

1 **Title:** The presence of heterospecific males causes an Allee effect.

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3 **Authors:** Daisuke Kyogoku\* and Takayoshi Nishida

4 \*Author for correspondence

5 Laboratory of Insect Ecology, Graduate School of Agriculture, Kyoto University,

6 Kyoto, Japan.

7 E-mail: kyogoku@kais.kyoto-u.ac.jp

8 Address: Kitashirakawa-oiwakecho, Sakyo, Kyoto, Kyoto 606-8502, Japan.

9 Takayoshi Nishida

10 Laboratory of Insect Ecology, Graduate School of Agriculture, Kyoto University,

11 Kyoto, Japan.

12 Present affiliation: Department of Ecosystem Studies, School of Environmental

13 Science, University of Shiga Prefecture, Hikone, Japan.

14 E-mail: nishida.t@ses.usp.ac.jp

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18

19 **Abstract**

20

21 The Allee effect is a positive causal relationship between any component of fitness and  
22 population density or size. Allee effects strongly affect the persistence of small or sparse  
23 populations. Predicting Allee effects remains a challenge, possibly because not all  
24 causal mechanisms are known. We hypothesized that reproductive interference (an  
25 interspecific reproductive interaction that reduces the fitness of the species involved)  
26 can generate an Allee effect. If the density of the interfering species is constant, an  
27 increase in the population of the species receiving interference may dilute the per capita  
28 effect of reproductive interference and may generate an Allee effect. To test this  
29 hypothesis, we examined the effect of heterospecific males on the relationship between  
30 per capita fecundity and conspecific density in *Callosobruchus chinensis* and *C.*  
31 *maculatus*. Of the two species, only *C. maculatus* females suffer reproductive  
32 interference from heterospecific males. Only *C. maculatus*, the species susceptible to  
33 reproductive interference, demonstrated an Allee effect, and only when heterospecific  
34 males were present. In contrast, *C. chinensis*, the species not susceptible to reproductive  
35 interference, demonstrated no Allee effect regardless of the presence of heterospecific

36 males. Our results show that reproductive interference in fact generated an Allee effect,  
37 suggesting the potential importance of interspecific sexual interactions especially in  
38 small or sparse populations, even in the absence of a shared resource. It may be possible  
39 to predict Allee effects produced by this mechanism *a priori* by testing reproductive  
40 interference between closely related species.

41

## 42 **Key words**

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44 *Callosobruchus* • Depensation • Dilution effect • Frequency dependence • Interspecific  
45 interaction • Reproductive interference

46

## 47 **Introduction**

48

49 The Allee effect is a positive causal relationship between any component of fitness and  
50 population density or size (Stephens et al. 1999). Allee effects have been broadly  
51 recognized as an important ecological phenomenon in conservation biology  
52 (Courchamp et al. 1999; Stephens and Sutherland 1999), biological invasion (Taylor and  
53 Hastings 2005; Liebhold and Tobin 2008), and biological control introductions (Hopper

54 and Roush 1993), because they can lead to the extinction of small or sparse populations.  
55 Various mechanisms have been suggested to generate Allee effects, including  
56 mate-finding difficulty, lack of predator satiation, and reduced foraging efficiency  
57 (Courchamp et al. 2008). However, though some species are unlikely to suffer from an  
58 Allee effect, other species with similar life history traits are subject to Allee effects (e.g.,  
59 Myers et al. 1995; Drake 2005; Fauvergue et al. 2007). Thus, predicting which species  
60 will be subject to an Allee effect remains a challenge. It is possibly because some of  
61 Allee effects are caused by an as yet unknown mechanism.

62         We propose the novel hypothesis that reproductive interference can generate an  
63 Allee effect, owing to its positive frequency dependence. Reproductive interference is  
64 a negative interspecific sexual interaction that reduces the fitness of at least one of the  
65 species involved (Gröning and Hochkirch 2008; Burdfield-Steel and Shuker 2011). For  
66 example, when a male of species A mistakenly recognizes a female of species B as a  
67 conspecific and attempts to mate with her, the female might suffer from an energy or  
68 time loss, reduced opportunities to mate with conspecifics, gamete wastage, genital  
69 damage, or hybrid formation (Gröning and Hochkirch 2008). Notably, in terms of its  
70 effect on population dynamics, reproductive interference seems to show frequency  
71 dependence (Kuno 1992; Gröning and Hochkirch 2008; Takakura et al. 2009): the

72 higher the frequency of species A, the greater the intensity of the reproductive  
73 interference on species B. If for a pair of species, reproductive interference is  
74 asymmetric (unidirectional) and the density of interfering species A is constant, the per  
75 capita level of interference with the interfered species B should become diluted as the  
76 density of species B increases. This dilution of reproductive interference should  
77 generate an Allee effect when it is accompanied by increased fitness of species B. Thus,  
78 reproductive interference may generate an Allee effect via a dilution effect, just as  
79 predation does (Gascoigne and Lipcius 2004).

80 To test this hypothesis, we examined the relationship between per capita  
81 fecundity and conspecific density when heterospecific males were present in a pair of  
82 bean weevil species, *Callosobruchus chinensis* (Linnaeus) and *C. maculatus* (Fabricius).  
83 Nishigaki (1963) measured copulation frequency in *C. chinensis* and reported that males  
84 had difficulty in finding mates. In typical laboratory conditions, however, both *C.*  
85 *chinensis* and *C. maculatus* show no positive relationship between population density  
86 and lifetime reproductive success in isolation from the other (e.g., Yoshida 1966). Kishi  
87 et al. (2009) showed that the fecundity of *C. maculatus*, but not that of *C. chinensis*, is  
88 asymmetrically reduced when heterospecific males are present; thus, they show  
89 asymmetric reproductive interference. We predict specifically that *C. maculatus*, the

90 species more susceptible to reproductive interference, will suffer an Allee effect only  
91 when heterospecific males are present, and that *C. chinensis*, the non-susceptible species,  
92 suffers no Allee effect.

93

#### 94 **Materials and methods**

95

96 We used the jC-F strain of *C. chinensis* (see Harano and Miyatake 2005) and the hQ  
97 strain of *C. maculatus* (see Miyatake and Matsumura 2004), both of which were derived  
98 from the stock cultures of Okayama University. We chose these strains, because Kishi et  
99 al. (2009) used these same strains to confirm unilateral reproductive interference by *C.*  
100 *chinensis* on *C. maculatus*. The stock cultures were maintained under laboratory  
101 conditions (25°C, 50–60% relative humidity, and 16L8D) and fed on adzuki beans,  
102 *Vigna angularis* (Willd.) cv. Dainagon. Mated females of *C. chinensis* and *C. maculatus*  
103 lay about 60 eggs on bean surfaces without feeding during their adult lifespan of around  
104 10 days. Males of both species try to copulate with either heterospecific or conspecific  
105 females indiscriminately even when conspecific females are present (Kishi et al. 2009).  
106 Interspecific copulation is sometimes observed, but no hybrid has been reported  
107 (Yamane and Miyatake 2010).

108 Virgin males and females of *C. chinensis* and *C. maculatus* were prepared for  
109 our experiments as follows. About 40 g of adzuki beans were placed in each of two  
110 plastic Petri dishes ( $\phi 90$  mm  $\times$  20 mm). Then, about 10 females of each species were  
111 randomly chosen from the stock cultures (200-400 individuals in each generation), and  
112 allowed to lay eggs on the beans in one of the dishes. Beans with one, two, or three eggs  
113 on them were selected and placed individually in wells of a 24-well plastic plate (each  
114 well,  $\phi 15.5$  mm  $\times$  17.3 mm). Beans with four or more eggs were discarded to avoid  
115 potential overcrowding effects. Each well was checked every day for newly emerged  
116 virgin individuals (i.e., individuals that emerged singly or in a unisexual group).

117 The effect of *C. chinensis* males on per capita fecundity (i.e., average number  
118 of hatched eggs per female) of *C. maculatus* was investigated under different *C.*  
119 *maculatus* density regimes as follows. From one to five *C. maculatus* pairs, each  
120 consisting of one male and one female individual, were housed with three *C. chinensis*  
121 males throughout their lives in a plastic Petri dish ( $\phi 70$  mm  $\times$  15 mm) containing 9.0 g  
122 of adzuki beans. Before the two species were put together, we allowed each *C.*  
123 *maculatus* female to copulate with a conspecific male to exclude the possibility that the  
124 presence of heterospecific males would lead to a potential mate-finding difficulty. We  
125 introduced only male heterospecifics, rather than heterospecific pairs, because we

126 wished to investigate how reproductive interference by heterospecific males affected the  
127 fecundity of the *C. maculatus* females. As the control, we also examined the per capita  
128 fecundity of one to five pairs of *C. maculatus* housed without any *C. chinensis* males.  
129 After 20 days, by which time all females had died, we counted the number of hatched  
130 eggs in each experimental replication and control treatment (we performed 16 or 17  
131 replications of each) in each density regime to determine the per capita fecundity of *C.*  
132 *maculatus* and the effect of reproductive interference by *C. chinensis* males. We  
133 similarly examined the effect of *C. maculatus* males on the relationship between  
134 fecundity and density in *C. chinensis*. All experiments were conducted at 25°C, 50–60%  
135 relative humidity, and 16L8D. We used analysis of covariance (ANCOVA) to examine  
136 the effects of the presence of heterospecific males, conspecific density, and their  
137 interactions on per capita fecundity. All statistical analyses were conducted with R  
138 software version 2.11.1 (R Development Core Team 2010). The significance level for  
139 the statistical tests was set at  $P = 0.05$ .

140

## 141 **Results**

142

143 The presence of *C. chinensis* males significantly reduced the per capita fecundity of *C.*



144 *maculatus*, and the impact was greater when the density of conspecifics was lower  
145 (ANCOVA: *C. chinensis* males,  $F_{1,165} = 130.05$ ,  $P < 0.001$ ; density,  $F_{1,165} = 18.71$ ,  $P <$   
146  $0.001$ ; interaction,  $F_{1,165} = 16.59$ ,  $P < 0.001$ ; Fig. 1a). In contrast, in the absence of *C.*  
147 *chinensis* males, density and fecundity of *C. maculatus* were not significantly related  
148 (linear regression analysis:  $F_{1,83} = 0.028$ ,  $P = 0.87$ ; Fig. 1a). The presence of *C.*  
149 *maculatus* males had no significant effect on per capita fecundity of *C. chinensis*, but *C.*  
150 *chinensis* density had a significant negative effect on the fecundity of that species  
151 (ANCOVA: *C. maculatus* males,  $F_{1,164} = 0.56$ ,  $P = 0.45$ ; density,  $F_{1,164} = 8.81$ ,  $P =$   
152  $0.003$ ; interaction,  $F_{1,164} = 2.79$ ,  $P = 0.10$ ; Fig. 1b).

153

## 154 **Discussion**

155

156 We suggest that reproductive interference by *C. chinensis* generated an Allee effect in *C.*  
157 *maculatus*. The presence of *C. chinensis* males reduced the per capita fecundity of *C.*  
158 *maculatus* (Fig. 1a), a result in accordance with the findings of a previous study that  
159 documented asymmetric reproductive interference by *C. chinensis* males on *C.*  
160 *maculatus* females (Kishi et al. 2009). A higher density of *C. maculatus*, however,  
161 mitigated the reduction in per capita fecundity, generating an Allee effect (Fig. 1a).

162 Although the presence of *C. chinensis* males reduced the per capita fecundity of *C.*  
163 *maculatus*, in the absence of *C. chinensis*, the fecundity of *C. maculatus* did not depend  
164 on density (Fig. 1a). Thus, we can attribute the observed fecundity reduction in *C.*  
165 *maculatus* exclusively to the presence of *C. chinensis* males. *Callosobruchus chinensis*  
166 males attempt to mate with females of either species indiscriminately (Kishi et al. 2009).  
167 In this study, the relative abundance of *C. chinensis* males to *C. maculatus* decreased as  
168 *C. maculatus* density increased. A single *C. maculatus* female was, therefore, expected  
169 to experience fewer mating attempts by *C. chinensis* males as the density of *C.*  
170 *maculatus* increased, as a result of the dilution effect. If this held true, the hypothesized  
171 reduction in the number of mating attempts by *C. chinensis* males can explain the  
172 enhanced per capita fecundity of *C. maculatus* with increasing *C. maculatus* density  
173 (Fig. 1a). We also observed no Allee effect in *C. chinensis*, which shows little  
174 susceptibility to reproductive interference by *C. maculatus*, a result consistent with our  
175 hypothesis (Fig. 1b).

176           The effects of the presence of heterospecific males on per capita fecundity were  
177 asymmetric; although *C. chinensis* males reduced the fecundity of *C. maculatus* (Fig.1a),  
178 *C. maculatus* males had no significant effect on the fecundity of *C. chinensis* (Fig. 1b).  
179 Interspecific copulation sometimes occurs between *C. chinensis* males and *C. maculatus*

180 females, but it seldom occurs between *C. maculatus* males and *C. chinensis* females  
181 (Yamane and Miyatake 2010). Thus, it is likely that the interspecific copulation between  
182 *C. chinensis* males and *C. maculatus* females plays an important role in the asymmetric  
183 reduction in fecundity. Yamane and Miyatake (2010), however, suggested that neither  
184 sperm nor seminal fluid was transferred during interspecific copulation between *C.*  
185 *chinensis* males and *C. maculatus* females. In *Callosobruchus*, male genitalia have  
186 spines, which injure the genital tracts of the females during intraspecific copulation (e.g.,  
187 Crudgington and Siva-Jothy 2000; Rönn et al. 2007). The genital spines of *C. chinensis*  
188 males may thus injure the genital tracts of *C. maculatus* females, and the putative injury  
189 might cause the fecundity reduction in *C. maculatus*. Another possibility is interspecific  
190 sexual harassment. Though males of both species attempt to mate with heterospecific  
191 females (Kishi et al. 2009), some behavioral differences between these species may  
192 cause asymmetric fecundity reduction. Further experiments are necessary to find out  
193 whether structural damage or behavioral interference is actually responsible for the  
194 asymmetric reproductive interference between *C. chinensis* and *C. maculatus*.

195         In our experiments, difficulty in finding mates was eliminated by allowing  
196 females to copulate with a conspecific male prior to experiments, and reproductive  
197 interference generated an Allee effect in *C. maculatus* even with the presence of

198 conspecific sperm (Fig. 1a). However, reproductive interference may cause lack of  
199 conspecific sperm. For example, heterospecific matting attempts may interfere with  
200 conspecific copulations (Liu et al. 2007). Thus, without copulation with a conspecific  
201 male prior to interaction with heterospecifics, the Allee effect might be enhanced. The  
202 interaction between reproductive interference and conventional mechanisms of Allee  
203 effects is an important topic for further research.

204           It is not clear to what extent reproductive interference causes Allee effects in  
205 nature. The importance of reproductive interference as a mechanism generating an Allee  
206 effect depends on the generality of reproductive interference itself. The role of  
207 reproductive interference in population dynamics, however, has not been appreciated  
208 until recently (Gröning and Hochkirch 2008). Reproductive interference is especially  
209 likely when a biological invasion occurs (Burdfield-Steel and Shuker 2011), that is,  
210 when originally allopatric species begin to interact with each other. Biological invasions,  
211 therefore, may be one of the most promising settings in nature for finding an Allee  
212 effect generated by reproductive interference. On the other hand, several studies have  
213 documented reproductive interference between originally sympatric species (e.g.,  
214 McLain and Pratt 1999; Hettyey and Pearman 2003; Van Gossum et al. 2007),  
215 suggesting that it has the potential to produce Allee effects in sympatric species as well.

216 Allee effects are increasingly appreciated for their importance in applied  
217 ecology problems, such as conservation and biological invasion (Taylor and Hastings  
218 2005; Courchamp et al. 2008; Tobin et al. 2011). Here we showed that reproductive  
219 interference can indeed generate an Allee effect. If reproductive interference can  
220 generate an Allee effect in nature, then programs dealing with small or sparse  
221 populations need to examine interactions between closely related species, even those  
222 that do not share a limited resource.

223

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225

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231

## 232 **References**

233

234 Burdfield-Steel ER, Shuker DM (2011) Reproductive interference. *Curr Biol*  
235 21:R450–R451  
236  
237 Courchamp F, Clutton-Brock T, Grenfell B (1999) Inverse density dependence and the  
238 Allee effect. *Trends Ecol Evol* 14:405–410  
239  
240 Courchamp F, Berec L, Gascoigne J (2008) Allee effects in ecology and conservation.  
241 Oxford University Press, Oxford  
242  
243 Crudgington HS, Siva-Jothy MT (2000) Genital damage, kicking and early death - the  
244 battle of the sexes takes a sinister turn in the bean weevil. *Nature* 407:855–856  
245  
246 Drake J (2005) Risk analysis for species introductions: Forecasting population growth  
247 of Eurasian ruffe (*Gymnocephalus cernuus*). *Can J Fish Aquat Sci*  
248 62:1053–1059  
249  
250 Fauvergue X, Malausa J, Giuge L, Courchamp F (2007) Invading parasitoids suffer no  
251 Allee effect: A manipulative field experiment. *Ecology* 88:2392–2403

252

253 Gascoigne JC, Lipcius RN (2004) Allee effects driven by predation. *J Appl Ecol*

254 41:801–810

255

256 Gröning J, Hochkirch A (2008) Reproductive interference between animal species. *Q*

257 *Rev Biol* 83:257–282

258

259 Harano T, Miyatake T (2005) Heritable variation in polyandry in *Callosobruchus*

260 *chinensis*. *Anim Behav* 70:299–304

261

262 Hettyey A, Pearman PB (2003) Social environment and reproductive interference affect

263 reproductive success in the frog *Rana latastei*. *Behav Ecol* 14:294–300

264

265 Hopper KR, Roush RT (1993) Mate finding, dispersal, number released, and the success

266 of biological-control introductions. *Ecol Entomol* 18:321–331

267

268 Kishi S, Nishida T, Tsubaki Y (2009) Reproductive interference determines persistence

269 and exclusion in species interactions. *J Anim Ecol* 78:1043–1049

270

271 Kuno E (1992) Competitive exclusion through reproductive interference. *Res Popul*

272 *Ecol* 34:275–284

273

274 Liebhold AM, Tobin PC (2008) Population ecology of insect invasions and their

275 management. *Annu Rev Entomol* 53:387–408

276

277 Liu S, De Barro PJ, Xu J, Luan J, Zang L, Ruan Y, Wan F (2007) Asymmetric mating

278 interactions drive widespread invasion and displacement in a whitefly. *Science*

279 318:1769–1772

280

281 McLain D, Pratt A (1999) The cost of sexual coercion and heterospecific sexual

282 harassment on the fecundity of a host-specific, seed-eating insect

283 (*Neacoryphus bicrucis*). *Behav Ecol Sociobiol* 46:164–170

284

285 Miyatake T, Matsumura F (2004) Intra-specific variation in female remating in

286 *Callosobruchus chinensis* and *C. maculatus*. *J Insect Physiol* 50:403–408

287



288 Myers RA, Barrowman NJ, Hutchings JA, Rosenberg AA (1995) Population-dynamics  
289 of exploited fish stocks at low population-levels. *Science* 269:1106–1108  
290

291 Nishigaki J (1963) The effect of low population density on the mating chance and the  
292 fecundity of the azuki bean weevil, *Callosobruchus chinensis* L. *Jpn J Ecol*  
293 13:178–184 (in Japanese)  
294

295 R Development Core Team (2010) R: a language and environment for statistical  
296 computing. R Foundation for Statistical Computing, Vienna, Austria. URL:  
297 <http://www.R-project.org>  
298

299 Rönn J, Katvala M, Arnqvist G (2007) Coevolution between harmful male genitalia and  
300 female resistance in seed beetles. *Proc Natl Acad Sci USA* 104:10921–10925  
301

302 Stephens PA, Sutherland WJ (1999) Consequences of the Allee effect for behaviour,  
303 ecology and conservation. *Trends Ecol Evol* 14:401–405  
304

305 Stephens PA, Sutherland WJ, Freckleton RP (1999) What is the Allee effect? *Oikos*

306                   87:185–190

307

308   Takakura K, Nishida T, Matsumoto T, Nishida S (2009) Alien dandelion reduces the

309                   seed-set of a native congener through frequency-dependent and one-sided

310                   effects. *Biol Invasions* 11:973–981

311

312   Taylor CM, Hastings A (2005) Allee effects in biological invasions. *Ecol Lett* 8:895–908

313

314   Tobin PC, Berec L, Liebhold AM (2011) Exploiting Allee effects for managing

315                   biological invasions. *Ecol Lett* 14:615–624

316

317   Van Gossum H, Beirinckx K, Forbes MR, Sherratt TN (2007) Reproductive interference

318                   between *Nehalennia* damselfly species. *Ecoscience* 14:1–7

319

320   Yamane T, Miyatake T (2010) Inhibition of female mating receptivity by male-derived

321                   extracts in two *Callosobruchus* species: Consequences for interspecific mating.

322                   *J Insect Physiol* 56:1565–1571

323

324 Yoshida, T (1966) Studies on the interspecific competition between bean weevils.  
325       Memoirs of the Faculty of Liberal Arts and Education, Miyazaki University,  
326       Miyazaki 20:59–98  
327  
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329

330 **Figure legend**

331

332 Fig. 1. Per capita fecundity of *C. maculatus* (a) and *C. chinensis* (b) in the absence  
333 (open circles) or presence (closed circles) of heterospecific males, plotted against  
334 population density. In (a), the broken line and the solid line are regression lines for  
335 the data in the absence or presence of *C. chinensis* males, respectively. In (b), the  
336 regression was calculated using all data, because the effect of *C. maculatus* males  
337 on *C. chinensis* fecundity was not significant.

Fig. 1.

