

Effect of oviposition substrate on female remating in *Callosobruchus chinensis* (Coleoptera: Bruchidae)

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Abstract

We investigated the effect of the presence of an oviposition substrate on female remating behavior in three strains of the adzuki bean beetle, *Callosobruchus chinensis*. In the two strains with low levels of female remating, females maintained with an oviposition substrate, and thus allowed to lay eggs, had a greater tendency to remate than females maintained without an oviposition substrate to prevent them from laying eggs. The oviposition substrate had no significant effect on female remating in the strain with a high level of female remating. The results indicate that female remating is influenced by the presence of an oviposition substrate, an environmental factor, in *C. chinensis*. This suggests that females adaptively change their receptivity to remate in response to environmental factors affecting the costs and benefits of remating in *C. chinensis*.

Key words: Polyandry; multiple mating; bean beetle; environmental factor; *Callosobruchus chinensis*

INTRODUCTION

Females of the majority of animal species are polyandrous and often mate with different males (Thornhill and Alcock, 1983; Ridley, 1988; Birkhead and Møller, 1998; Birkhead, 2000). However, the adaptive significance for females of multiple mating or polyandry is unclear because the classic model of sexual selection predicts that male reproductive success increases with the number of mates, whereas female reproductive success does not increase monotonically with the number of mates (Bateman, 1948). Therefore, the evolution of female multiple mating or polyandry has been the subject of a large number of theoretical and empirical studies (Thornhill and Alcock, 1983; Yasui, 1997, 1998; Arnqvist and Nilsson, 2000).

The adzuki bean beetle, *Callosobruchus chinensis*, is a cosmopolitan pest of stored legumes, such as the adzuki bean, *Vigna angularis*, and the cowpea, *Vigna unguiculata* (Kiritani, 1961). Marked variation in female remating frequency has been found between different strains of *C. chinensis*, implying genetic variation in polyandry (Miyatake and Matsumura, 2004; Harano and Miyatake,

2005). As this intraspecific variation is interesting, *C. chinensis* has recently become a model species for the study of female multiple mating (e.g., Miyatake and Matsumura, 2004; Harano and Miyatake, 2005, 2007; Yamane and Miyatake, 2005; Harano et al., 2006).

The variation in female remating might be caused by not only genetic factors but also environmental factors (Harshman et al., 1988); however, we have no information about the effects of environmental factors on female remating in *C. chinensis*. Females of this beetle lay their eggs on the surface of host beans, and rarely lay eggs without an oviposition substrate (Umeya, 1987). Thus, the availability of an oviposition substrate greatly influences the reproductive behavior of *C. chinensis* females. In a closely related species, *C. maculatus*, in which most females have multiple mates (Eady, 1991; Arnqvist et al., 2005), females allowed access to an oviposition substrate more readily remate with courting males (Fox and Hickman, 1994). The proportion of polyandrous females in *C. chinensis* (range 6–40%; Harano and Miyatake, 2005) is considerably less than that in *C. maculatus* (85%; Eady, 1991). Therefore, it is unclear whether

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C. chinensis is similar to *C. maculatus* in the effect of an oviposition substrate on female remating behavior. In this study, we investigated the effect of the presence of an oviposition substrate on female remating behavior in *C. chinensis*.

MATERIALS AND METHODS

Insects and cultures. We used three strains of *C. chinensis*. The first, referred to as the jC-S strain, was established with beetles collected in Kyoto City, Japan in 1936 (Utida, 1941a, b). The second, referred to as the mC strain, was established with beetles collected in Morioka City, Japan in the 1960s (Nakamura, 1969; see also Harano and Miyatake, 2005). The third, referred to as the isC strain, was established with about 200 adults collected in Ishigaki City, Japan in 1997 (Yanagi and Miyatake, 2003). In a prior study (Harano and Miyatake, 2005), less than 10% of females remated in jC-S and mC strains, whereas approximately 40% of females remated in the isC strain.

All beetles used for this study were reared from eggs laid by parents collected randomly from stock cultures of each strain. The parent beetles were allowed to lay five to ten eggs per adzuki bean. Virgin adults that emerged from these beans were kept in separate-sex groups of ten to fifteen beetles in plastic cups (2.8 cm high, 7 cm in diameter) and given water and adult food (1:2 yeast extract:sugar). At the age of 2–5 d, they were used for the following experiments. All rearing and subsequent experiments were conducted in a chamber maintained at 25°C and 60% relative humidity under a photoperiod cycle of 16 h light:8 h dark.

Remating in females with and without oviposition substrate. We placed one virgin female and one virgin male from each strain in a glass vial (4.4 cm high, 1.7 cm in diameter), and the female was allowed to copulate once. After copulation, the male was removed, and the mated female was randomly assigned to either the with-beans treatment or the without-beans treatment. In the with-beans treatment, the females were maintained in groups of five beetles in plastic cups containing water, adult food, and ten adzuki beans per female as an oviposition substrate to be allowed to lay eggs. The beans were replaced with fresh ones on days 1 and 5 after the first mating. In the without-beans treatment, the females were maintained in groups of

five beetles in plastic cups containing only water and adult food to prevent egg laying.

Female remating was observed 1, 5, and 10 d after the first mating. To determine whether females remate, we placed one mated female and one virgin male from the same strain as the female in a glass vial, and observed them each day until either the female had remated once or 1 h had passed. Once a female remated, we stopped observing and discarded the females. For each female, we recorded the score, either 'remated on day 1', 'remated on day 5', 'remated on day 10,' or 'never remated', ranked in descending order. We assessed the level of female remating as the score. A few females (6/150 females in the with-beans treatment and 0/150 females in the without-beans treatment in the jC-S strain, 4/150 females in the with-beans treatment and 3/150 females in the without-beans treatment in the mC strain, 3/150 females in the with-beans treatment and 0/150 females in the without-beans treatment in the isC strain) died before we recorded the score of remating and were excluded from the analysis. To compare the level of female remating between the two treatments, Mann-Whitney *U* test was carried out using SPSS version 11.0 (SPSS Institute, 2001).

RESULTS

In the jC-S strain, 16% of females remated in the with-beans treatment, whereas only 3% of females remated in the without-beans treatment during the 10 d after the first mating (Table 1). In the mC strain, 16% of females remated in the with-beans treatment, whereas only 6% of females remated in the without-beans treatment during the 10 d after the first mating (Table 1). In these two strains, there were significant differences in the level of female remating between the two treatments (Table 1). On the other hand, in the isC strain, 39% of females in the with-beans treatment and 33% of females in the without-beans treatment remated during the 10 d after the first mating, and there was no statistically significant difference in the level of female remating between the two treatments (Table 1).

DISCUSSION

In the two strains of *C. chinensis* with low levels of polyandry, the jC-S and mC strains, females

Table 1. Comparison of the tendency of females to remate between oviposition treatments

Strain	Beans	N	Number of remated females on each day after first mating				Mann-Whitney <i>U</i> test		
			1	5	10	None	Mean rank ^a	Z-value	<i>p</i>
jC-S	With	144	18	5	0	121	157.0	3.678	0.0002
	Without	150	4	1	0	145	138.4		
mC	With	146	11	5	8	122	154.5	2.752	0.0059
	Without	147	4	4	1	138	139.6		
isC	With	147	21	26	10	90	152.8	0.859	0.3904
	Without	150	21	20	9	100	145.3		

^a We gave the values, 3, 2, 1, and 0 for the scores of female remating, 'remated on 1 d', 'remated on 5 d', 'remated on 10 d', and 'not remated at all', respectively, and calculated the mean rank of the values for each treatment within each strain.

maintained with an oviposition substrate had a greater tendency to remate than females maintained without an oviposition substrate (Table 1). This indicates that oviposition promotes female remating in these strains. On the other hand, in the isC strain that showed a high level of polyandry, an oviposition substrate had no significant effect on female remating (Table 1). Very few females in the jC-S and mC strains remate when prevented from laying eggs, whereas relatively more females in the isC strain remate even if they have not laid eggs, and thereby oviposition may not have an obvious effect on female remating behavior in the isC strain. This study presents the first evidence that the effect of an oviposition substrate on female remating differs between strains within a species. Three strains examined in this study differ in their geographic origins (see Materials and Methods). This may account for the difference in the effect of an oviposition substrate on female remating between the strains in *C. chinensis*. The strains also differ in the rearing periods in the laboratory. The two strains with low levels of polyandry, the jC-S and mC strains, have been reared long term in the laboratory, whereas the strain with a high level of polyandry, the isC strain, was established recently (see Materials and Methods). Under the rearing conditions, some selection may directly or indirectly affect female remating traits in *C. chinensis* (Miyatake and Matsumura, 2004; Harano and Miyatake, 2005, 2007). A random genetic drift may also occur in the laboratory cultures in *C. chinensis* (Miyatake and Matsumura, 2004; Harano and Mi-

yatake, 2007). These may cause differences in the effect of an oviposition substrate on female remating between strains reared long and short term in *C. chinensis*.

One possible explanation for the promotion effect of oviposition on female remating is that females remate to replenish sperm supplies diminished by oviposition (Fox and Hickman, 1994). If females that laid eggs remate to replenish sperm, then females that were allowed to remate should have higher fertility than females that were not allowed to remate, and females that laid more eggs may remate more readily. There were no positive effects of female remating on fertility in the isC strain that showed a high level of polyandry of *C. chinensis* (Harano et al., 2006). This is inconsistent with the hypothesis that females remate to replenish diminishing sperm supplies. In the future, we need to investigate the effect of female remating on fertility in strains with low levels of polyandry because the effect may differ between strains in *C. chinensis*. We also need to quantify the relation between the number of eggs laid by females and their tendency to remate in *C. chinensis*.

In addition, at least two hypotheses may explain why females maintained with an oviposition substrate have a greater tendency to remate than females maintained without an oviposition substrate. First, if females remate to increase the genetic diversity of their offspring (Watson, 1991; Yasui, 1998; Fox and Rauter, 2003), females would have to remate with second males after they have laid some eggs sired by first males to secure this benefit

from remating when the P2 value, sperm precedence of the second male mated, is high (Fox and Hickman, 1994). Second, females may remate to reduce sexual harassment by males, which is referred to as convenience polyandry (Thornhill and Alcock, 1983), and females that engage in laying eggs may be more sensitive to harassment than females that are prevented from laying eggs (Fox and Hickman, 1994). An environment with an oviposition substrate is suitable for females. Thus, females that engage in laying eggs may accept remating to retain a suitable environment because females fleeing from courting males to avoid remating may lose this environment. In contrast, females that have no access to an oviposition substrate may not benefit from remating by staying there.

Mating brings various costs and benefits to females (Thornhill and Alcock, 1983; Yasui, 1998; Arnqvist and Nilsson, 2000). This study indicates that female remating in *C. chinensis* is influenced by not only genetic factors but also the presence of an oviposition substrate, one of various environmental factors. This suggests that females of this species adaptively change their receptivity to remating in response to environmental factors that affect the costs and benefits of remating.

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