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Mating refusal and its significance in females of the ladybird beetle, *Harmonia axyridis*

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ABSTRACT. Mating receptivity in females of the ladybird, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), was studied with reference to internal and environmental conditions. Females sometimes reject copulation: they run away, or shake off the male and bend the abdomen upwards. Newly emerged females show a greater likelihood of accepting copulation with an advance in the stage of ovarian development, this being related to age. Females usually mate repeatedly, but frequently show refusal behaviour under unfavourable food conditions. Dissections reveal that females which accept copulation have a greater number of mature eggs in the ovary than do rejectors. Results also suggest that the food conditions are a major environmental factor, directly controlling mating receptivity.

Key words. Coleoptera, Coccinellidae, ladybird, mating refusal, food condition.

Introduction

The aphidophagous ladybird, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), is a multiple-mating species, in which both sexes mate repeatedly. In the course of a study on mating behaviour and sperm transfer in this species (Obata, 1987), it was observed that the male's copulatory attempt occasionally resulted in failure due to a refusal by the female to mate. Mating refusal in a female consisted of the following three phases, which correspond to each step of the male's behaviour: running away from the chasing male; shaking off the male from her back when mounted; and bending the last abdominal segments upwards to prevent genital insertion. In some instances females discontinued rejection and finally accepted the copulation attempt, but in others females firmly rejected males until they eventually gave up. Hodek (1973), quoting from a Ph.D. thesis by Ming (1971), described newly emerged female coccinellids as rejecting copulation in the same manner as *H.axyridis* described here. In *H.axyridis*, however, mating refusal was not restricted to young, virgin females but was also seen in mature, mated females.

The present paper describes the internal and environmental conditions which induce mating refusal in female *H.axyridis*. The factors which control mating receptivity are also discussed with special reference to the food conditions.

Materials and Methods

Pupae and prepupae of *Harmonia axyridis* attached to leaves of Japanese pittosporum, *Pittosporum tobira*, were collected at Takaragaike in Kyoto, Japan, from late May to early June 1985. Following emergence, adult beetles were

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reared in separate unisexual groups of five to six individuals in 200 ml round plastic cups under room conditions (20–25°C, approximately LD 16:8 h) until required for experiments. Aphids, *Aphis citricola*, infesting their host plant, *Erigeron anuus*, were given to beetles as food everyday.

Virgin females were used to examine the change in mating receptivity with the age after emergence. A female of known age (days 1–7 and 10) was placed in a round plastic case (8 cm in diameter, 2.5 cm in height) together with an aphid-infested shoot of *Erigeron anuus*. After 1 h a 10-day-old virgin male which was ready to copulate was introduced into the cage containing the female, and their behaviour was observed for 1 h. A female performing either of the following two types of response was recorded as having rejected copulation: one of three types of mating refusal behaviour (escape, shaking off and abdominal bending, see introduction) was performed three times during the 1 h observation period; the female, mounted by the male, continued abdominal bending for more than 10 min. The female was considered to have no sexual attraction if the male ignored her in spite of frequent contact, but instead attempted to copulate with a new mature female presented right after a 1 h period.

In order to clarify the relationship between ovarian development and mating receptivity, virgin females were dissected at various ages (days 3, 5, 7 and 10). The ovarioles were examined under a stereoscopic microscope and the developmental stage was recorded as one of six stages shown in Fig. 1. Each ovary is composed of about thirty (twenty-seven to thirty-nine; Robertson, 1961) ovarioles, and their development is not always synchronized but ranges at most within two grades in Fig. 1. The advanced ovarioles were used to designate the developmental stage.

To investigate mating receptivity in mated females, fourteen females which had undergone the first copulation at 10 days after emergence were prepared. After the first copulation they were individually maintained under one of the following three types of food conditions: the female was given an aphid-infested shoot of *Erigeron anuus* (A) every day, (B) every other day, and (C) every third day. For the females belonging to groups B and C, in the absence of aphids, a 10% honey solution soaked in a 1 cm^3 sponge piece was provided as an alternative diet. A 10-day-old virgin male and single female were introduced into a new cage with freshly provided diet at the same time each day (12.00–13.00 hours) and the female's acceptance or rejection of copulation was noted. Since males and females encountered each other on a leaf or twig more frequently than on the wall of the cage during the observation period, a healthy shoot of *E.anuus* was placed in the cage of females given honey diet. Criteria for mating refusal were as adopted for virgin females. When the female was receptive the copulation was not interrupted but allowed to continue to the end (for 2 h in most cases). The male was removed after the observation period in the case of refusal, or after genital separation in the case of acceptance. Mating receptivity in each female was tested daily for 10 days after the first copulation and the number of eggs laid by the female were also counted each day.

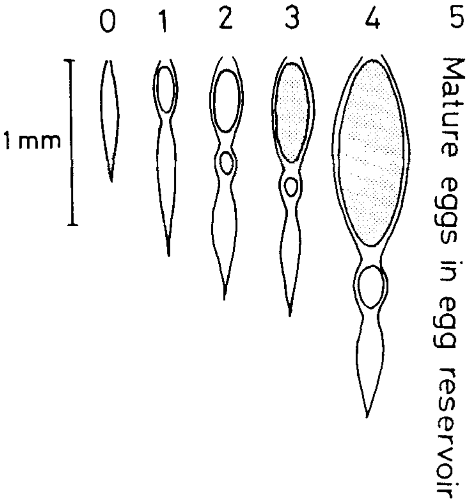

FIG. 1. Developmental stages of oocytes in adult *H.axyridis*. Stage 0: no oocytes in ovarioles (the stage on the day of emergence). Stage 1: one oocyte in ovariole. Stage 2: two whitish oocytes. Stage 3: two oocytes in ovariole; the basal one filled with yellow yolk. Stage 4: the basal oocyte in ovariole grows to the maximum size. Stage 5: egg reservoir contains mature eggs.

In the second series of experiments a comparison was made between the stage of ovarian development of the mated females which accepted the further copulation and those which rejected it. The female was allowed to copulate at 10 days old and, on the next day (termed 'I'), or 2 days after (termed 'II'), she was introduced into the cage together with a 10-day-old male. The female was observed for 1 h and the acceptance or rejection of the second copulation was recorded. Females which showed mating refusal were killed in a freezing box soon after the observation and dissected to examine the ovary, while females which accepted the copulation were treated identically immediately following genital separation. The developmental stage of the ovarioles was determined not only by the six stages shown in Fig. 1, but also by counting the number of mature eggs (1 mm in major axis) in the egg reservoir and ovarioles, and of smaller eggs with yellow yolk (0.7 mm in major axis) in the ovarioles. The number of eggs laid by each female between the first copulation and the dissection was also recorded.

Results

The mating receptivity of virgin females changed with female ages (Table 1). No female induced copulatory behaviour by males on day 1 and day 2. Most females began to attract the male on day 3 or day 4, but mating refusal behaviour was observed frequently when the male attempted to copulate. The rate of rejection by the female decreased with an increase in age, and the 7- or 10-day-old females accepted the copulation without exception. Table 2 shows the developmental stage of ovarioles in relation to the age of virgin females. Production of one oocyte started 3 days after adult emergence. By day 5 the oocyte had grown bigger but was still whitish with no yolk. A sudden increase in the rate of ovarian development was detected in the 7-day-old females, which usually had mature eggs filled with yellow yolk in their ovarioles. Since most females at 10 days old had several mature eggs in the egg reservoir, it seemed likely that they could lay eggs immediately after the first copulation.

TABLE 2. Ovarian development in virgin female *H.axyridis* with the age after adult emergence. See Fig. 1 for stages of development.

Age of female (days)	Stage of development						No. of females
	0	1	2	3	4	5	
3	0	10	0	0	0	0	10
5	1	18	4	0	0	0	23
7	0	5	7	6	5	4	27
10	0	1	0	1	3	12	17

The mating receptivity in non-virgin females was related to food conditions (Fig. 2). Females which were kept on an aphid diet every day accepted copulation in almost all cases, whereas those which were given aphid and honey diet alternately refused mating much more frequently. The rate of mating refusal increased and the number of eggs laid per female decreased with decreasing aphid diet (Fig. 2). Mating receptivity, however, was not always related to the number of eggs laid; some females accepted copulation but laid no eggs, whereas others laid many eggs in spite of mating refusal.

The data shown in Fig. 2 were therefore re-analysed. Mating receptivity and the number of eggs laid each day were rearranged according to the food situation on the last 3 days, including the day of observation (sometimes including 1 or 2 days prior to the experiment). Table 3 shows the rate of mating refusal and the mean number of eggs in relation to six different food situations. The rate of mating refusal increased with the period of honey diet during the last 3 days, and was also influenced by the order of diet: the rate of mating refusal of females on HAH, HHA and AHH food regimes were different from one another. Females laid more eggs when at least 2

TABLE 1. Mating receptivity in virgin female *H.axyridis* at various ages after adult emergence.

	Age of female (days)							
	1	2	3	4	5	6	7	10
No. accepted	0	0	4	3	6	6	6	10
No. rejected	0	0	3	5	3	3	0	0
No. no sexual attraction	5	5	2	0	0	1	0	0
Total	5	5	9	8	9	10	6	10
Rate of successful copulation (%)	0	0	44.4	37.5	66.7	60.0	100.0	100.0

Day after the first copulation (diet)	1(A)	2(A)	3(A)	4(A)	5(A)	6(A)	7(A)	8(A)	9(A)	10(A)	Rate of mating refusal	Number of eggs laid for 10 days
Group A - 1	○56	○50	○57	○0	○30	○52	●16	○7	○32	○23	0.10	323
A - 2	○44	○42	○53	○53	○43	○38	○29	○7	○0	○56	0.00	365
A - 3	○0	○67	○49	○84	●7	○71	○31	○6	○26	○33	0.10	374
A - 4	○4	○76	○56	○74	○39	○0	○56	○3	○0	○43	0.00	351
							mean per female				0.05	353.3

	1(H)	2(A)	3(H)	4(A)	5(H)	6(A)	7(H)	8(A)	9(H)	10(A)		
Group B - 1	○0	○54	○0	●46	○30	●24	○0	●23	○0	○38	0.30	215
B - 2	○28	○34	●0	○63	○33	●37	●38	●28	●0	●34	0.60	295
B - 3	○44	○59	○15	○38	○0	○59	●0	○25	●17	●12	0.30	269
B - 4	○56	○37	●0	○48	●0	○30	○0	○39	●23	○7	0.30	240
B - 5	●27	●34	○24	○27	○14	○14	○58	●0	●0	○0	0.40	198
							mean per female				0.38	243.5

	1(H)	2(H)	3(A)	4(H)	5(H)	6(A)	7(H)	8(H)	9(A)	10(H)		
Group C - 1	○41	○40	●23	○27	○0	○18	●0	●0	○7	○48	0.30	204
C - 2	○0	●0	●23	○32	●16	●0	○36	●22	●0	●0	0.70	129
C - 3	●47	●49	●61	●2	●37	●49	●23	●0	○26	●0	0.90	294
C - 4	●97	●9	●0	○30	●0	○8	○38	●13	●0	●21	0.70	216
C - 5	○30	○0	●16	●18	●0	●3	●0	●0	●0	○0	0.70	67
							mean per female				0.66	182.0

FIG. 2. Mating receptivity in mated females for 10 days after the first copulation under the various food conditions. The food conditions on each day are shown in parentheses: 'A' and 'H' mean aphid and honey diet, respectively. The female was given aphid diet every day (Group A), every other day (Group B) or every third day (Group C). The number of eggs laid for each day is shown together with the circles, indicating mating acceptance (○) and mating refusal (●).

TABLE 3. Re-analysis of the data shown in Fig. 2. The rate of mating refusal and the mean number of eggs laid per day are shown for each food situation on the last 3 days, including the day of observation. A: aphid diet; H: honey diet.

Food situation on the last 3 days	Rate of refusal	Mean no. of eggs per day ($\bar{x}$ ±SD)	*n*
AAA	0.05	35.3±24.4	40
AAH	0.30	37.0±26.6	10
AHA	0.36	32.4±16.9	25
HAH	0.46	15.1±16.6	35
HHA	0.67	15.5±17.9	15
AHH	0.80	12.4±16.4	15

days of aphid diet were included within the last 3 days, when compared with those where only 1 day of aphid diet was included. The order of diet, however, had no influence on the number of eggs: the value for HAH, HHA and AHH were almost identical.

The stage of ovarian development was compared between mated females which accepted a second copulation and those which rejected it (Table 4). It was confirmed that females which accepted further copulation had significantly more mature eggs in the ovary than those which rejected it, whereas no significant difference was detected between the acceptors and the rejectors in the number of smaller yellow eggs and of eggs laid per day (Table 4).

Discussion

One- or 2-day-old females of *Harmonia axyridis* did not induce a copulatory attempt by males.

TABLE 4. Comparison of ovarian development between mated females which accepted a second copulation and those which rejected it. The female copulated once at 10 days after emergence, and on the next day (I), or 2 days after (II), their mating receptivity was tested.

No. females		No. mature eggs ($\bar{x}$ ±SD)	No. smaller yellow eggs ($\bar{x}$ ±SD)	No. eggs laid per day ($\bar{x}$ ±SD)
I	Acceptor (11)	25.00±16.36	21.64±9.02	13.91±19.33
	Rejector (9)	10.67±8.54	13.00±7.65	22.33±16.49
II	Acceptor (10)	23.50±13.90	17.40±10.57	13.90±17.74
	Rejector (8)	14.88±14.48	14.88±8.51	14.94±10.87
Total	Acceptor (21)	24.29±15.26	19.62±10.01	13.91±18.59
	Rejector (17)	12.06±11.31	13.88±8.12	18.85±14.60

Analysis of variance, Acceptors (21) *v*. Rejectors (17):
No. mature eggs $F=7.158$, $P<0.05$
No. smaller yellow eggs $F=3.452$, $0.05<P<0.10$
No. eggs laid per day $F=0.761$, $P>0.10$

Obata (1987) suggested that a chemical factor on the female body surface releases the male's copulatory behaviour in this species and the young females may have an inadequate amount of this sexual attractant. It takes 3–4 days after emergence for the body to harden and darken in a newly emerged adult of *H.axyridis*, and this process is related probably to the production of sexual attractant. On day 3 or day 4, females attracted males and some of them accepted copulation. It is therefore likely that mating acceptance coincides with the start of oocyte development. However, mating refusal seen frequently in young, virgin females seemed to be related to insufficient ovarian development, because 7- or 10-day-old females, which had mature eggs in the ovary, accepted copulation without exception. In the Coccinellidae the females of some species copulated soon or within 2–3 days after emergence (Clausen, 1915; Richards, 1980), and in these species the first copulation may trigger ovarian development. In other species, however, the first copulation occurs 7–10 days after emergence, when the ovary has sufficiently developed (Hariri, 1966). *H.axyridis* belongs to the latter group, in which the ovary develops without the stimulus of copulation. The first copulation, however, was considered as the stimulus for normal oviposition. A 10-day-old virgin female laid a few scattered eggs, whereas she laid many eggs in batches soon after the first copulation.

In *H.axyridis*, mating refusal was not restricted to young virgin females but was sometimes performed by mated females, despite being a multiple-mating species. Many investigators have reported that mating receptivity of a female was reduced during a certain period after the copulation, even in multiple-mating species (Thornhill & Alcock, 1983). The presence of spermatophores or sperm in the reproductive organ is considered as one of the factors which regulate mating receptivity in females (Manning, 1967; Nakagawa *et al.*, 1971; Riddiford & Ashenhurst, 1973; Obara *et al.*, 1975; Rutowski, 1980; Rutowski *et al.*, 1981). Sugawara (1979) has shown that in *Pieris rapae*, stretch receptors provide information about the volume of materials contained within the bursa copulatrix. Dissection of a mated female revealed that in *H.axyridis* the bursa copulatrix was filled up with one spermatophore and that the tube between the bursa and the spermatheca was inserted within the spermatophore. Therefore it is impossible for a female to receive a further spermatophore as long as the bursa contains the former one. No females were observed to accept further copulation within a short interval. However, it is unlikely that mating refusal the next day is due to the presence of a spermatophore in the bursa copulatrix, because a female *H.axyridis* ejects a spermatophore sac within an hour after genital separation (Obata & Hidaka, 1987). Females which were continuously given an aphid diet accepted copulation every day, while females which were sometimes given honey solution rejected copulation more frequently. This suggests that mating refusal is not inevitable in this species, but is induced under unfavourable food conditions. This characteristic differs from that of the grasshopper, *Chorthippus curtipennis*, in which all females reject copulation for about 1 day between spermatophore ejection and oviposition (Hartmann & Loher, 1974).

It has been confirmed for several species of entomophagous coccinellids that females kept on some alternative or artificial diet laid no or few eggs, in spite of the long life span (Smith, 1965; Sundby, 1968). This study also showed a honey diet to induce a decrease in the number of eggs laid by female *H.axyridis*. Probably an aphid diet is indispensable for egg production and maturation in the ovary. It is possible that the decline in egg production under such unfavourable food conditions causes the female to reject copulation. This hypothesis was supported by the comparison of the number of eggs within the ovary between mating acceptors and rejectors. The acceptors had a greater number of mature eggs, which were regarded as eggs whose oviposition was imminent, than the rejectors. A few studies have experimentally confirmed that mating receptivity in female insects is controlled by the presence of mature eggs within the ovary. Thornhill & Alcock (1983) described a special case where some female Odonata accept copulation only when she has mature eggs in her ovary.

The food conditions indirectly controlled mating receptivity in female *H.axyridis* through the internal development of mature eggs and in some cases, seemed to directly influence the rejection of copulation. The three different food regimes provided over the last 3 days, HAH, HHA and AHH, induced the same degree of reduction in the number of eggs laid by females. Nevertheless, the rate of mating refusal under the three food regimes were different from one another. Obata (1986) showed that adult *H.axyridis* were capable of finding an aphid by visual and olfactory cues and it is likely that the female can detect small changes in food availability or quality by sensory cues and/or the hunger level, and then controls mating receptivity.

Entomophagous coccinellids respond sharply to prey density and migrate to a new habitat when the prey decrease in number (Banks, 1955; Wright & Laing, 1980). Under unfavourable food conditions, copulation represents only a loss of time and energy, because it delays the female from searching for and migrating to a new habitat. Perhaps this is the explanation for mating refusal by mated females in *H.axyridis*. Under good food conditions, the female may accept copulation every day even though one copulation is generally reported as sufficient for her permanent fertility (Hodek, 1973).

Many authors have discussed the benefits of multiple mating for the insect female (Thornhill & Alcock, 1983), and as also seen in *Adalia bipunctata* (Hodek, 1973) the number of eggs laid by *H.axyridis* may increase following each copulation. There may be other benefits of multiple mating for the female in this species, but the theory of 'convenience polyandry' (Thornhill & Alcock, 1983), whereby it is advantageous for a female to accept copulation if rejection of the male's copulatory attempt results in a considerable loss of time and energy, is also applicable. Copulating females of *H.axyridis* can feed on aphids in the same manner as when not *in copula*. Under sufficient food conditions there is no reason for mating refusal, which costs time and energy. It is notable that the response of female *H.axyridis* is sharply switched from convenience polyandry to strict mating refusal according to the food conditions, and such a feature may be characteristic to predaceous insects depending on an unstable prey resource.

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References

Banks, C.J. (1955) An ecological study of Coccinellidae (Col.) associated with *Aphis fabae* Scop. on *Vicia faba*. *Bulletin of Entomological Research*, **46**, 561–574.

Clausen, C.P. (1915) A comparative study of a series of aphid-feeding Coccinellidae. *Journal of Economic Entomology*, **8**, 487–491.

Hariri, G. (1966) Laboratory studies on the reproduction of *Adalia bipunctata* (Coleoptera: Coccinellidae). *Entomologia Experimentalis et Applicata*, **9**, 200–204.

Hartmann, R. & Loher, W. (1974) Control of sexual behaviour pattern 'secondary defence' in the grasshopper, *Chorthippus curtipennis*. *Journal of Insect Physiology*, **20**, 1713–1728.

Hodek, I. (1973) *Biology of Coccinellidae*. Academia, Prague.

Manning, A. (1967) The control of sexual receptivity in female *Drosophila*. *Animal Behaviour*, **15**, 239–250.

Nakagawa, S., Farias, G.J., Suda, D., Cunningham, R.T. & Chambers, D.L. (1971) Reproduction of the Mediterranean fruitfly; frequency of mating in the laboratory. *Annals of the Entomological Society of America*, **65**, 1364–1367.

Obara, Y., Tateda, H. & Kuwabara, M. (1975) Mating behavior of the cabbage butterfly, *Pieris rapae crucivora* Boisduval. V. Copulatory stimuli inducing changes of female response patterns. *Zoological Magazine, Tokyo*, **84**, 71–76.

Obata, S. (1986) Mechanisms of prey finding in the aphidophagous ladybird beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Entomophaga*, **31**, 303–311.

Obata, S. (1987) Mating behavior and sperm transfer in the ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). *Applied Entomology and Zoology*, **22**, 434–442.

Obata, S. & Hidaka, T. (1987) Ejection and ingestion of the spermatophore by the female ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). *Canadian Entomologist*, **119**, 603–604.

Richards, A.M. (1980) Sexual selection, guarding and sexual conflict in a species of Coccinellidae (Coleoptera). *Journal of the Australian Entomological Society*, **19**, 26.

Riddiford, L.M. & Ashenhurst, J. (1973) The switchover from virgin to mated behavior in female *Cecropia* moths: the role of the bursa copulatrix. *Biological Bulletin*, **144**, 162–171.

Robertson, J.G. (1961) Ovariole numbers in Coleoptera. *Canadian Journal of Zoology*, **39**, 245–263.

Rutowski, R.L. (1980) Courtship solicitation by female of the checkered white butterfly, *Pieris protodice*. *Behavioural Ecology and Sociobiology*, **7**, 113–117.

Rutowski, R.L., Long, C.E., Marshall, L.D. & Vetter, R.S. (1981) Courtship solicitation by *Colias* females (Lepidoptera: Pieridae). *American Midland Naturalist*, **105**, 334–340.

Smith, B.C. (1965) Effects of food on the longevity, fecundity, and development of adult Coccinellidae (Coleoptera: Coccinellidae). *Canadian Entomologist*, **97**, 910–919.

Sugawara, T. (1979) Stretch reception in the bursa copulatrix of the butterfly, *Pieris rapae crucivora* and its role in behavior. *Journal of Comparative Physiology*, **130**, 191–199.

Sundby, R.A. (1968) Some factors influencing the reproduction and longevity of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *Entomophaga*, **13**, 197–202.

Thornhill, R. & Alcock, J. (1983) *The Evolution of Insect Mating Systems*. Harvard University Press, Cambridge.

Wright, E.J. & Laing, J.E. (1980) Numerical response of coccinellids to aphids in corn in southern Ontario. *Canadian Entomologist*, **112**, 977–988.

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