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Egg and cluster size in ladybird beetles (Coleoptera: Coccinellidae): The direct and indirect effects of aphid abundance

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Aphid abundance, Coccinellidae, cannibalism, clutch size, egg size, ovarioles

Abstract. Food stress experienced during larval and adult life in *Coccinella septempunctata* L. results in variation in the rate of production of eggs per unit time and in the size of egg clusters but not in egg size. This lends support to the suggestion that egg size is constrained by the minimum size at which first instar larvae can capture active prey and/or complete their development before prey become scarce. When supplied with an excess of food large beetles produce larger clusters of eggs than small beetles. This is associated with the large females having more ovarioles in their gonads than small females. As the proportion of eggs that give rise to larvae remains the same for clusters of from 6 to 60 eggs there appear to be no costs, in terms of increased predispersal sibling cannibalism, to laying larger clusters of eggs.

INTRODUCTION

Although there has been much interest in insect reproductive tactics, in particular how many eggs should be laid in a patch (e.g. Godfray, 1987; Mangel, 1987; Parker & Courtney, 1984), little attention has been given to the factors that determine egg and cluster size in predatory insects. Ladybird beetles lay their eggs in clusters, which vary in size both within and between species. Previous studies have shown that variation in egg size between clusters is independent of cluster size within a species and the average number of eggs laid in a cluster by a species is approximately half the number of ovarioles that species has in its gonads. It has been suggested that egg size is possibly constrained by the minimum size at which first instar larvae can capture active prey and complete their development before prey becomes scarce (Stewart et al., 1991a,b). This leads to the prediction that within species of ladybirds, as in birds (cf. Lack, 1986), egg size is likely to be the least variable reproductive trait and cluster size the most variable.

Clusters of eggs are better defended chemically against predation than are single eggs (Agarwala & Dixon, 1993). For a better understanding of the evolution of egg clustering in ladybird beetles, however, one also needs to know whether there are any costs associated with laying eggs in clusters, e.g., an increase in the incidence of sibling cannibalism. Similarly, information on whether all the eggs in a cluster are equally vulnerable to sibling cannibalism is needed for a better understanding of the evolution of egg cannibalism.

This paper tests the above prediction by determining the direct and indirect effects of aphid abundance on egg and cluster size in the seven spot ladybird, *Coccinella septempunctata* L. In addition the viability and hatch time of eggs in clusters of eggs of different sizes and the hatch times of eggs of different sizes are determined.

MATERIAL AND METHODS

The larvae and adults of the seven spot ladybird beetle, *Coccinella septempunctata* L. were fed pea aphids, *Acyrthosiphon pisum* Harris. The stock cultures of ladybirds were kept in clear plastic boxes (6 ×

17.5 × 11.5 cm), which contained damp paper tissue and a piece of corrugated filter paper (10 × 15 cm). All experiments were done at 20°C and a 16L : 8D photoperiod.

Direct effects of aphid abundance. Sixteen pairs of ladybirds were removed from the stock culture and each pair was placed in a plastic specimen tube (7.9 × 3.4 cm) stoppered with a tissue paper covered plastic sponge plug. Each tube was lined with filter paper and a piece of corrugated paper (2.5 × 6 cm), for the beetle to rest and lay eggs on, was added to each tube. Each pair was fed an excess of pea aphids until they had laid 5 clusters of eggs after which they were fed 15 mg of aphids per day, which is approximately a third of the amount consumed when offered excess food. This was continued until they laid a further five clusters of eggs after which they were again offered an excess of aphids each day until they had laid another five clusters of eggs. That is, each pair was fed a high, followed by a low, followed by a high level of food and produced five clusters of eggs in each of the feeding regimes.

The aphids and paper linings of each tube were changed daily and any clusters of eggs removed and the number of eggs in the cluster noted. For ten of the pairs four eggs from each of the clusters laid in each of the feeding regimes were individually weighed, i.e., a total of eighty eggs were weighed for the ten pairs in the high, low and high feeding regimes. The eggs were easily separated from one another by dampening with water the tissue the eggs had been laid on. Five pairs of beetles, fed continuously with an excess of pea aphids were used as the control. These beetles were observed for the same length of time as the experimental beetles and the total period was divided into three equal periods. Egg weight in each of the three periods was determined by weighing four eggs from one cluster from each of the pairs, i.e., a total of twenty eggs for each period.

Indirect effect of aphid abundance. Newly hatched larvae were divided into two equal sized groups, which were fed different amounts of aphids. This resulted in the production of markedly different sized adults. They were then kept collectively in 14 cm Petri dishes and fed on excess of aphids. Beetles observed mating were removed from the dishes as a pair and placed in plastic tubes (3.4 × 7.9 cm) as in the previous experiment. Each pair was fed on excess of aphids. Numbers of eggs and clusters of eggs were collected and removed daily and four eggs from each cluster were weighed. After laying at least 5 clusters of eggs the females were anaesthetized with carbon dioxide and their minimum length and breadth determined, which was used to calculate a body size index (cf. Dixon, 1959). The females were then weighed and dissected and the number of ovarioles in their ovaries counted.

Viability and Hatch Time of eggs in clusters of different sizes. The number of larvae that hatched and dispersed from 52 clusters of eggs varying in size from less than 10 to slightly less than 70 eggs was observed. In addition the time of egg hatch was determined for another 20 clusters by removing the larvae with fine forceps and noting the time as each larva hatched.

Hatch times of eggs of different sizes. Eggs from five clusters were weighed individually and then put on to numbered pieces of filter paper in a Petri dish. A piece of dampened filter paper was added to each dish every day until the eggs hatched. The time when each larva left the egg shell was noted.

RESULTS

Direct effects of aphid abundance

Both the number of eggs laid per day and the size of the individual clusters of eggs were greatly affected by the availability of food. When aphids were supplied in excess the beetles laid twice as many eggs per day as when the aphid supply was reduced. In addition the size of the average cluster of eggs when the aphid supply was reduced was half that when aphids were supplied in excess. Over the same period of time the control beetles fed continuously an excess of aphids showed no significant variation in either the number of eggs laid per day or in the average size of their clusters of eggs (Tables 1 and 2).

In marked contrast the average size of the eggs laid by individual females was not significantly affected by the range of aphid abundance used in this study (Table 2). Thus as predicted, when food supply varies cluster size is more likely to vary than egg size.

However, between individuals there is a lot of variability in the average sizes of the egg clusters and of the eggs, which remains to be accounted for.

TABLE 1. The average sizes of five clusters (± S.E.) of eggs laid by (A) each of 16 females of *C. septempunctata* fed first a high, then a low and finally a high abundance of aphids and (B) of 5 females fed continuously an abundance of aphids. Figures in the same row followed by the same letter are not significantly different from one another, $P < 0.05$.

A

Pair	Aphid abundance					
	High		Low		High	
1	29.2 ± 8.4	(a)	28.8 ± 7.2	(a)	28.8 ± 9.0	(a)
2	25.8 ± 12.5	(a)	16.8 ± 5.8	(a)	26.0 ± 7.4	(a)
3	40.8 ± 15.8	(a)	27.6 ± 3.9	(a)	30.6 ± 11.2	(a)
4	38.2 ± 6.2	(b)	12.8 ± 2.2	(a)	35.4 ± 4.8	(b)
5	44.6 ± 10.0	(b)	22.2 ± 9.9	(a)	43.0 ± 8.7	(b)
6	49.6 ± 13.8	(b)	22.4 ± 5.3	(a)	42.4 ± 7.3	(b)
7	45.2 ± 12.6	(a)	38.2 ± 9.9	(a)	45.8 ± 10.0	(a)
8	42.0 ± 9.0	(b)	23.0 ± 13.5	(a)	39.4 ± 3.9	(b)
9	34.2 ± 16.6	(ba)	27.0 ± 3.7	(a)	41.6 ± 3.3	(b)
10	49.6 ± 12.2	(b)	27.8 ± 9.7	(a)	44.6 ± 15.7	(b)
11	49.0 ± 7.9	(b)	28.6 ± 9.2	(a)	46.6 ± 4.3	(b)
12	54.4 ± 5.9	(b)	26.2 ± 6.4	(a)	52.4 ± 12.0	(b)
13	45.6 ± 11.7	(b)	30.0 ± 8.0	(a)	50.0 ± 12.1	(b)
14	42.0 ± 16.8	(ba)	24.8 ± 11.5	(a)	46.8 ± 10.1	(b)
15	31.6 ± 7.0	(b)	15.6 ± 5.9	(a)	37.4 ± 7.0	(b)
16	44.4 ± 18.1	(b)	20.2 ± 13.9	(a)	46.6 ± 12.0	(b)
	41.6 ± 8.0	(a)	24.5 ± 6.3	(b)	41.1 ± 7.7	(a)

B

	First period		Second period		Third period	
1–5	36.6 ± 16.0	(a)	36.0 ± 10.1	(a)	34.7 ± 12.1	(a)

TABLE 2. The average number of eggs laid per day (± S.E.) and the average egg sizes of females of *C. septempunctata* (A), fed first a high, then a low and finally a high abundance of aphids, and (B) fed continuously an abundance of aphids. Figures in the same row followed by the same letter are not significantly different from one another, $P < 0.05$.

A

	High aphid abundance	N	Low aphid abundance	N	High aphid abundance	N
Eggs/day	34.5 ± 9.4 (a)	16	17.7 ± 6.9 (b)	16	34.2 ± 12.5 (a)	16
Egg size	0.210 ± 0.011 (a)	80	0.207 ± 0.015 (a)	80	0.211 ± 0.013 (a)	80

B

	First period	N	Second period	N	Third period	N
Eggs/day	37.8 ± 11.2 (a)	5	38.8 ± 5.1 (a)	5	39.5 ± 8.6 (a)	5
Egg size	0.246 ± 0.016 (a)	20	0.247 ± 0.007 (a)	20	0.245 ± 0.014 (a)	20

Indirect effects of aphid abundance

When fed an excess of aphids individual bettles consistently laid either small or large clusters of eggs, with large individuals laying significantly larger clusters than small individuals (Fig. 1A). Large beetles also had significantly more ovarioles than small beetles (Fig. 1C) and beetles with a large number of ovarioles laid significantly larger clusters of eggs than beetles with few ovarioles (Fig. 1B). Therefore, not surprisingly, there is a relationship between the residuals about the relationship between cluster size and adult size (Fig. 1D) and the ovariole number of the individual beetles. The weight of individual female beetles varies greatly dependent on the state of development of their gonads. This temporal variability in adult weight was largely avoided by using the length to the tip of the elytra and the widest width as a measure of size. However, there is nevertheless a significant relationship between adult weight (y) and the size index (x) ($y = 1.29x - 10.76$, $r^2 = 0.42$, $n = 20$).

This analysis reveals that previous aphid abundance, through its effect on beetle size has an indirect affect on the response of beetles to current aphid abundance. Large beetles

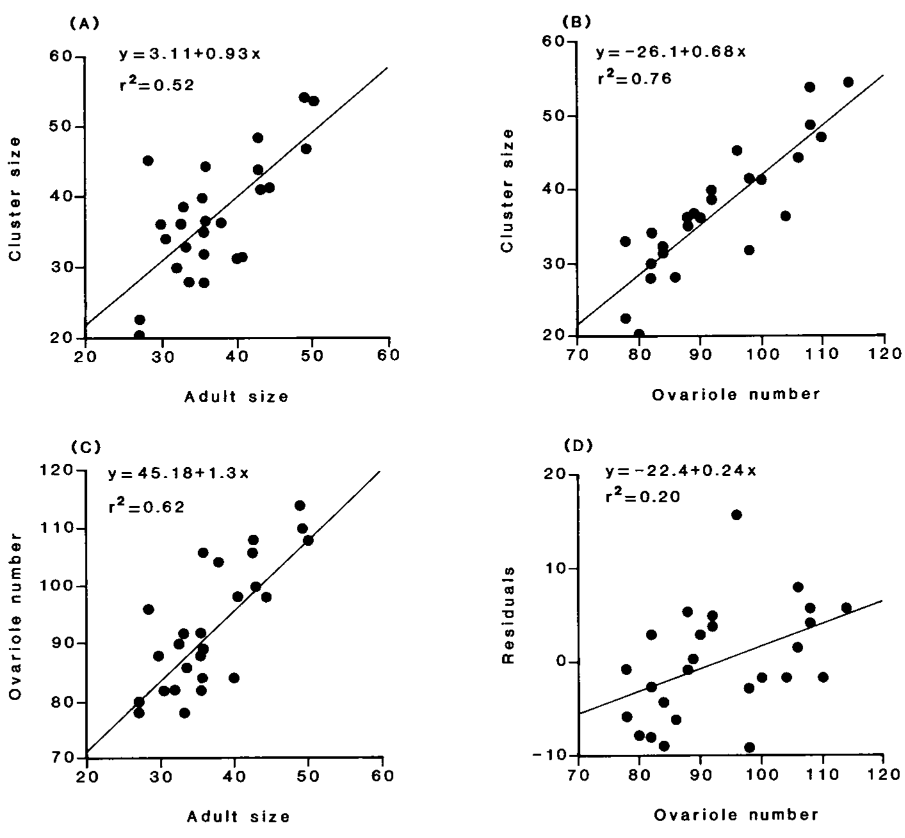

Fig. 1. Egg cluster size in relation to (A) adult size and (B) ovariole number, ovariole number in relation to adult size (C) and the residuals in relationships (A) in relation to ovariole number (D).

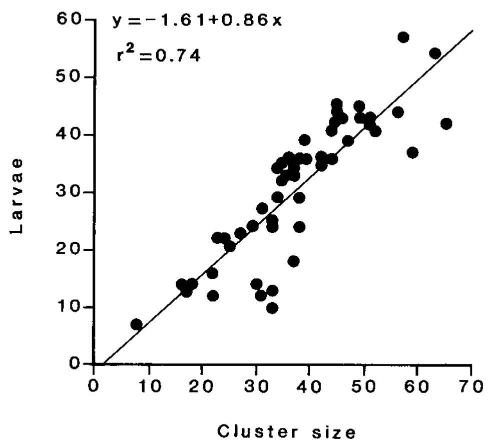

Fig 2. The number of larvae dispersing in relation to the size of the egg cluster.

have a greater number of ovarioles and are potentially capable of laying larger clusters of eggs than small beetles. However, there was no relationship between average egg weight and cluster size (n = 20, r = 0.13), ovariole number (r = 0.16) or adult weight (r = 0.04).

Viability and hatch times of eggs in different sized clusters

For the range of sizes of clusters of eggs observed there was a linear and directly proportional relationship between the number of larvae that dispersed and the size of the cluster of eggs from which they hatched (Fig. 2). Thus under the conditions prevailing in this experiment there appears to be no costs, in terms of egg infertility or a greater predispersal sibling cannibalism, associated with laying large clusters of eggs.

Although there was a lot of variability in the average time from the onset to the completion of egg hatch, between clusters, there was no significant trend between the average time of egg hatch and cluster size (n = 11, r^2 = 0.021).

Hatch time and egg size

To determine whether the hatch time of individual eggs was determined by their size relative to the other eggs in the cluster the relative egg hatch times and relative egg sizes were plotted (Fig. 3). The range in relative egg hatch times does not appear to show a trend with relative egg size. Thus the time at which an egg hatches does not appear to be determined by its relative size.

DISCUSSION

The birth size of ladybird beetles is important as it affects both the developmental time and the ability to capture active prey. If relatively small at birth it will take longer to complete development and be inefficient at capturing prey (Stewart et al., 1991b). If these constraints are limiting then it follows that in times of food stress ladybirds should vary the

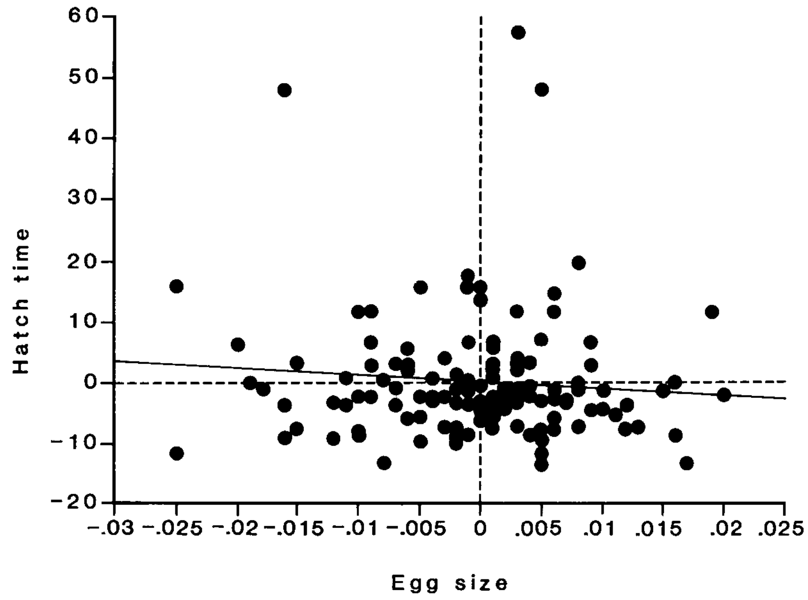

Fig 3. Hatch time of eggs expressed as a percentage of the mean hatch time in relation to egg size expressed as a percentage of mean egg size.

size of their clusters of eggs rather than the size of their eggs. This prediction is born out by this study.

The range (1.5 ×) in average egg size laid by different females does not appear to be related to any of the adult size dependent features measured. Thus it is likely that the variation between females is mainly genetically determined. The range in egg size might indicate the variability, both within and between seasons, in the availability of easily caught prey and the period for which aphid colonies remain suitable for exploitation, both of which are likely to determine the fitness of ladybird beetles.

As previously indicated egg production in ladybirds is determined by the availability of prey (Dixon, 1959; Evans & Dixon, 1986). However, the availability of prey during the development of ladybirds, through its effects an adult size, can also affect egg production. Even when prey is abundant small beetles are less fecund than large beetles. This is likely to be determined by the smaller number of ovarioles in the gonads of small beetles and the likelihood that the speed with which eggs are produced by each ovariole is rate limited. Thus potential egg production is affected by both the aphid abundance experienced by a beetle during its larval development and that available to it as an adult (Fig. 4).

Sibling cannibalism, in which recently hatched larvae eat their emerging or unhatched sibs, has been widely reported for ladybirds (c.f. Osawa, 1989). In this study 83% of the eggs gave rise to larvae that dispersed from the egg clusters, and the percentage that was killed did not vary significantly with the size of the clusters. Thus over a wide range in size there appears to be no cost in terms of poor viability or survival of predispersal larvae, in laying large clusters of eggs. As it is the last eggs in a cluster to hatch that are at

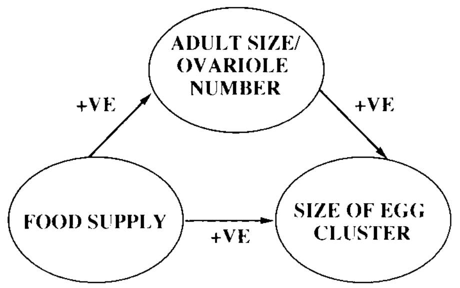

Fig 4. Schematic diagram of the direct and indirect effects of food supply on the size of egg clusters laid by ladybird beetles.

greatest risk from sibling cannibalism it is of interest to know whether these eggs are the smallest. However, relative hatch time does not appear to be correlated with relative egg weight within a cluster.

The numerical response of ladybirds is determined both by the aphid abundance they experienced as larvae and current aphid abundance. The flexibility of the response is achieved by varying the size and frequency of production of clusters of eggs, but not by varying the size of individual eggs. Sibling cannibalism does not appear to impose an upper limit on the size of the egg clusters laid by *C. septempunctata*.

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