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Interpopulation variation in female remating is attributable to female and male effects in *Callosobruchus chinensis*

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18

19 **Abstract**

20 The evolution of female multiple mating is best understood from a consideration of male and
21 female reproductive perspectives. Generally, females should be selected to remate at their
22 optimal frequencies, whereas males should be selected to manipulate female remating to their
23 advantage. Therefore, female remating behavior may be changed by variation in male as well
24 as female traits. In this study, our aim was to separate the effects of female and male strains on
25 female remating in the adzuki bean beetle, *Callosobruchus chinensis*, which have the
26 interstrain variation in the female remating frequency. We found that the interstrain variation in
27 female remating is primarily attributable to female traits, suggesting genetic variation in
28 female receptivity to remating in *C. chinensis*. However, some interstrain variation in female
29 remating propensity was attributable to an interaction between female and male strains, with
30 the males of some strains being good at inducing nonreceptivity in females from one
31 high-remating strain, whereas others were good at inducing copulation in nonvirgin females
32 from the high-remating strain. Thus, there is interstrain variation in male ability to deter
33 females from remating and in male ability to mate successfully with nonvirgin females. These
34 results suggest that mating traits have evolved along different trajectories within different
35 strains of *C. chinensis*.

36

37

38 **Key words** polyandry · multiple mating · sexual conflict · sexual selection · genetic

39 variation · *Callosobruchus chinensis*

40

41 **Introduction**

42

43 For females of most animal species, a single mating is indispensable to reproduction, but the
44 fitness advantages of multiple mating are not easily understood. This is because the classic
45 model of sexual selection predicts that, unlike that of males, female reproductive success does
46 not increase monotonically with the number of mates (Bateman 1948). Moreover, superfluous
47 mating may decrease female fitness because mating involves various costs to females
48 (Thornhill and Alcock 1983; Arnqvist and Nilsson 2000). However, females of the majority of
49 animal species do mate multiply (Thornhill and Alcock 1983; Ridley 1988; Birkhead and
50 Møller 1998; Birkhead 2000). Thus, a variety of the benefits to females of remating have been
51 proposed to account for the evolution of female multiple mating (Thornhill and Alcock 1983;
52 Yasui 1998; Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Zeh and Zeh 2003). Many
53 studies have shown that female fitness increases with mating frequency to some extent (Ridley
54 1988; Arnqvist and Nilsson 2000). Therefore, the relationship between female mating
55 frequency and fitness is often more complex than that predicted in the classic model, and
56 females should be selected to remate at their optimal frequencies (Arnqvist and Nilsson 2000;
57 Arnqvist et al. 2005).

58 The evolution of female remating behavior cannot be understood only from the perspective of

59 female benefits because males may manipulate female remating in favor of them (Parker 1979;
60 Holland and Rice 1998; Arnqvist and Rowe 2002, 2005; Chapman et al. 2003; Pizzari and
61 Snook 2003). Under conditions of polyandry, males should benefit through increased
62 fertilization success by inducing nonreceptivity in females after mating, and they also benefit
63 from mating with nonvirgin females via sperm mixing in the spermatheca or displacement of
64 sperm from previous mates. The male manipulation of female remating may coincide with the
65 interests of females. In this case, coevolution of male traits and female traits may be driven by
66 selection on males to manipulate female mating behavior and on females to acquire direct or
67 indirect benefits from preferring the males with manipulative traits (Andersson 1994; Eberhard
68 1996; Jennions and Petrie 2000; Cordero and Eberhard 2003; Kokko et al. 2003). In contrast,
69 the male manipulation of female remating may conflict with the interests of females. Thus,
70 males may induce females to remate less frequently than the optima of females (Pitnick et al.
71 2001; Montrose et al. 2004), or they may seduce or coerce females to remate more frequently
72 than the optima of females (Clutton-Brock and Parker 1995; Arnqvist 1997; Holland and Rice
73 1998). It has been suggested that the conflict of interests of a female, her previous mate and
74 her potential future mates results in the evolution of male manipulation of female remating and
75 the evolution of female counteradaptation to prevent the manipulation (Holland and Rice 1998;
76 Rice 1998; Arnqvist and Nilsson 2000; Gavrilets et al. 2001; Arnqvist and Rowe 2002, 2005;

77 Chapman et al. 2003; Pizzari and Snook 2003; Härdling and Kaitala 2005). Both type of
78 male-female coevolution will affect the evolution of female remating behavior.

79 In the adzuki bean beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae), remating
80 reduces female fecundity, suggesting that there is sexual conflict between reluctant females
81 and persistent males over female remating (Harano et al. 2006). This leads us to the prediction
82 that female remating behavior has been subjected to the selection that stems from sexual
83 conflict in *C. chinensis*. Marked variation in female remating frequency has been found
84 between different strains of *C. chinensis* (Miyatake and Matsumura 2004; Harano and
85 Miyatake 2005). This implies that there is genetic variation in female remating, as has been
86 shown by using artificial selection in a related species, *C. maculatus* (Eady et al. 2004). The
87 variation in female remating might be attributable to female genetic traits and/or male genetic
88 traits affecting female remating. The inheritance of female and male traits related to female
89 remating behavior has been studied extensively in *Drosophila melanogaster*. In this species,
90 artificial selection showed genetic variation in the female traits that control female remating
91 speed (Gromko and Newport 1988; Sgró et al. 1998). Moreover, there is evidence for genetic
92 variation in the ability of first males to deter females from remating (Service and Vossbrink
93 1996; Sgró et al. 1998). Under the removal of sexual selection through experimentally forced
94 monogamy in *D. melanogaster*, a naturally promiscuous species, males evolved to have

95 reduced deterrence of female remating (Pitnick et al. 2001). When females were prevented
96 from evolving and males were allowed to adapt to the female phenotype in an experimental
97 population, the ability of males to increase the rate of female remating evolved within the
98 population (Rice 1996). These findings suggest that genetic variation in female and/or male
99 traits potentially causes the difference in female remating behavior.

100 The interstrain variation for female remating frequency in *C. chinensis* may be a result of the
101 difference in (1) female traits, (2) the ability of the first males to inhibit female remating
102 through their ejaculates and/or the physical effects of copulation or (3) the ability of the second
103 males to promote female remating through their courtship behavior, or (4) a combination of the
104 above. To distinguish these different scenarios, we first determined whether the interstrain
105 variation in female remating behavior is attributable to genetic traits of females, males or both
106 in *C. chinensis*. Here, we predict that, if the interstrain variation for female remating depends
107 entirely on female traits, then female remating behavior should not be influenced by a
108 difference in the strain of origin of the males that mate with the females, whereas if there is
109 variation in male traits affecting female remating between strains, then female remating
110 behavior should be influenced by the strain of origin of the males. Second, we compared the
111 ability of first males to deter females from remating after copulation and the ability of second
112 males to mate successfully with already mated females between strains of this species.

113

114 **Materials and Methods**

115

116 Insects and culture

117

118 We used four strains of *C. chinensis* (see Table 1 for detailed information). We classified the
119 isC and yoC02 as high female remating strains and the jC-S and rdaCmrkt as low female
120 remating strains. The classification was done with the help of existing data on the frequency of
121 female remating of the strains (Harano and Miyatake 2005, T. Harano unpublished). According
122 to the classification, we refer to the isC, yoC02, jC-S and rdaCmrkt as the High-1, High-2,
123 Low-1 and Low-2 strains, respectively. Stock cultures of these strains had been maintained as
124 mass cultures.

125 All beetles used for this study were reared from eggs laid by parents collected randomly from
126 stock cultures of each strain. The parent beetles were allowed to lay up to five eggs per adzuki
127 bean, *Vigna angularis* in any strain. Virgin adults emerging from these beans were kept in
128 separate-sex groups of up to 10 adults in plastic cups (2.8 cm high, 7 cm in diameter) and
129 given water and adult food (1:2 yeast extract:sugar). At the age of 2-5 days, female and male
130 adults were used for the following experiments. Umeya and Shimizu (1968) have reported that

131 mean longevity of female adults equals to 58 days under the rearing condition, which is similar
132 to this study. Thus, adults were used early in their life for the experiments in this study. All
133 rearing and subsequent experiments were conducted in a chamber maintained at 25°C and 50%
134 relative humidity under a photoperiod cycle of 14:10 light: dark.

135

136 Experiment 1: effects of female and male strains on female remating

137

138 In this experiment, we used the High-1 and Low-1 strains (Table 1). To examine the effects of
139 female and male strains separately on female remating, we created four treatments of mating
140 pairs (High-1 female × High-1 male, High-1 female × Low-1 male, Low-1 female × High-1
141 male and Low-1 female × Low-1 male).

142 To confirm female first mating, we placed one virgin female and one virgin male in a glass
143 vial (4.4 cm high, 1.7 cm in diameter), and observed their mating for 1 h. After copulation, the
144 male was removed, and the female was maintained in groups of up to 10 beetles in plastic cups
145 and given water and adult food. Female remating was observed on days 1, 3 and 5 after the
146 first mating. To determine whether the female remates, we placed the female and another
147 virgin male from the same strain as the first mate in a glass vial, and observed them each day
148 either until females had remated once or 1 h had passed. Remated females were not observed

149 further. For each female, we recorded ‘remated on day 1’, ‘remated on day 3’, ‘remated on day
150 5’ or ‘not remated at all’ as the score of the tendency of females to remate.

151 We compared the frequency of female first mating, calculated as the percentage of virgin
152 females mated with males presented to them, between the treatments. The score of the
153 tendency of females to remate was ranked in the descending order of ‘remated on day 1’,
154 ‘remated on day 3’, ‘remated on day 5’ and ‘not remated at all’. We assessed the level of
155 female remating as the ranked score, and compared the level of female remating between the
156 treatments of mating pairs.

157

158 Experiment 2: comparison of the effects of first and second males on female remating between
159 strains

160

161 We examined variation in the ability of first males to deter females from remating and the
162 ability of second males to promote female remating in the females from the High-1 strain,
163 remating of which was influenced by the strains of origin of their mates in the experiment 1
164 (see Results). The abilities of first and second males were separately compared between four
165 strains: High-1, High-2, Low-1 and Low-2 (Table 1).

166

167 *Effects of first male*

168

169 A virgin female from the High-1 strain was mated first with a virgin male from any one of four
170 strains; then she was given opportunities to remate with a virgin male from the High-1 strain,
171 and the remating was observed in the same way as the experiment 1. To examine first male
172 deterrence of female remating, we compared the level of female remating between the strains
173 of origin of the males that females mated with first.

174

175 *Effects of second male*

176

177 A virgin female from the High-1 strain was mated first with a virgin male from the High-1
178 strain; then she was given opportunities to remate with a virgin male from any one of four
179 strains, and the remating was observed as described above. To examine the ability of second
180 males to mate successfully with mated females, we compared the level of female remating
181 between the strains of origin of the males that the females were paired with at remating.

182

183 Statistical analyses

184

185 To compare the frequency of female first mating between the treatments of mating pairs in
186 experiment 1, we applied the *G* test using Williams's correction (Sokal and Rohlf 1995) and
187 corrected the significance level ($\alpha=0.05$) by the sequential Bonferroni method (Rice 1989). To
188 test for the effects of female strain and male strain on the level of female remating in the
189 experiment 1, we used a non-parametric two-way ANOVA according to Scheirer-Ray Hare
190 extension of the Kruskal-Wallis test (Sokal and Rohlf 1995). To compare the level of female
191 remating between male strains in experiment 2, one-way Kruskal-Wallis test was carried out
192 using SPSS version 11.0 (SPSS Institute 2001). Pairwise comparisons between the treatments
193 of mating pairs in the experiment 1 and between male strains in the experiment 2 were
194 performed using the non-parametric multiple comparison, Steel-Dwass method (Dwass 1960;
195 Steel 1960) if the non-parametric two-way ANOVA showed a significant interaction between
196 female strain and male strain in the experiment 1 or the Kruskal-Wallis test showed a
197 significant difference in the experiment 2.

198

199 **Results**

200

201 Experiment 1: effects of female and male strains on female remating

202

203 Approximately 70% of virgin females from the High-1 strain and more than 80% of virgin
204 females from the Low-1 strain mated, regardless of the strains of origin of the males that the
205 females were paired with (Table 2). There were no significant differences between male strains
206 in the first mating frequency of the High-1 females ($G_{adj}=0.16$, $P>0.05$; Table 2) and in that of
207 the Low-1 females ($G_{adj}=5.64$, $P>0.05$; Table 2). The first mating frequency was significantly
208 higher in the Low-1 females than in the High-1 females when the females were paired with the
209 High-1 males ($G_{adj}=18.88$, $P<0.05$; Table 2), but it did not differ significantly between female
210 strains when the females were paired with the Low-1 males ($G_{adj}=6.05$, $P>0.05$; Table 2).

211 Overall the level of female remating was significantly affected by female strain ($df=1$,
212 $SS=673450.40$, $H=80.37$, $P<0.001$) and male strain ($df=1$, $SS=110776.27$, $H=13.22$, $P<0.001$),
213 and there was a significant interaction between female strain and male strain ($df=1$,
214 $SS=103297.40$, $H=12.33$, $P<0.001$). Therefore, we performed pairwise comparison between
215 the treatments of mating pairs. The High-1 females had significantly higher levels of remating
216 than the Low-1 females when paired with the High-1 males (test statistic=7.78, $P<0.01$; Table
217 2), and they also did so when paired with the Low-1 males (test statistic=4.55, $P<0.01$; Table
218 2). The effects of male strain on the level of female remating depended on the female strain.
219 Almost none of the Low-1 females remated either when paired with the High-1 males or when
220 paired with the Low-1 males, and the remating level of the Low-1 females did not differ

221 significantly between the male strains (test statistic=0.00, $P>0.05$; Table 2). On the other hand,
222 the remating level of the High-1 females paired with the High-1 males was significantly higher
223 than those paired with the Low-1 males (test statistic=4.60, $P<0.01$; Table 2).

224

225 Experiment 2: comparison of the effects of first and second males on female remating between
226 strains

227

228 *Effects of first male*

229

230 The cumulative remating frequency of the High-1 females ranged from 42.5 to 61.5% during
231 the 5 days after the first mating among the strains of origin of first males (Table 3). There was
232 a significant difference in the level of the female remating between the strains of first males
233 ($H_3=11.17$, $P=0.011$). The remating level of the females mated first with the Low-1 males was
234 significantly lower than that of females mated first with the High-2 males (test statistic=3.06,
235 $P<0.05$; Table 3), and it was marginally but not significantly lower than that of females mated
236 first with the High-1 males (test statistics=2.56, critical value at significance level set to 0.05
237 =2.57; Table 3).

238

239 *Effects of second male*

240

241 The cumulative remating frequency of the High-1 females varied more among the strains of
242 origin of second males, ranging from 22.4 to 59.2% during the 5 days after the first mating
243 (Table 4), than among the strains of origin the first males (Table 3). There was a significant
244 difference in the level of the female remating between the strains of second males ($H_3=32.84$,
245 $P<0.001$). The remating level of females given opportunities to remate with the High-1 males
246 was significantly higher than that of females given opportunities to remate with the High-2
247 (test statistic=2.97, $P<0.05$; Table 4), Low-1 (test statistic=5.79, $P<0.01$; Table 4) and Low-2
248 (test statistic=2.92, $P<0.05$; Table 4) males, and the remating level was significantly higher in
249 females given opportunities to remate with the High-2 and Low-2 males than in females given
250 opportunities to remate with the Low-1 males (test statistic=2.86, $P<0.05$ and test
251 statistic=2.81, $P<0.05$, respectively; Table 4).

252

253 **Discussion**

254

255 The difference in the level of female remating between females derived from strains with high
256 and low frequencies of female remating, the High-1 and Low-1 strains, was consistently

257 significant across the strains of origin of the males that females paired with (Table 2). This
258 indicates that the genetic variation in female remating between strains of *C. chinensis* is
259 primarily attributable to the differences in female receptivity to remating (see also Miyatake
260 and Matsumura 2004; Harano and Miyatake 2005).

261 The effects of male strain on the level of female remating depended on female strain. Most of
262 the Low-1 females mated indiscriminately with the first male they encountered and then
263 became nonreceptive, regardless of the male strain (Table 2). On the other hand, the High-1
264 females showed some receptivity after their first mating (Table 2). The remating levels of the
265 High-1 females were influenced by the strain of origin of the first male (Table 3), suggesting
266 genetic variation in male ability to inhibit female remating through ejaculate or the physical
267 effects of copulation in *C. chinensis*. The remating levels of the High-1 females were also
268 influenced by the strain of origin of the second male (Table 4), suggesting genetic variation in
269 male ability to mate successfully with mated females through courtship behavior in *C.*
270 *chinensis*.

271 Studies with population crosses have shown the effects of interaction between male and
272 female genotypes on male induction of female nonreceptivity to remating in some insect
273 species (Andrés and Arnqvist 2001; Brown and Eady 2001; Nilsson et al. 2003). These suggest
274 that female traits may shape the pattern of sexual selection on acting males (Nilsson et al.

275 2003). The present study also showed significant male-female interaction effects on female
276 remating behavior in *C. chinensis*, such that the effects of male strain on female remating
277 differed between the strains of origin of females. In the *C. chinensis* populations with high
278 levels of female remating, the variation in male traits influences the level of female remating,
279 in other words, whether a female remates (Table 2). Therefore, males that have superior ability
280 to deter females from remating after copulation or to mate successfully with already mated
281 females can achieve higher reproductive success in the high-remating populations. In the
282 populations with low levels of female remating, in contrast, male traits do not influence
283 whether a female remate (Table 2). Therefore, sexual selection on the male traits affecting
284 female remating may be strong in the high-remating populations, whereas such selection may
285 be weak or absent in the low-remating populations.

286 The remating level of females derived from the High-1 strain mated first with males from one
287 low-remating population, the Low-1 strain, was lower than that of females mated first with
288 males from two high-remating populations, the High-2 and High-1 strains, although the
289 difference with the latter strain was statistically marginal (Table 3). However, the remating
290 level of females mated first with males from the other low-remating population, the Low-2
291 strain, do not differ from that of females mated first with males from the High-1 and High-2
292 strains (Table 3). This result indicates that the males only from the Low-1 strain exert superior

293 ability to deter females from remating than the males from the High-1 and High-2 strains do.
294 This is not consistent with the hypothesis that differences in sexual selection generate the
295 variation in the ability of first males to inhibit female remating between populations. On the
296 other hand, the level of female remating was highest when females were given opportunities to
297 remate with the males from the High-1 strain, intermediate when offered the opportunity to
298 remate with the males from the High-2 and Low-2 strains and lowest with males from the
299 Low-1 strain (Table 4). This result indicates that the ability of second males to mate
300 successfully with mated females is most superior in males from one high-remating population
301 and worst in males from one low-remating population, although this pattern was not entirely
302 consistent across high- and low-remating populations. Further study using more numerous
303 populations is needed to confirm the hypothesis that differences in sexual selection generate
304 the variation in the male ability between populations because we used a small number of
305 populations in the present study.

306 Another possible explanation for the interstrain variation in male traits affecting female
307 remating behavior is differences between strains in rearing periods in the laboratory. Rearing
308 condition may generate selection on some traits of beetles. If the male traits affecting female
309 remating are genetically correlated with any other traits, then they may have changed as a
310 result of inadvertent selection acting on the correlated traits, such as body size or courtship

311 activity, under the rearing for successive generations (Miyatake 1998). Among strains of *C.*
312 *chinensis* used for the present study, males of the Low-1 strain, which has been maintained in
313 the laboratory for more than 60 years (Table 1), have a larger body size and a lower courtship
314 activity (unpublished). In a seed beetle, *Stator limbatus*, the body size of the first male
315 influences female remating, such that females mated first with larger males were less likely to
316 remate (Savalli and Fox 1998). In *C. chinensis*, larger body size in males from the Low-1
317 strain may account for the males being good at inducing nonreceptivity in females (Table 3).
318 Male body size may also influence mating success with reluctant females (Day and Gilburn
319 1997; Crean and Gilburn 1998; Ortigosa and Rowe 2002; Shuker and Day 2002; Maklakov et
320 al. 2003). In *Drosophila melanogaster*, larger males court more often than smaller males, and
321 females remate more rapidly when courted by larger males (Pitnick 1991; Friberg and Arnqvist
322 2003). The interstrain variation in the male traits affecting female remating might be generated
323 as a result of selection acting on male body size and/or courtship activity in *C. chinensis*.
324 Moreover, it is also possible that a random genetic drift occurs under the rearing condition.
325 The genetic drift might have influenced male traits affecting female remating in *C. chinensis*.
326 Males and females typically maximize their reproductive success in different ways (Arnqvist
327 and Rowe 2005). Females should generally be selected to remate at their optimal frequencies
328 (Arnqvist and Nilsson 2000; Arnqvist et al. 2005), whereas males should generally be selected

329 to manipulate female remating to their advantage (Parker 1979; Holland and Rice 1998;
330 Arnqvist and Rowe 2002, 2005; Chapman et al. 2003; Pizzari and Snook 2003). As an
331 evolutionary consequence of this, female remating behavior may be affected not only by
332 female traits but also by male traits: male abilities to inhibit female remating and to mate
333 successfully with females already mated to other males. The strains of *C. chinensis* used in this
334 study have diverged in female receptivity to remating and the male abilities to manipulate
335 female remating behavior. This suggests that the female and male traits have evolved along
336 different evolutionary trajectories between strains of *C. chinensis*. Coevolution of female traits
337 and male traits affecting female remating would either be driven by selection on individuals of
338 both sexes to acquire benefits from an interaction with each other (Andersson 1994; Eberhard
339 1996; Jennions and Petrie 2000; Cordero and Eberhard 2003; Kokko et al. 2003) or by
340 sexually antagonistic selection that stems from conflict between the interests of the two sexes
341 (Holland and Rice 1998; Arnqvist and Nilsson 2000; Gavrilets et al. 2001; Arnqvist and Rowe
342 2002, 2005; Chapman et al. 2003; Pizzari and Snook 2003). The latter selection mechanism is
343 more likely in the evolution of female traits of resistance to remating and male traits of
344 persistence in mating in *C. chinensis* because remating reduces female fecundity, suggesting
345 that there is sexual conflict over female remating in this species (Harano et al. 2006).
346

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Table 1. The rearing history and female remating frequency of each strain of *Callosobruchus chinensis* used in this study.

| Strain | Collection | | Number of founder adults | % Female remating * |
|------------------|------------|--------------------------|--------------------------|---------------------|
| | year | Locality of population | | |
| High-1 (isC) | 1997 | Ishigaki, Okinawa, Japan | More than 200 | 38.4 ** |
| High-2 (yoC02) | 2002 | Yoshii, Okayama, Japan | 26 | 32.7 ** |
| Low-1 (jC-S) | 1936 | Kyoto, Kyoto, Japan | No information | 8.8 ** |
| Low-2 (rdaCmrkt) | 1998 | Rajshahi, Bangladesh | More than 50 | 7.5 *** |

Reference to each strain: isC to Yanagi and Miyatake (2003); yoC02 to Harano and Miyatake (2005); jC-S to Utida (1941a, b); rdaCmrkt to Toquenaga Y. (personal communication).

*The data represents the cumulative frequency of female remating for 5 day after first mating.

The data from Harano and Miyatake (2005). * The data from T. Harano (Unpublished); it was examined followed by the method of Harano and Miyatake (2005).

Table 2. Frequency of female first mating, cumulative frequency of female remating after first mating and the level of female remating in each mating pair.

| Mating pair | | First mating | | Remating | | | | | Rank of the level of female remating | |
|-------------|--------|--------------|--------------------|----------|-------------------------|------|------|---------------|--------------------------------------|--|
| | | | | <i>n</i> | % Female remating | | | Mean ± SE | | |
| ♀ | ♂ | <i>n</i> | <i>%</i> | | Days after first mating | | | | | |
| | | | | 1 | 3 | 5 | | | | |
| High-1 | High-1 | 149 | 70.5 ^a | 103 | 31.1 | 49.5 | 56.3 | 284.3 ± 10.69 | ^a | |
| High-1 | Low-1 | 187 | 68.4 ^a | 125 | 12.8 | 20.0 | 27.2 | 220.5 ± 8.46 | ^b | |
| Low-1 | High-1 | 109 | 91.7 ^b | 100 | 4.0 | 4.0 | 4.0 | 172.9 ± 4.64 | ^c | |
| Low-1 | Low-1 | 121 | 81.0 ^{ab} | 98 | 2.0 | 2.0 | 4.1 | 171.8 ± 4.14 | ^c | |

The frequency of female first mating was compared by the *G*-test using Williams's correction (Sokal and Rohlf 1995); the significance level was corrected by the sequential Bonferroni method (Rice 1989). The level of female remating (see Materials and Methods) was compared by Steel-Dwass method (Dwass 1960; Steel 1960). The different letters indicate significant difference between mating pairs at $P < 0.05$.

Table 3. Cumulative remating frequency of the High-1 females after the first mating and the level of female remating in females that were mated first with males from each strain. Females received opportunities to remate with the High-1 males.

| Strain of first males | <i>n</i> | % Female remating | | | Rank of the level of female remating | |
|--------------------------|----------|-------------------------|------|------|---|------|
| | | Days after first mating | | | Mean ± SE | |
| | | 1 | 3 | 5 | | |
| High-1 | 125 | 32.0 | 49.6 | 59.2 | 257.3 ± 11.64 | ab * |
| High-2 | 109 | 38.5 | 53.2 | 61.5 | 268.6 ± 12.79 | a |
| Low-1 | 146 | 24.0 | 35.6 | 42.5 | 217.2 ± 10.64 | b * |
| Low-2 | 113 | 33.6 | 47.8 | 54.9 | 251.3 ± 12.64 | ab |

The different letters indicate significant difference in the level of female remating (see Materials and Methods) between male strains at $P < 0.05$ by Steel-Dwass method (Dwass 1960; Steel 1960). *The difference in the remating level between the females mated first with the Low-1 males and with the High-1 males was statistically marginal (test statistics=2.56, critical value at significance level set to 0.05 =2.57).

Table 4. Cumulative remating frequency of the High-1 females after the first mating and the level of female remating in females that were paired with males from each strain at remating. The females were mated first with the High-1 males.

| Strain of second males | <i>n</i> | % Female remating | | | Rank of the level of female remating | |
|---------------------------|----------|-------------------------|------|------|---|--------------------|
| | | Days after first mating | | | Mean | ± SE |
| | | 1 | 3 | 5 | | |
| High-1 | 125 | 32.0 | 49.6 | 59.2 | 326.9 ± | 12.33 ^a |
| High-2 | 133 | 24.8 | 30.8 | 38.3 | 273.2 ± | 11.96 ^b |
| Low-1 | 107 | 15.9 | 18.7 | 22.4 | 229.3 ± | 9.99 ^c |
| Low-2 | 153 | 27.5 | 32.0 | 37.3 | 273.9 ± | 11.41 ^b |

The same letters indicate no significant difference in the level of female remating (see Materials and Methods) between male strains at $P < 0.05$ by Steel-Dwass method (Dwass 1960; Steel 1960).