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Mating Behavior and Sperm Transfer in the Ladybird Beetle, *Harmonia axyridis* PALLAS (Coleoptera: Coccinellidae)

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The behavior of male *Harmonia axyridis* prior to and during copulation was studied. First, in order to investigate mate recognition by males, several kinds of models were presented to males and their response was observed. It was inferred that visual factors such as body size and shape were involved in mate recognition by males in a close distance. Some chemical factor was suggested to be the main factor to release male copulatory behavior. Second, "body shaking", a characteristic behavior of a male during copulation, was analyzed in detail. This behavior was regular and rigid, and such characteristics suggested the direct control by the nervous system. By the experimental interruption of copulation, it was confirmed that body shaking was the process of sperm transfer. The mode of sperm transfer by spermatophore formation was also clarified.

INTRODUCTION

Harmonia axyridis PALLAS is one of the two most common species of aphidophagous ladybird in Japan, and is known to be polymorphic in elytral patterns, like the European *Adalia bipunctata*.

Much work has been done on entomophagous coccinellids and their prey-searching behavior in relation to their importance as agents of biological control, but relatively few studies have analyzed the reproductive behavior patterns of this group experimentally. This is probably due to the inherent difficulties in rearing these beetles under laboratory conditions with sufficient food supply and of obtaining insects of known age and whether virgin or copulated. No description of mating behavior is given in the studies on reproduction (HARIRI, 1966) and sexual selection (MUGGLETON, 1979; O'DONALD and MUGGLETON, 1979) in *Adalia bipunctata*, nor in studies on the spermatophore in several species of *Chilocorus* (FISHER, 1959) and the elementary points, such as the sequence of mating behavior and mate recognition by the male, have not yet been clarified. In this study, the behavior of male *Harmonia axyridis* prior to and during copulation was observed and analyzed. The mode of sperm transfer was also described from inferences made during ethological observation and manipulation.

MATERIALS AND METHODS

Animals. Pupae and prepupae of *Harmonia axyridis* attached to leaves of Japanese pittosporum, *Pittosporum tobira*, were collected at Takaragaike in Kyoto from late May

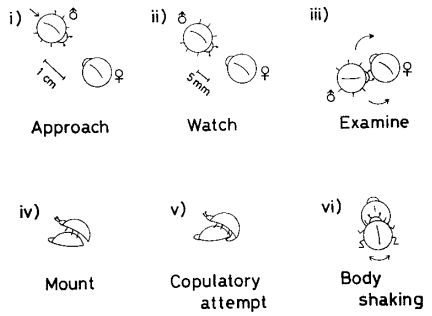

Fig. 1. Behavioral components of male *H. axyridis* prior to and during copulation.

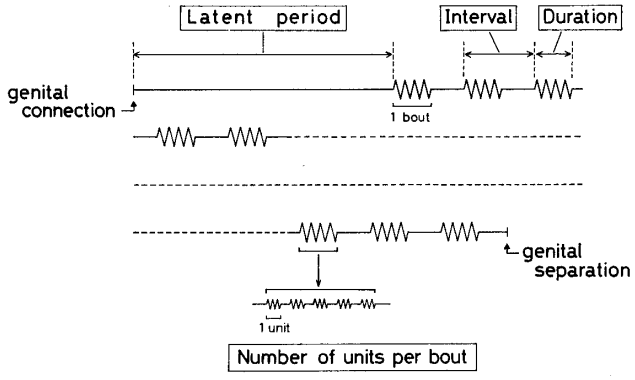

Fig. 2. Schematic presentation of body shaking and the four phases (enclosed by a square) measured for analysis. Another two phases, duration of copulation and number of bouts throughout the entire copulation, were also measured (not shown in the scheme).

to early June. Following emergence, adult beetles were reared under room conditions (20–25°C, about 16L8D) in separate unisexual groups of 5 to 6 individuals in 200 m*l* round plastic cups until required for experiments. Aphids, *Aphis citricola*, infesting their host plant, *Erigeron anuus*, were given to beetles as food every day or two. Observations and experiments were carried out in the daytime (11:00–17:00), the time that the beetles were usually active.

Preliminary observations. By 10 days after emergence most males were ready to copulate. A 10-day-old virgin male was introduced into a round plastic cage (8 cm in diam., 2.5 cm in height) together with the dead body of a female within one day after natural death, and the sequence of mating behavior was observed. A dead female body was used because it was difficult to observe the mating behavior of males with live females due to the male's fast and erratic motion and the female's activity. The behavior of a male prior to copulation was divided into five steps (Fig. 1, i–v): "Approach" (turning to the dead body from a distance of 1 cm or less), "Watch" (pausing at a distance of about 0.5 cm without any body contact), "Examine" (touching with his antennae and forelegs), and "Mount" and "Copulatory attempt" (bending the tip of his abdomen downwards). Sometimes either watch or examine or both of these behaviors were not performed. These five steps were used in the experiment for investigating mate recognition by males.

In the second series of experiments, a male and female 10 days after emergence were introduced into the plastic cage and "real" copulation was observed. Beetles soon copulated when they were placed in the cage together with aphid-infested leaves. The male response to a live female was slightly different from that to a dead female: after approaching, genital connection usually took place within a short time, without watch and examine. In all observations the male shook his body laterally during copulation (Fig. 1, vi). This behavior of the male during copulation was called "body shaking" and was analyzed in detail as described below.

Mate recognition by males. In order to examine which cues help the male in recognizing a female as a mate, several kinds of models were presented to males as a substitute for a live female. A 10-day-old virgin male was introduced into a plastic cage,

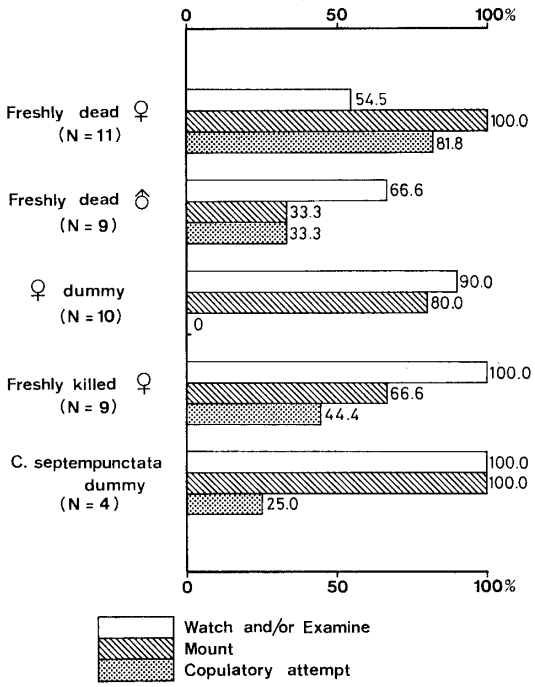

Fig. 3. Behavioral response of male *H. axyridis* to each type of model. Frequencies of each step are represented as the relative percentage to that of approach.

at the center of which the model was placed and the male's response to the model was observed for 1 hr. When the male approached the model, the occurrence or absence of the following steps of precopulatory behavior (see preliminary observations) were recorded. Five kinds of models were presented: a dead body of a female within one day after natural death (freshly dead ♀), a dead body of a male within one day after natural death (freshly dead ♂), a freshly dead body of a female killed in a freezing box (freshly killed ♀), a dead body of a female which had been kept in a refrigerator for two years until it became completely dry (♀ dummy), and a dead body of a female *Coccinella septempunctata* which had been kept in the same way as a ♀ dummy (*C. septempunctata* dummy). *C. septempunctata* was used because it was almost the same size as *H. axyridis* and they both coexisted in the same habitat.

Analysis of body shaking. The male in copulation began body shaking about 35 minutes after genital connection (see Fig. 1). Body shaking was usually repeated about 200 times during the course of copulation at constant intervals (Fig. 2). One bout of body shaking includes several units separated by very short time intervals. The characteristics and meaning of body shaking were investigated experimentally. For mating pairs, the duration of the following six phases were recorded: 1) duration of copulation (between genital connection and separation), 2) latent period (duration of male's standstill between genital connection and first bout of body shaking), 3) "interval" between the start of one bout of body shaking and the start of the next bout, 4) "duration" of one bout, 5) frequency of units of shaking per one bout and 6) number of bouts throughout the entire copulation. In order to clarify the meaning of body shaking, further experiments were performed. For several pairs, copulation was interrupted 30 min after genital connection before any body shaking had been seen. After the interrupted copulation, the female was individually maintained on an aphid diet for 10

Table 1. Duration of copulation and latent period of body shaking

Pair			Duration of copulation (min. $x \pm$ s.d.)	Latent period of body shaking (min. $x \pm$ s.d.)	Number of pairs
Male	Female				
virgin	virgin		119.08±16.82	34.55±2.43	21
virgin	copulated	(A)[a]	116.29±15.75	36.90±2.85	6
		(B)	133.31±15.95	35.21±2.19	6
copulated	virgin	(a)[b]	108.20±16.08	47.59±13.66	5
		(b)	103.44± 7.71	34.69± 9.52	4
		(c)	92.00± 7.48	32.67±2.49	3

[a] (A): second, and (B): more than third copulation for female.

[b] The second copulation for male was conducted on: (a) the same day, (b) the next day and (c) two days after the first copulation.

Analysis of variance

Comparison			F cal	
			Duration of copulation	Latent period of body shaking
virgin × virgin	vs.	virgin × copulated ♀	1.739 (N.S.)	1.927 (N.S.)
virgin × virgin	vs.	copulated ♂ × virgin	3.262 ($p<0.05$)	5.057 ($p<0.01$)

days and the number of eggs laid and hatched were noted. Identical treatment and observations were performed on females which had undergone one full copulation, and those where copulation was interrupted 30 min after its start and one bout of body shaking had finished.

RESULTS

Mate recognition by male. Figure 3 shows the entire behavioral response of males to each type of model. Frequencies of watch and/or examine, of mount and of copulatory attempt are represented as the relative percentage to that of approach. For freshly dead ♀, all males mounted the model and copulatory attempt was frequently seen. Since watch and/or examine were sometimes omitted, the percentage of these steps was rather low for freshly dead ♀. Freshly dead ♂ was sometimes inspected by males, but mount and copulatory attempt were seen less frequently than in the case of freshly dead ♀. Although males usually mounted the ♀ dummy after inspection, no copulatory attempt was observed. The male's response to the *C. septempunctata* dummy was similar to that for the ♀ dummy. It is notable that the result for freshly dead and freshly killed ♀ differed from each other. For freshly killed ♀, the frequency of watch and/or examine was high whereas that of mount and of copulatory attempt were conspicuously low.

Characteristics of body shaking. Table 1 shows the duration of copulation and the latent period of body shaking. In the case of the first copulation for the male, he

Table 2. Interval of body shaking

Pair		Interval (sec.) $\bar{x}\pm$s.d. (range)	Number of bouts
	1	25.774±3.984 (20–36)	209
	2	27.432±5.589 (13–40)	184
virgin male	3	24.095±4.962 (14–40)	233
×	4	27.393±2.797 (23–38)	179
virgin female	5	25.579±2.123 (19–32)	123
	6	25.712±3.402 (13–38)	209
	7	25.156±2.642 (18–39)	168
virgin male	11	28.059±6.173 (17–39)	223
×	12	25.244±2.671 (18–34)	165
copulated female	13	30.011±4.270 (18–67)	187
copulated male	21	23.102±4.116 (18–42)	177
×	22	36.370±6.068 (24–60)	82
virgin female	23	27.433±2.717 (19–42)	128

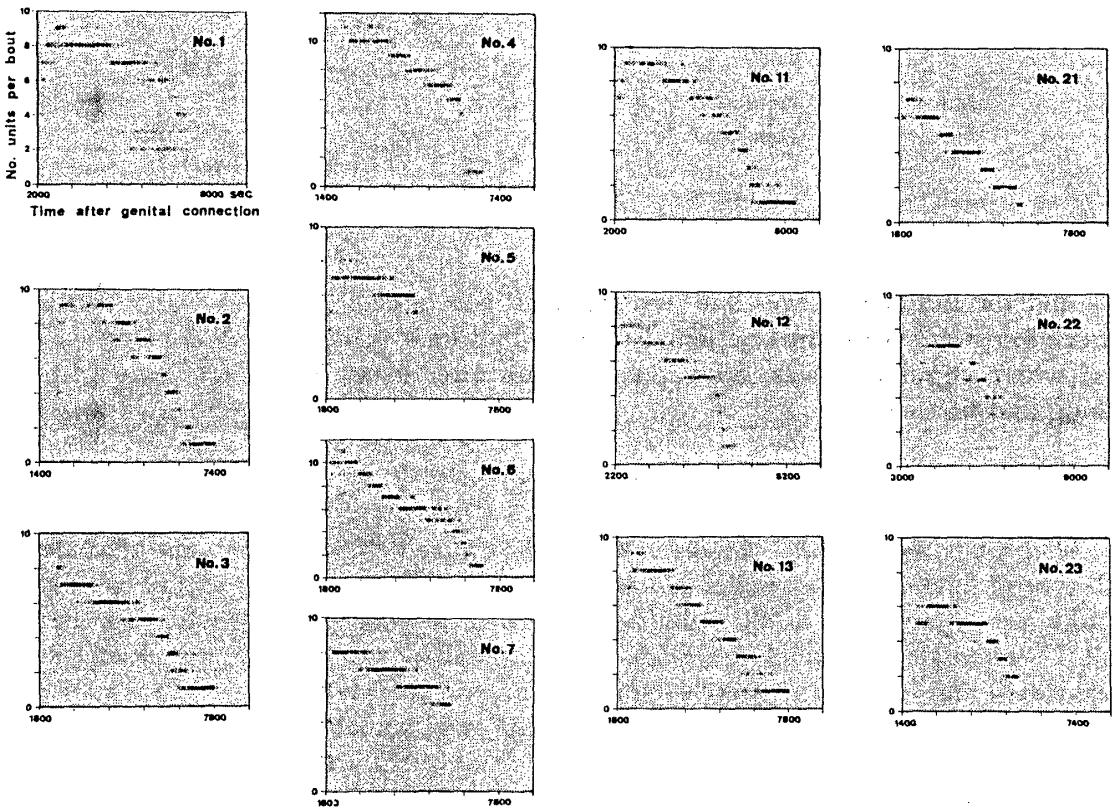

Fig. 4. Temporal change in the frequency of units of body shaking per one bout for the 13 copulating pairs. The number of each pairs corresponds to the number in Table 2.

began body shaking about 35 minutes after genital connection, regardless of the female's sexual status and of the length of copulation. Sometimes a longer latent period was observed in the male's second copulation which took place after the first. An analysis of variance revealed a significant difference between virgin and non-virgin males in

Table 3. Comparisons of number of eggs laid and hatched 10 days after full or interrupted copulation

Treatment		Number of eggs		Hatching rate (%)
		laid	hatched	
full copulation	1	346	282	81.5
	2	407	160	39.3
	3	388	335	86.3
	4	269	185	68.8
	5	361	295	81.7
30 min-interrupted copulation (without body shaking)	1	83	0	0
	2	92	0	0
	3	27	0	0
	4	9	0	0
	5	39	0	0
	6	38	0	0
30 min-interrupted copulation (after 1 body shaking)	1	210	105	50.0
	2	223	31	13.9

both duration of copulation and latent period of body shaking (Table 1). Interval of body shaking is presented in Table 2 for 13 cases which were observed throughout the copulation. The duration of interval was relatively constant with little variation, irrespective of individuals or the background of both sexes. For the same 13 pairs, frequency of units of shaking per one bout was counted, and its temporal change is shown in Fig. 4. It clearly decreased stepwise, which resulted in the bout consisting of only one unit in most cases. Duration of one bout ranged from 6 to 15 sec and was roughly proportional to the frequency of units of shaking (data not shown).

Table 3 shows the total number of eggs laid and hatched 10 days after full or interrupted copulation. In the case where copulation was interrupted at 30 min when no body shaking was seen, the female laid fewer eggs and no eggs hatched. These eggs were not laid in a batch but were scattered. Even 5 days after oviposition, microscopic examination revealed no indication of embryonic development in these eggs. When copulation was interrupted after 30 min when the first bout of body shaking had been completed, the female laid rather more eggs in batches, and some of them hatched.

DISCUSSION

Males of *Harmonia axyridis* attempted to copulate with the dead body of a female within one day after natural death. Sometimes they jumped to mount it soon after approaching without watch and examine, similar to the response seen to a live female. This suggests the presence of a chemical factor on the female body surface that releases a male's copulatory behavior. Probably this chemical factor was still present on the surface of the freshly dead body. It is likely that the chemical factor remained near the body as the odor of female, because in some cases the male seemed to recognize it as a potential mate without any contact. For the ♀ dummy of the same or other species

(*Coccinella septempunctate*) which was kept for over two years in a refrigerator, the male mounted it after comparatively persistent examination. In this case, however, most males stopped mating behavior at this step and did not show any copulatory attempt. This may be explained by the dummy being similar to the intact mate in body size and shape but being removed chemical factor to some extent by keeping in a refrigerator. Both chemical factor and the size or shape of the body seem to be involved in mate recognition by males of *H. axyridis*. Body size and shape may be recognized by antennal or tarsal touching as well as visually, since males sometimes examined models before mounting. The chemical factor, however, seems to be the key stimulus necessary for a copulatory attempt. Presentation of the body of a male within one day after natural death, or a freshly killed female resulted in fewer males mounting it compared with the response to the freshly dead female and the ♀ dummy. Male's mounting to the freshly dead male is probably due to the same size or shape of the body as female, but the chemical substance on the body surface of males may differ from that of females to easily facilitate sex recognition of individual conspecifics. The peculiar smelling orange-colored fluid secreted by most species of Coccinellidae when surprised or killed, probably has a negative effect on the male's copulatory behavior. Males of *H. axyridis* could recognize a female as a potential mate using visual and chemical cues, but no long-distance orientation was observed either in the field or laboratory. Therefore, it is considered that males must arrive near aphid-infested plants, where females of the same species are likely to live, prior to engaging in mate searching. Adults of *H. axyridis* are attracted to aphid-infested plants by cues such as its smell and appearance (OBATA, 1986) and the tendency seems to be significant not only in prey-searching but also in mate searching.

Analysis of body shaking revealed a regular pattern characterized by a constant latent period, a constant interval and a stepwise decrease in the number of units of shaking per bout. Such characteristics of body shaking suggest that it is a rigid process controlled directly by the nervous system. Furthermore, this behavior is probably related to the physiological state of the copulating male, because the pattern is not transformed by a female's sexual condition (virgin or copulated) or her behavior (walking or resting, feeding or not, etc.). Just what is the function of body shaking? The 30-min copulation interrupted before the first bout of body shaking resulted in unfertilized eggs, whereas in the case of the 30-min copulation interrupted after one bout of body shaking, the female laid some fertilized eggs, though fewer than those laid by the female which engaged in a full copulation. This indicates two points about the copulation of *H. axyridis*: 1) no sperm is transferred from male to female until body shaking starts, even if the genitalia of both sexes are connected, and 2) body shaking itself is the process of sperm transfer. Sperm transfer in coccinellid beetles has been considered in only two studies (FISHER (1959) for *Chilocorus*; KATAKURA (1985) for *Henosepilachna vigintioctomaculata*), both of which described sperm transfer as starting 40–60 min after genital connection and that material derived from the male's accessory gland was injected into the bursa copulatrix of the female prior to actual insemination. In *Chilocorus* the material later packed sperm and formed a spermatophore, while in *H. vigintioctomaculata* spermatophore formation did not occur. From the analogy of these two examples, it may be expected that also in *H. axyridis* some material is injected into the female's body prior to insemination during the latent period of body shaking. Only when sufficient material is transferred to the female, will the male start body shaking,

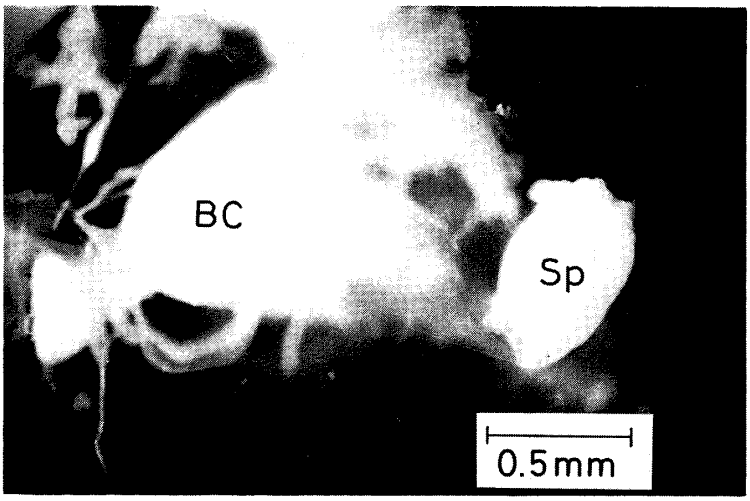

Fig. 5. Binocular vision of the spermatophore of *H. axyridis* brought out from the bursa copulatrix of the female dissected soon after genital separation. Sp: spermatophore, BC: bursa copulatrix.

which is the actual process of sperm transfer. The longer latent period observed when the male repeated copulations at rather short intervals, is perhaps due to consumption of the material in the male's accessory gland.

Several studies have reported on spermatophore formation in other species of Coleoptera besides *Chilocorus*: GERBER et al. (1971) for *Litta nuttalli* (Meloidae) and ZACHARUK (1958) for *Ctenicera aeripennis destructor* (Elateridae). In *H. axyridis*, a spermatophore was confirmed to be formed in the bursa copulatrix by means of dissection of females soon after genital separation (Fig. 5). The shape of the spermatophore is elliptical, which is similar to that of *Chilocorus discoideus*. Perhaps the process of spermatophore formation in this species, though it has not been investigated in detail, is very similar to that in *C. discoideus*: the material injected prior to insemination packs the sperm and forms a spermatophore. The method of spermatophore formation in *H. axyridis* seems to be an example of "first female-determined type" proposed by GERBER (1970), in which the spermatophore is formed in the female reproductive system during copulation. FISHER (1959) does not give a full explanation of the function of the spermatophore in *Chilocorus*. For *H. axyridis*, the spermatophore may play an important role concerned with the migration of sperm to the spermatheca. The female reproductive system of *H. axyridis* is similar in many respects to that of *Coccinella novemnotata* described by WILLIAMS (1945), except for the accessory glands. The bursa copulatrix is an expanded part of the vagina and is connected with the chitinous spermatheca by a sclerotized tube, which is inserted within the distal end of the bursa. Such a connection between the bursa and spermatheca is a common structure in many coccinellid species (HODEK, 1973). Dissection of mated females confirmed that the bursa copulatrix was filled up with one spermatophore and that the tube between the bursa and the spermatheca was inserted within the spermatophore. If under this condition the wall of the bursa can contract, the spermatophore will be compressed and the entire sperm will quickly migrate to the spermatheca. DAVEY (1958) described, quoting from a PhD thesis by KHALIFA (1948), that in *Coccinella* the spermatophore was possibly emptied by muscular contractions in the bursa. Although the significance has not

been confirmed, this system for sperm migration to the spermatheca may be adopted by many species of entomophagous coccinellids.

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