Eur. J. Entomol. **90**: 45–50, 1993
ISSN 1210–5759

Kin recognition: egg and larval cannibalism in *Adalia bipunctata* (Coleoptera: Coccinellidae)

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Eco-ethology, ladybird beetles, cannibalism, kin recognition

Abstract. Adult female two spot ladybirds *Adalia bipunctata* (L.) were reluctant to eat their own eggs. Similarly, second instar larvae avoided eating sibling eggs. However, adult males did not appear to avoid eating eggs they had sired and hungry third instar larvae showed no reluctance to consume younger sibling larvae. These results indicate that female beetles and young larvae are capable of kin recognition. The adaptive significance of this is discussed.

INTRODUCTION

Egg cannibalism has been widely reported in ladybirds (coccinellids) and the studies of Mills (1982) and Osawa (1989) have shown that it is very important in the field. The aggregative response of ladybird beetles to aphids leads them to spend more time and lay eggs in the vicinity of high concentrations of aphids (Dixon, 1959; Evans & Dixon, 1986). However, as ladybird larvae pose a considerable threat to the survival of eggs (Osawa, 1989), it is advantageous for adult ladybirds to leave an aphid patch once the eggs there begin to hatch (Hemptinne et al., 1992). Although the window in time when both adults and eggs occur together in a patch is relatively short (Hemptinne et al., 1992), nevertheless, it is highly likely that beetles will encounter their own eggs and those of other individuals of the same species. Similarly larvae are also likely to encounter eggs and particularly larvae. If in these circumstances ladybirds can use sensory information to avoid eating their kin they exhibit kin recognition (Grafen, 1990). In this paper we address the question – can ladybirds recognize and so avoid eating their own kin and is there any difference between larvae, adults and the sexes in this respect?

MATERIALS AND METHODS

Experiments were carried out on *Adalia bipunctata* (L.). A stock culture of this ladybird was kept at 15 ± 1°C and a photoperiod of 16L : 8D, and fed on pea aphid, *Acyrthosiphon pisum* (Harris). Eggs were removed from this culture on alternate days and the larvae reared at 20 ± 1°C and a photoperiod of 16L : 8D.

Unmated females and males were raised by individually confining advanced fourth instar larvae in 5 cm diameter Petri dishes the bottom of the lower half of which was lined with filter paper. All experiments were carried out in new 5 cm diameter Petri dishes, each lined with fresh filter paper. The incidence of cannibalism was monitored continuously for the first hour, then every 30 minutes for a further four hours and then finally at the end of 24 hours.

CAN A FEMALE RECOGNIZE HER OWN EGGS?

Newly emerged adults were kept isolated for 16–18 days and fed an excess of aphids after which a male and female were placed in each Petri dish. After mating the females were isolated from the males and kept separately in Petri dishes without food for 24 hours. In most cases these females laid a clutch of eggs (mean 13.8) on the filter paper at the bottom of each dish. These eggs were removed from the filter paper by cutting close around the base of each egg cluster. Each batch of eggs, and a cluster of similar size, treatment and age (24h) from another (alien) pair of ladybirds were transferred to a new and freshly lined Petri dish. Clutch size is variable so it was not always possible to match the size of the clutches exactly; of the 37 pairs of clutches used 76% were the same size, 16% differed by 1 egg, 5% by 2 eggs and 3% by 3 eggs. The individual females were then transferred to these Petri dishes and confined with their own and another pairs (alien) eggs for 24 hours. Nine female s were used and each female was tested at 2–3 day intervals at least twice and as many as 7 times. The number of their own and alien eggs consumed was noted after 1, 5 and 24 hours.

CAN MALES RECOGNIZE EGGS THEY HAVE FERTILIZED?

The procedure adopted was similar to that outlined above, but males rather than females were confined with the eggs. Four males were used and each was tested at 2–3 day intervals either 4 times (2 males) or 5 times (2 males). The number of their own and alien eggs consumed was noted after 1, 5 and 24 hours.

CAN LARVAE RECOGNIZE THEIR SIBS IN THE EGG STAGE?

Twenty, two day old second instar larvae were starved for 20h and then each larva was presented for one hour with an equal number of 24h old eggs produced by its own parents and by an alien pair in a 5cm Petri dish. The larvae were the offspring of 5 pairs of beetles; 1 pair supplied 5, 3 pairs 4 each, and 1pair 3 larvae.

CAN LARVAE RECOGNIZE SIBLING LARVAE?

Forty, three day old third instar larvae, which had each been starved for 24h, were each presented in a 5cm Petri dish with a well fed 2 day old second instar sibling larva and a similar aged larva raised under identical conditions, but produced by different parents. The experimental third instar larvae were the offspring of 6 pairs of beetles; 1 pair supplied 9, 3 pairs 7 each, and 2 pairs 5 larvae. The number of larvae of the two kinds eaten was noted after 1h. The second instar larvae from the two sets of parents were distinguished by a spot of coloured paint on their heads applied by means of a fine needle.

RESULTS

CAN FEMALES RECOGNIZE THEIR OWN EGGS?

Over the first 5 hours hungry females were markedly reluctant to eat their own eggs. (Table 1, Fig. 1) (Mann Witney: 1h, $U = 78$, $P = 0.0009$; 5h, $U = 76.5$, $P = 0.0015$). Even after 24 hours, when they had nearly consumed all the alien eggs, they were still reluctant to eat their own eggs (24h, $U = 65.5$, $P = 0.033$). Thus when a choice is available, females will eat the eggs of other females before their own.

CAN MALES RECOGNIZE EGGS THEY HAVE FERTLIZED?

In marked contrast to females, hungry males showed no reluctance to eat eggs they had fertilized (Table 2).

CAN LARVAE RECOGNIZE THEIR SIBS IN THE EGG STAGE?

Hungry second instar larvae, when given a choice, preferred to eat the eggs of alien parents than those laid by their own parents (Table 3, Mann Whitney $U = 23$, $P < 0.01$).

CAN LARVAE RECOGNIZE SIBLING LARVAE?

Third instar larvae showed no reluctance to eat second instar sibling larvae (sibling 0.17 ± 0.38, alien 0.25 ± 0.43, $x^2 = 0.18$, N.S).

TABLE 1. Average number of their own and alien eggs eaten by female *Adalia bipunctata* after 1, 5 and 24 hours.

Female beetle	Times tested	Mean number of eggs							
		Own				Alien			
		Supplied	Eaten after			Supplied	Eaten after		
			1 h	5 h	24 h		1 h	5 h	24 h
1	4	11.5	3.5	7.5	11.3	12.25	6.0	9.3	12.3
2	3	13.3	1.7	8.3	12.3	14.3	12.0	14.0	14.3
3	5	14.0	3.2	6.8	11.8	14.2	9.0	13.2	14.2
4	2	13.0	0	1.0	9.0	13.5	5.0	7.5	9.5
5	2	15.0	3	5.5	12.5	15.5	10.0	10.0	15.5
6	5	15.2	0	2.4	14.4	15.2	5.2	11.0	14.2
7	7	13.0	1.4	1.9	10.0	13.4	5.9	7.9	12.9
8	5	13.4	0.8	2.4	10.2	13.4	5.8	10.4	13.2
9	4	13.8	0.3	1.8	12.0	14.0	2.8	7.0	13.0
$\overline{X}$		13.58	1.54	4.18	11.5	14.0	6.85	10.0	13.2
S.E.		0.37	0.47	0.94	0.54	0.33	0.96	0.81	0.57

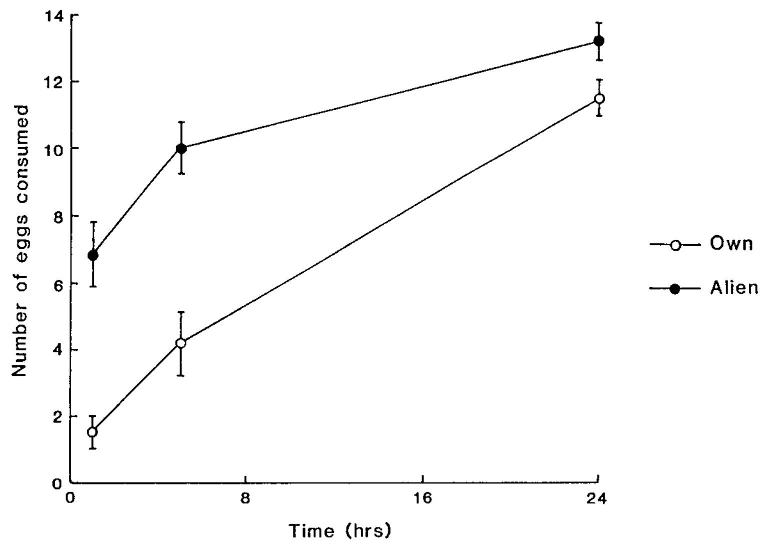

Fig 1. The mean number (± S.E.) of their own and alien eggs eaten by female *Adalia bipunctata* after 1, 5 and 24 hours.

TABLE 2. Average number of eggs fertilized by themselves (own) and other males (alien), eaten by male *Adalia bipunctata*.

Period of observation (hours)	Mean number of eggs (± S.D.)				Mann Whitney test of the average results for each of the males
	Supplied		Eaten		
	Own	Alien	Own	Alien	
	16.77 ± 0.56	16.72 ± 0.53			U
1			3.61 ± 4.4	3.61 ± 4.0	6 N.S.
5			7.39 ± 4.8	7.56 ± 5.3	9 N.S.
24			13.56 ± 5.6	13.11 ± 5.9	8.5 N.S.

TABLE 3. Number of sibling and non sibling eggs eaten by second instar larvae of *Adalia bipunctata* after 1 hour.

Pair of beetles	Larvae	Mean number of eggs			
		Sibling		Non-sibling	
		Supplied	Eaten	Supplied	Eaten
1	1	6	0	5	2
	2	6	0	6	3
	3	5	0	6	2
2	1	7	2	7	0
	2	7	1	7	0
	3	6	0	6	2
	4	6	0	6	2
3	1	6	0	6	3
	2	8	0	8	3
	3	8	0	8	2
	4	7	2	7	0
4	1	7	0	7	1
	2	8	2	8	1
	3	8	1	8	2
	4	6	1	6	1
	5	6	2	6	0
5	1	7	0	7	2
	2	7	0	7	2
	3	8	0	8	2
	4	8	0	7	1
$\overline{X}$		6.85	0.55	6.80	1.55
S.E.		0.21	0.18	0.20	0.22

DISCUSSION

In the laboratory egg and larval cannibalism in ladybirds is dependent on aphid abundance (Agarwala & Dixon, 1991, 1993), and egg cannibalism has frequently been observed in the field (Mills, 1982; Osawa, 1989). Weight for weight conspecific eggs and larvae are more nutritious than aphids, and young eggs more nutritious than old eggs (Agarwala, 1991; Agarwala & Dixon, 1993). Thus if adults and larvae could avoid eating their kin, cannibalism would be an effective means by which adults could fuel their reproductive effort. In addition, by cannibalism adults and larvae could eliminate potential competitors and ensure future availability of food for themselves and their offspring. Thus the marked reluctance shown by female beetles to eat their own eggs and larvae to eat sibling eggs is likely to reduce the incidence of kin cannibalism under natural conditions. Although there are no records of kin recognition in ladybirds it has been recorded for other invertebrates (Fletcher & Michener, 1987; Nummelin, 1989).

That male ladybirds were not reluctant to eat their own offspring is interesting. This could imply that females might recognize their own eggs because they carry their 'odour' or that the selective pressure on males to develop this ability has been markedly less because they invest very little energy in each egg and tend to leave a female after mating. The priority for males is to seek out and fertilize females whereas that of females is more to locate and oviposit close to suitable aphid colonies. Similarly, the inability of hungry late stage larvae to recognize sibling larvae might indicate that the best strategy at that stage is for such larvae to eliminate all potential competitors and so secure their future food supply.

Although it is unrealistic to equate laboratory and field conditions the results nevertheless complement what is known of the natural history of ladybird beetles. Egg cannibalism is strongly density dependent (Mills, 1982), prey recognition occurs mainly on physical contact (Carter & Dixon, 1982) and adults tend to remain and continue ovipositing in areas of high aphid abundance until eggs begin to hatch (Dixon, 1959; Hemptinne et al., 1992). Therefore, larval and adult ladybirds are likely to encounter their kin. In such circumstances to be able to recognize and so avoid eating one's kin would have a very strong adaptive significance.

ACKNOWLEDGMENTS. This work was funded through a Commonwealth Fellowship to the senior author who is indebted to the Vice-Chancellor, Tripura University, for nominating him for an award. We are indebted to Aulay Mackenzie for help with the statistical analysis.

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Received August 18, 1992; accepted September 28, 1992