

The Occurrence of Multiple Mating in a Wild Population of the Ladybird Beetle *Harmonia axyridis* PALLAS (Coleoptera: Coccinellidae)

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Female insects of many species often have multiple copulations, while a single copulation usually can provide sufficient sperm quantity and quality for lifetime fertilization. This controversial phenomenon has recently received much attention (Parker 1984; Curtsinger 1991; Halliday & Arnold 1987; Harvey & May 1989; Knowlton & Greenwell 1984). In some insect taxa, a lifetime copulation number can be easily estimated by counting number of spermatophores remaining in the spermatheca (e.g. Matsumoto & Suzuki 1992), but more clarification is needed on the actual conditions for multiple mating in a wild population. In coccinellid ladybird beetles, mating behavior was observed in laboratory conditions by Obata (1987, 1988). However, mating behavior in field conditions was only partially interpreted.

This study reports the occurrence of multiple mating of the ladybird beetle *Harmonia axyridis* PALLAS in a wild population, with laboratory observations on mating behavior concerning multiple mating.

Materials and Methods

Field observation

Observations were made in the Botanical Garden (C. 10,000 m²) of Kyoto University (35°02'N135°47'W), in central Japan. Every day from early April to early August in 1985, 1987, and 1988, adults were captured and individually marked with quickly drying paints (see Osawa 1991 and Osawa & Nishida, 1992). Presence or absence of mating of newly and recaptured beetles was also recorded, and a generations were distinguished (see Osawa & Nishida 1992). All the captured adults were

released at the same site where they were caught. The overwintering generation emerging from hibernation was defined as the spring generation, the following one was called the summer generation.

Laboratory observation

On 26 May 1991, summer generation *H. axyridis* pupae were collected from *Zelkova serrata* and kept in 25°C, 16L-8D conditions. After emergence, 8 of each virgin males and females (N = 16) were separately maintained as mass stock cultures in unisexual groups with surplus aphids (*Myzus varians*) in the laboratory conditions of 25°C, 16L-8D) for 14 days. Four male and female beetles were randomly chosen from the stock cultures and introduced to a Petri dish (9 cm dia. 2 cm ht.) to observe mating behavior and its duration until all mating was finished.

Obata (1987) categorized mating behavior of *H. axyridis* males into 6 behavioral components: approach, watch, examination, mount, copulation attempt, and body shaking (Obata 1987). Obata and Hidaka (1987) also showed that sperm transfer in *H. axyridis* is performed by a spermatophore, with the specific "body shaking" that is the male behavior of sperm transfer. In this paper, I divided the mating behavior of male and female *H. axyridis* into 2 categories; mating (mount, copulation, and male body shaking) and mating refusal (mount and copulation attempt, but no body shaking). And the duration of each behavior was recorded.

Result and Discussion

Field observation on the occurrence of multiple mating

Figure 1 shows the number of copulations in the spring and summer generations in the field. A total of 394 beetles were observed mating, among which 13.8% ($n = 46$) performed multiple mating. No differences were observed between sex (spring generation; $P > 0.5$, summer generation; $P > 0.5$, spring and summer combined; $P > 0.5$, χ^2 -test) and generations (male; $P > 0.2$, female; $P > 0.2$, male and female combined; $P > 0.2$, χ^2 -test) in the frequency of multiple mating. The distribution pattern of the copulation number was significantly different from the Poisson distribution (χ^2 -test $\chi^2 = 27.41$, $P < 0.001$) (Fig. 2). This was largely due to many beetles being observed in the third and fourth copulations. This may be because

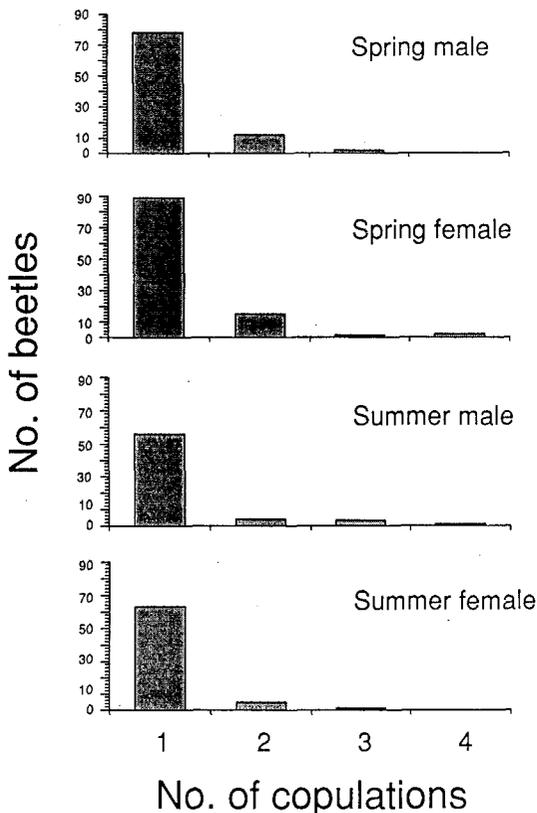


Fig. 1. Mating frequency of *H. axyridis* in the spring and summer generations in a field.

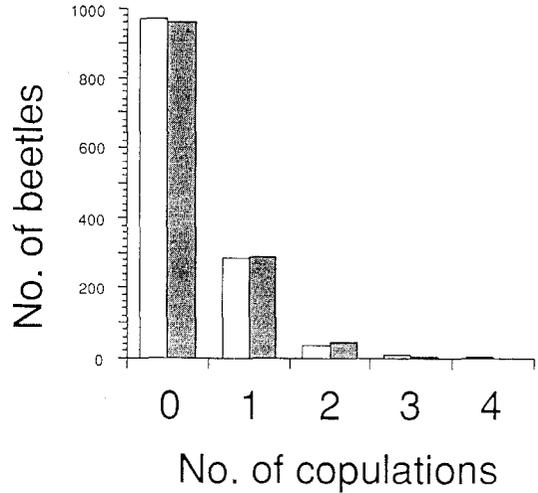


Fig. 2. Mating frequency of *H. axyridis*. Open bars indicate observed values and dotted bars estimated values from the Poisson distribution.

i) *H. axyridis* had good prey searching ability, therefore leading to the observed beetle concentration in habitat patches where aphid density was high and food quality was suitable (Osawa 1991); ii) staying time in the habitat was dependent on prey density species (Osawa 1991). These caused a higher probability of multiple mating both for males and females in patches with good food conditions.

Laboratory observation on duration of matings and mate refusing behavior

The duration of mating without “body shaking”, regarded as a signal of sperm transfer (Obata, 1987), was much shorter (78.24 ± 34.71 s, mean \pm S.E.) than that with “body shaking” (114.62 ± 6.66 min, mean \pm S.E.) (Fig. 3). Two types of female mate refusing behavior possibly existed: one was pre-copulatory mating refusal, which in most cases occurred within 15s (60.9% in total mating refusal). The other was interrupted genital contact, usually occurring after 2 min from the beginning of a mating trial (39.1%). These results suggested that the female decision to mate and the male decision to give up the female were made in a short time. Ovary development greatly influenced female mate acceptance

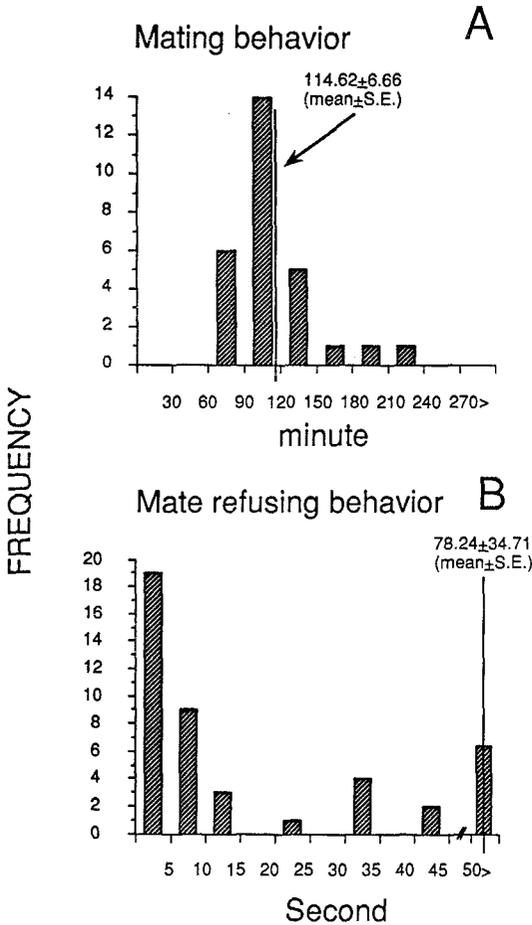


Fig. 3. The duration of mating and mate refusing behavior of *H. axyridis*.

(Obata 1988), indicating that in the pre-copulatory mating behavior the male detected the stage of female ovary development shortly before contact. This suggests that the smell of the female may be one of the cues of ovary development detection.

Laboratory observation on age-specific changes of mating activities

Figure 4 shows the changes of mating activities with age. The percentage of successful mating (the number of matings attained/the number of mating behaviors) was the highest during the age of around 15 days after emergence, gradually decreasing toward the age of

17 days, and thereafter remaining rather constant (Fig. 4a). Matings were frequently interrupted by other males at ages around 15-17 days (Fig. 4b). Moreover, mating trials gradually increased toward the age of 20 days after emergence and thereafter decreased (Fig. 4c). These results suggest that females preferentially accepted younger males with "low" mating activities, and that "middle-aged" males attained the "highest" age-specific mating activities. The high mating activities of older-males may result in enhancing the possibility of multiple mating.

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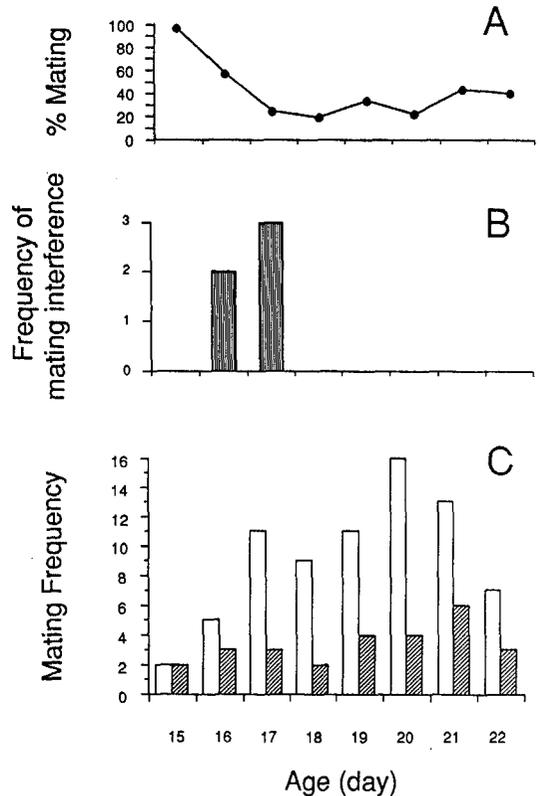


Fig. 4. Percentage of mating (frequency of mating attained/frequency of male mating behaviors) (a), frequency of mating interference by other males (b), and frequency of mating attained (dashed) and mating behaviors (open) (c), in *H. axyridis*.

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