & Uetz, 2004). Each arena was cleaned after each  
138 trial with 70% ethanol and left to air dry. Videos were analysed using Observer v. 4.1 (Noldus  
139 Information Technology, Wageningen, The Netherlands), a software package for behavioural  
140 data analysis. On the basis of work reported in Wu et al. (2007, 2008) and Jiao et al. (2009),  
141 the following courtship behaviours were analysed: (a) time to the start of body shaking and  
142 (b) foreleg raising; (c) The number of body shaking and (d) foreleg raising events per minute.

143 Data from all trials was analysed to determine whether the likelihood of courting  
144 behavior was affected by female relatedness. For analysis of specific courtship behaviors,  
145 data from trials where such behaviors did not occur within five minutes were excluded from  
146 analysis.

## 147 **Experiment 2: Male and female responses to siblings and non-siblings**

148 We paired individual virgin females ( $N = 120$ ; 12 spiders from each family) in their  
149 third day of adulthood with individual virgin males 5-10 days into adulthood; 60 male-female

150 pairs were siblings, and the other sixty pairs were non-siblings. All spiders belonged to one of  
151 the ten families. We recorded behavioural data on male courtship as per experiment #1, and  
152 also whether mating occurred within 30 minutes. Data for replicates in which no mating  
153 occurred was used to analyse mating likelihood in treatments but not included in other  
154 mating-dependent analyses (described below). Forty-two females mated with non-sibling  
155 males and 31 females with sibling males; each mating produced an eggsac. The unit of  
156 replication for analysis of mating behavior was individual mating pairs ( $N = 73$ ).

### 157 **Experiment 3: Impact of inbreeding on female fecundity and offspring survival**

158 We held mated females individually under the conditions described above, and  
159 checked daily for an eggsac. Although all 73 females produced eggsacs, 20 cannibalized their  
160 eggsacs prior to hatching; eggsacs from the remaining 53 spiders (26 sibling and 27 non-  
161 sibling) hatched successfully. The size (measured as carapace width) of female spiders did  
162 not differ between treatments ( $t_{45} = 0.51$ ,  $P = 0.61$ ). We removed the eggsacs of five  
163 randomly-chosen sibling-mated spiders and eight non-sibling-mated spiders for an unrelated  
164 experiment, leaving a total of 40 eggsac-producing females (21 sibling and 19 non-sibling,  
165 representing all ten families). For each female, we recorded time (days) from mating to first  
166 eggsac production and from first eggsac production to hatching. After the first eggsac was  
167 produced, each female was kept alive and fed *ad libitum* until death to measure their lifespan  
168 and see if they produced additional eggsacs. Offspring from these eggsacs plus the number of  
169 offspring from the first eggsac determined total offspring production per female.

170 After recording the number of offspring emerging from the first eggsac, we preserved  
171 five randomly-selected offspring from it in 70% alcohol for carapace width measurements.

172 We divided the remaining offspring of the first eggsac into three groups. Spiderlings  
173 were kept in 1.5-cm diameter glass tubes with no water and held at one of three temperatures  
174 (15, 25 and 30 °C) without food nor water ( $60 \pm 10\%$  RH, 14:10 light:dark cycle). These  
175 temperatures were chosen to reflect the mean, high, and absolute highest temperatures spiders  
176 might experience at this point in the year. While 25° C temperatures are ideal for spider  
177 development when water is provided *ad libitum*, in the absence of water such high  
178 temperatures speed desiccation and death. Survival was checked twice daily. The survival of  
179 all offspring of a female at a given temperature was averaged; the unit of replication was  
180 mean offspring survival per female per temperature ( $N = 120$ ).

#### 181 **Ethical note**

182 Animal care in all experiments complied with the current laws and standards of China  
183 (Bayne & Wang, 2014).

#### 184 **Data analysis**

185 Data were analyzed by fitting a generalized linear mixed model (glmm) with the  
186 appropriate link function (e.g. Gaussian, Poisson, binomial) using penalized quasi-likelihood  
187 (PQL) ('glmmPQL' function in MASS package, Venables & Ripley, 2002) in R (R  
188 Development Core Team, 2017). Family nested within treatment (i.e. sibling and non-sibling)  
189 was used in all models as a random effect to account for the non-independence of multiple  
190 individuals from a given family. A Wald  $\chi^2$  test was used to extract  $\chi^2$  and  $P$ -values on the  
191 glmm model using the 'Anova' function in the 'car' package (Fox & Weisberg, 2011).  
192 Additionally, data on mean offspring size from experiment three was analyzed by including  
193 mating treatment in all models as a fixed effect with female carapace width (a proxy for body

194 size) as a covariate. Data on mean offspring survival from experiment three was also  
195 analyzed as above but with the addition of a fixed main effect (temperature) and a  
196 temperature\*mating interaction.

## 197 **Results**

198 Twenty-one of 33 males responded to sibling silk, and 22 of 32 males responded to  
199 non-sibling silk; the proportion of non-responding males did not differ between treatments  
200 ( $\chi^2_1 = 0.50$ ,  $P = 0.48$ ). Males did not differentiate between sibling and non-sibling females  
201 when exposed to either silk cues (Fig. 1, top panel) or directly to the females themselves (Fig.  
202 1, bottom panel). The start of courtship behaviors such as foreleg raising or body shaking was  
203 unaffected by female relatedness, whether conveyed via silkborne cue (Figs. 1A and 1B,  
204 respectively;  $\chi^2_1$ , both  $P > 0.5$ ) or direct female exposure (Figs. 1E and 1F; both  $P > 0.5$ ).  
205 There were also no treatment differences in the frequency of courtship behaviors in both the  
206 silk-cue (Figs. 1C and 1D; both  $P > 0.4$ ) and direct exposure (Figs. 1G and 1H; both  $P > 0.3$ )  
207 experiments.

208 Despite similar male courtship behavior, mating occurred more often between  
209 unrelated individuals (70% of pairings) than between siblings (52%;  $\chi^2_1 = 4.26$ ,  $P = 0.039$ ).  
210 The time from mating to first eggsac production (Fig. 2A) and from production to hatching  
211 (Fig. 2B) was similar for both sibling and non-sibling pairings ( $\chi^2_1 = 0.43$  and 0.31,  
212 respectively, both  $P > 0.05$ ). The fecundity of sibling-mated females, however, was much  
213 lower than that of non-sibling mated ones: they produced 41% fewer offspring in their first  
214 eggsac (Fig. 2C;  $\chi^2_1 = 24.8$ ,  $P < 0.001$ ) and 44% fewer offspring in total (Fig. 2D;  $\chi^2_1 = 34.2$ ,  
215  $P < 0.001$ ). Five of 27 non-sibling mated females produced a second eggsac, while only two

216 of 26 sibling-mated females did so; this difference was not, however, significant ( $\chi^2_1 = 1.40$ ,  $P$   
217 = 0.24). There were no treatment-level differences in the longevity of mated adult females  
218 ( $\chi^2_1 = 0.07$ ,  $P = 0.80$ ).

219 The offspring of sibling and non-sibling pairings were of similar size ( $1.28 \pm 0.006$   
220 [SE] and  $1.27 \pm 0.007$  mm carapace width, respectively;  $\chi^2_1 = 1.79$ ,  $P = 0.18$ ). Offspring in  
221 the sibling treatment survived an average of 23% longer ( $9.3 \pm 0.20$  [SE] and  $7.1 \pm 0.13$  days;  
222  $\chi^2_1 = 33.0$ ,  $P < 0.001$ ) across all three temperature treatments than those in the non-sibling  
223 treatment (Fig. 3). Spiderling survival declined as temperature increased ( $\chi^2_2 = 111$ ,  $P <$   
224  $0.001$ ), and there was a significant mating\*temperature interaction  $\chi^2_2 = 10.3$ ,  $P = 0.006$ ).  
225 This interaction reflected the fact that the survival advantage of inbred offspring generally  
226 decreased as temperature increased; inbred offspring survived 28% longer in the 15°C  
227 treatment, 19% longer in 25°C, and 22% longer in 30°C (Fig. 3).

## 228 Discussion

229 Contrary to our predictions, we found only weak pre-copulatory inbreeding avoidance  
230 in *P. astrigera*. Male spiders, by not responding differently to silk or courting female cues,  
231 showed no evidence of kin discrimination (Fig. 1). Female spiders mated at a higher rate with  
232 unrelated individuals, but over half still mated successfully with male siblings. While weak  
233 sibling avoidance suggests a minimal cost to inbreeding, the fecundity of sibling-mated  
234 females was reduced (Fig. 2). Experimental assessment of their offspring, however, revealed  
235 that although they were the same size as their outbred congeners, the offspring of sibling-  
236 mated females survived ~20% longer under a range of environmental conditions (Fig. 3).  
237 These findings highlight the importance of assessing both parental and offspring fitness when

238 exploring the costs of inbreeding.

239           The fact that females bred less often with sibling males demonstrates their ability to  
240 detect relatedness via chemical or other cues; mate recognition via such cues often plays a  
241 key role in inbreeding avoidance (Geiselhardt et al., 2009; Herzner et al., 2006; Lihoreau &  
242 Rivault, 2010; Thomas & Simmons, 2011). In many spider species, males employ silk-  
243 mediated cues for species, sex, and mating status recognition (Gaskett, 2007; Xiao et al.,  
244 2015). Given this, we were surprised to find no evidence for male pre-copulatory mate choice  
245 in response to either females or their silk. This result likely reflects the fact that male *P.*  
246 *astrigera* are polygynous and compete fiercely with each other for mating opportunities (Jiao  
247 et al., 2011). Because the males can remate, they have little to lose from inbreeding and  
248 should seek to maximize mating opportunities even under strong inbreeding depression  
249 (Duthie et al., 2016).

250           The inbreeding-related decline in female fecundity is consistent with results from a  
251 wide range of taxa (Charlesworth & Charlesworth, 1987; Hedrick & Garcia-Dorado, 2016;  
252 Pusey & Wolf, 1996). The >40% reduction in offspring number is especially harmful in a  
253 monandrous species like *P. astrigera*, since females cannot compensate via subsequent  
254 matings with higher-quality partners. Given these high costs, it may seem surprising that over  
255 half of the females in the sibling group chose to mate. One explanation for this result may  
256 involve our decision to employ a no-choice design in our mating assays. A recent meta-  
257 analysis (Dougherty & Shuker, 2015) found stronger mating preferences in choice  
258 experiments where females were exposed to different mates. If this is the case in *P. astrigera*,  
259 our results may underestimate the strength of female mate preference. Alternately, sex-biased

260 dispersal prior to reproductive maturity has been shown to reduce the likelihood of  
261 inbreeding in some species (Keane, 1990; Pusey & Wolf, 1996; Smith et al., 2016). If such  
262 sex-biased dispersal occurs in this species, it may reduce the need for females to strongly  
263 discriminate against related individuals. Finally, the weak sibling avoidance we observed may  
264 highlight the importance of viewing the costs of inbreeding depression within the larger  
265 context of female inclusive fitness - and specifically, the higher survival of inbred offspring.

266         There is considerable evidence that the offspring of sibling matings are equally or  
267 more sensitive to environmental variation than their outbred congeners, presumably because  
268 the stress associated with that variation increases the expression of deleterious recessive  
269 alleles (Armbruster & Reed, 2005; Fox, Stillwell, Wallin, Curtis, & Reed, 2011; Kristensen et  
270 al., 2008). We were thus surprised to find that inbred offspring survived longer than outbred  
271 ones across a range of temperatures (Fig. 3). One explanation for this pattern, the idea that  
272 density-dependent resource competition may disproportionately affect spiderlings from larger  
273 clutches (Wise, 2006), is unlikely since hatched spiderlings were immediately confined to  
274 individual glass tubes.

275         One likely explanation for our results involves the trade-off between offspring number  
276 and per-offspring investment predicted for sibling matings (Duthie et al., 2016). Since inbred  
277 offspring share more alleles with their parents than outbred offspring, each successful inbred  
278 offspring increases parental inclusive fitness more than its outbred congener and is thus more  
279 'worthy' of parental resource investment. As a consequence, the inclusive fitness of  
280 inbreeding parents that invest resources in fewer offspring may equal or exceed that of  
281 outbreeding parents that produce more less-provisioned offspring (Duthie et al., 2016). Were

282 this the case, we might expect offspring size to differ. Although spiderling carapace width  
283 was negatively correlated with offspring per eggsac, there were no between-treatment  
284 differences. Inbreeding parents may allocate more nutrients to eggs (Wilder, 2011) or employ  
285 other forms of investment (e.g., parental care; Pilakouta & Smiseth, 2016). Future research  
286 might address whether such alternate forms of parental provisioning occur in this system.

287         Our results are also consistent with the hypothesis that inbreeding in *P. astrigera*,  
288 while harmful to parental fecundity, benefits one or more traits that prolong offspring  
289 survival. The effects of inbreeding are often trait-specific, with some traits strongly affected  
290 and others remaining similar to those found in outbred congeners (Kristensen et al., 2008;  
291 Pilakouta & Smiseth, 2016; Valtonen, Roff, & Rantala, 2011). Given this, it is unsurprising  
292 that inbreeding can increase the benefit of some life history traits. In the cricket *Teleogryllus*  
293 *commodus*, for example, inbred individuals exhibit higher macroparasitic immunity than  
294 outbred individuals (Gershman et al., 2010). Similarly, male *Litoria peronii* frogs that mate  
295 with sibling females sire more offspring in sperm competition (Sherman, Wapstra, Uller, &  
296 Olsson, 2008). These benefits can also be sex-specific: inbreeding in the beetle  
297 *Callosobruchus maculatus* increases male - but shortens female - lifespan (Bilde, Maklakov,  
298 Meisner, la Guardia, & Friberg, 2009). In our case, an increase in desiccation tolerance or  
299 modifications to similar traits might provide inbred spiderlings a survival advantage  
300 consistent with our results.

301         While inbreeding is generally harmful, its costs can vary substantially both between  
302 and within species (Aviles & Bukowski, 2006; Szulkin et al., 2013); theory predicts an  
303 optimal balance between inbreeding and outbreeding (Kokko & Ots, 2006; Puurtinen, 2011;



304 Richard, Losdat, Lecomte, de Fraipont, & Clobert, 2009). Our results reveal unexpectedly  
305 weak inbreeding avoidance in a monandrous spider and demonstrate that sibling mating  
306 reduces maternal fecundity but increases offspring survival in a range of environmental  
307 conditions. These findings highlight the importance of viewing maternal fecundity in the  
308 larger context of inclusive fitness; a relatively low degree of inbreeding avoidance may  
309 reflect a trade-off between parental and offspring fitness. This is especially important for  
310 monandrous organisms that, by definition, cannot employ post-copulatory mechanisms to  
311 reduce the impact of inbreeding. In such species, weak sibling avoidance may be indicative of  
312 inbreeding-related tradeoffs: future research should explore both the conditions that  
313 necessitate pre-copulatory mate choice strategies and determine its strength.

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458 **Figure Legends**

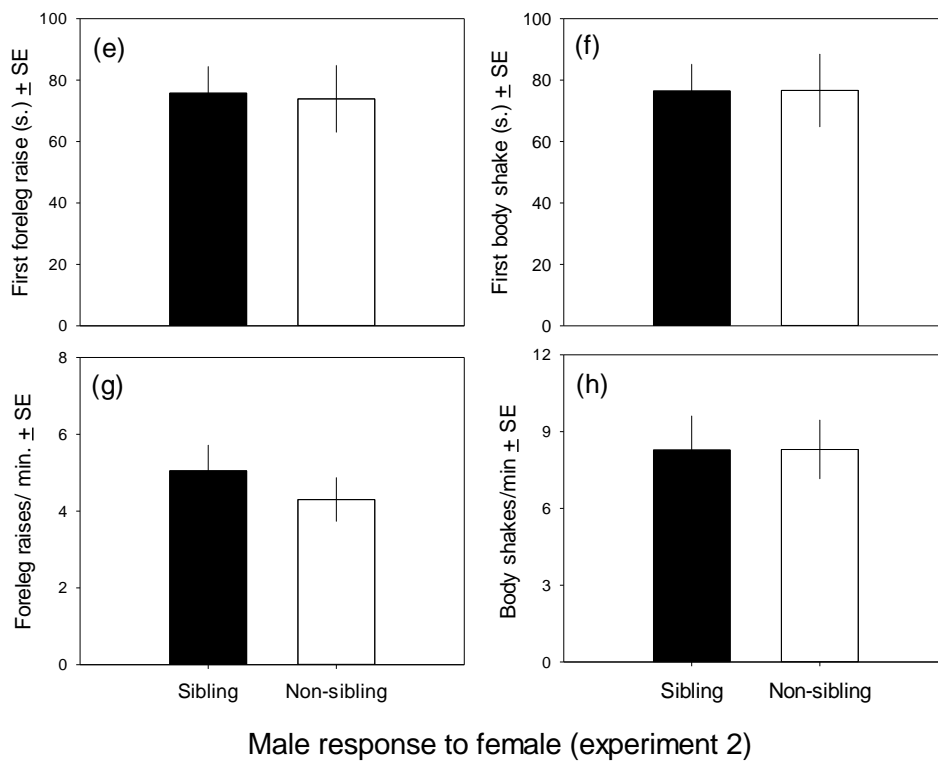
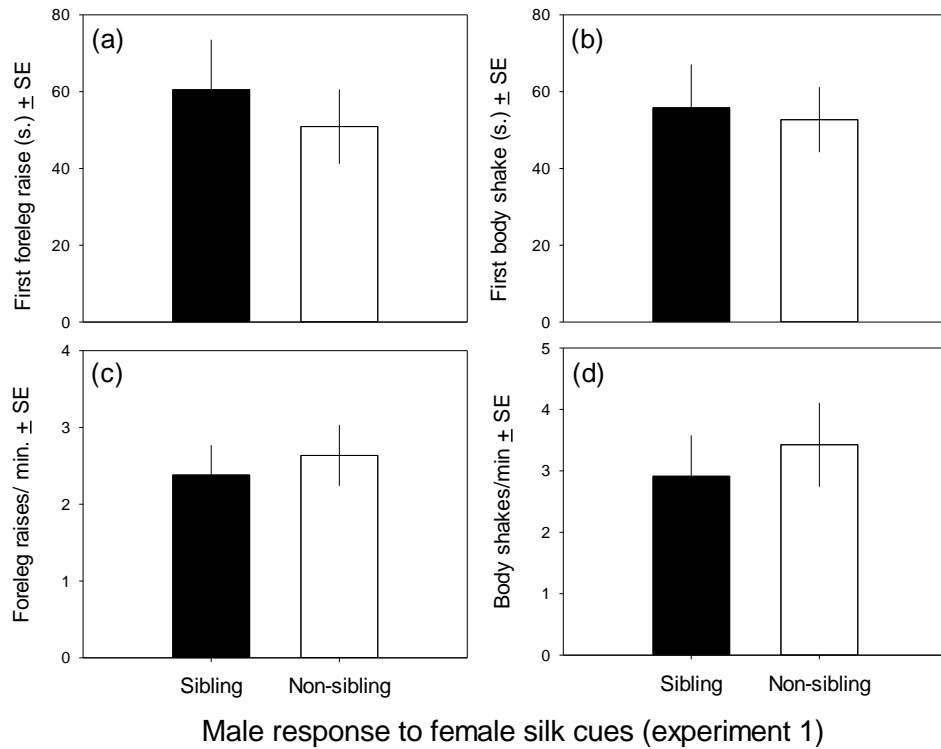
459 Figure 1. Male courtship behaviors in response to sibling versus non-sibling females.

460 Panels A-D: courtship in the presence of silkborne cues produced by sibling (filled bars) and  
461 non-sibling (open bars) females. Panels E-H: courtship in the physical presence of sibling and  
462 non-sibling females.

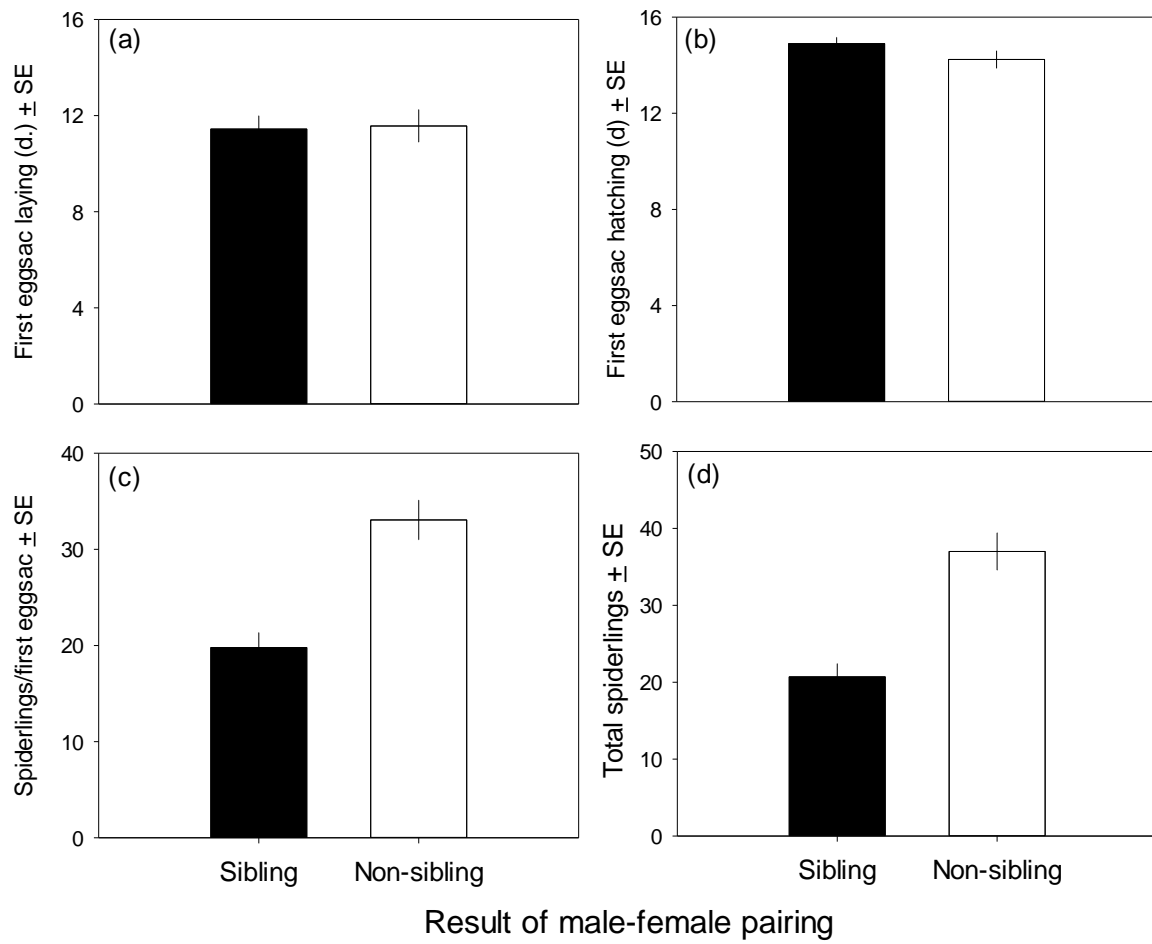
463 Figure 2. Female reproduction (A-B) and fecundity (C-D) following mating with  
464 sibling (filled bars) and non-sibling (open bars) males.

465 Figure 3. Survival of offspring (days) from sibling-mated females (filled circles) and  
466 non-sibling mated females (open triangles) held without food or water at 15, 25, and 30 °C.





470 Figure 2.



471

472

473 Figure 3.

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