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Short communication

Social feeding in ladybird beetles: adaptive significance and mechanism

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Summary. Young larvae of *Adalia bipunctata* search an area more intensively when exposed to the odour from other larvae feeding on aphids than when exposed only to the odour of aphids. In an olfactometer young larvae were significantly attracted either to the odour of crushed aphids or larvae feeding on aphids, but not to that of aphids, larvae, larvae plus aphids or larvae feeding on an artificial diet. That is, the change in searching behaviour appears in response to a volatile released by aphids when attacked. The odour released by crushed aphids is made up entirely of aphid alarm pheromone, β-farnesene. It is likely that the adaptive significance of this response is that it increases the ability of larvae to locate larvae that have already caught prey. By sharing the aphid kill of another larva it is likely that a first instar ladybird larva greatly increases its probability of surviving to the next instar. It is suggested that this social feeding is facilitated by egg clustering, which also may additionally account for why aphidophagous ladybirds lay their eggs in clusters.

Key words. *Adalia bipunctata* – aphid alarm pheromone – ladybirds – social feeding

Introduction

The larvae of aphidophagous ladybirds have to pursue and subdue their prey, and because of the relatively poor powers of pursuit and capture of small larvae they require a population density of aphids for survival many times greater than that required by large larvae. Thus aphidophagous ladybirds are likely to maximise their fitness by ovipositing in high density young colonies of aphids, where there is a preponderance of small and more easily captured aphids. At a constant aphid population density, the probability of a first-instar larva capturing its second aphid is considerably greater than the probability of capturing its first aphid. That is, the capture of the first aphid is critically important for the survival of a ladybird larva (Dixon 1959).

Most aphidophagous ladybirds lay their eggs in clusters. The first larvae to hatch have frequently been observed to eat the late-emerging larvae and unhatched eggs. Although there is debate about the extent to which this sibling cannibalism occurs in the field (Dixon 1959; Dimetry 1974; Mills 1982), the study of Osawa (1992) clearly indicates that cannibals have a greatly increased chance of surviving to the next instar. From a mother's point of view however sibling cannibalism is wasteful. This raises the question of why most aphidophagous ladybirds lay their eggs in clusters. Why do they not lay eggs singly like most of the coccidophagous ladybirds? Although the chemical protection of eggs is more effective against predation when they are laid in clusters than singly, clusters of eggs would nevertheless appear to be at a greater potential risk of cannibalism as they are more likely to be encountered than single eggs (Agarwala & Dixon 1993). That is, there are costs and benefits associated with laying eggs singly or in clusters, some of which may not have been identified. The overall effect these factors have on larval survival and whether they are important in the evolution of egg clustering is unknown.

Observations made on ladybird larvae dispersing from egg clusters revealed that they appeared to be attracted to sibs that were feeding on an aphid. This results in a high incidence of social feeding in first-instar larvae at a time when they are particularly vulnerable to death from starvation. The objective of the study reported here was to determine whether first-instar larvae are attracted to larvae feeding on aphids and the mechanism by which this is achieved.

Material and methods

Ladybird culture

Two-spot ladybirds *Adalia bipunctata* (L.) were reared at 15 ± 1°C and a LD 16:8 h photoperiod. Groups of ≈ 20 males and 20 females

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were kept in 5 l plastic boxes, which also contained a piece of corrugated filter paper to increase the surface area. Every other day, the ladybirds were fed an excess of pea aphids *Acyrthosiphon pisum* (Harris). A section of a stem of broad bean *Vicia faba* L. was provided as a source of humidity and to keep the aphids alive for as long as possible. Once a week, the ladybirds were transferred to clean containers to stimulate egg laying.

The eggs laid on the corrugated filter paper were collected every day. Depending on experimental requirements, they were either used immediately or incubated in 175-cm^3 plastic boxes under the same laboratory conditions as the adults. In order to reduce the risk of cannibalism, the number of larvae per box never exceeded 15.

Is the searching behaviour of first-instar larvae of Adalia bipunctata *affected by the presence of larvae eating aphids?*

Approximately 40 mg of pea aphids were placed in a circular plastic dish (diameter: 4.2 cm, height: 1.3 cm), the lid of which had a central hole (diameter: 2.6 cm) covered with metallic mesh (diameter of threads: 0.18 mm; mesh size: 0.3 × 0.3 mm). A Fluon® coating on the inner walls prevented aphids from climbing onto the lid of the dish. A first-instar larva, which had just hatched, was gently transferred to the centre of the lid, using a triangular piece of filter paper, and the time it remained on the mesh was recorded. This was repeated 10 times and constituted the control. The experimental dishes contained approximately 40 mg of pea aphids plus 10 first-instar larvae of *A. bipunctata*. In this case, a larva was only placed on the mesh when several of the larvae within each dish were eating aphids. This was also repeated 10 times.

Do first-instar larvae of Adalia bipunctata *react to volatiles released by aphids or feeding larvae?*

In order to investigate the role of volatile substances in the location of prey by first-instar larvae of *A. bipunctata*, an olfactometer was built following the specifications given by Vet *et al.* (1983). This olfactometer consisted of a quadrilateral central chamber into which air was drawn in via an entry port at each corner. Prior to an experiment, vapour of NH_4Cl was sucked into the olfactometer through the entry port in order to visualise the gas fields. The four flow rates were then adjusted to give four fields of vapour of equal size. Dry air was first purified by sucking it through a 250-ml vial filled with activated charcoal, and then along each of four tubes at a flow rate of 60 $ml \cdot min^{-1}$. The air in each of the tubes first passed through a 50-ml vial of distilled water before entering a 1-cm diameter × 5-cm long glass tube containing the odour source. Larvae were introduced into the central chamber via the same hole in the centre of the chamber as the air was sucked out of by means of a vacuum pump. Three circular concentric zones of increasing diameter, which were centred on the centre of the olfactometer, were delimited by faint lines traced on the floor of the central chamber.

Response to aphid odour
A 24-h-old first-instar larva that had been starved since birth was gently transferred using a paintbrush to the centre of the olfactometer. It was exposed to two odour fields of air that had passed through tubes each containing 40 mg of pea aphid and to two fields of uncontaminated air, the control. The two control and two experimental air flows arrived in the exposure chamber from opposite corners. The larva was allowed to search for up to 30 min but observations ceased as soon as a larva reached the outer of the three concentric zones and stayed there for at least 3 min. This was repeated 20 times. After each test, the exposure chamber was washed with pure 90% ethanol, rinsed with distilled water and dried with clean tissue. The positions of the control and experimental tubes were alternated every five tests. The aphids were weighed on a Sartorius microbalance.

Response to the odour of feeding larvae, crushed aphids, and larvae plus aphids
As above but the larvae were exposed to the following stimuli: the odour of 10 previously unfed 24-h-old first-instar larvae feeding on 40 mg of aphids, or 40 mg of aphids crushed with a glass rod, or five previously unfed 24-h-old first-instar larvae feeding on small cubes of an agar-based diet (Majerus 1989).

In a further experiment, each entry port of the olfactometer was connected to two glass tubes in sequence: one with 40 mg of pea aphid, the other with 10 previously unfed 24-h-old first-instar larvae. In 20 replicates, the tube with the larvae was downwind of that with the aphids; in another 20 replicates, the tubes were reversed.

Chemical analysis of the volatiles released by aphids

Forty micrograms of pea aphid were crushed for 10 s with a glass rod in a 50-ml vial, which was then quickly sealed with a septum-type cap. The volatiles liberated were sampled by solid phase micro-extraction (SPME) with a PDMS fibre (polydimethylsiloxane-100 μm) previously conditioned at 250°C for 1 h. The fibre was left in the vapour above the crushed aphids for 90 min at 25°C (Zhang & Pawliszyn 1993), then removed and directly inserted into the injection port of a Gas Chromatography Mass Spectrum. Gas Chromatography Mass Spectrum analyses were performed on a MS Hewlett Packard 5972 coupled to a Hewlett Packard 5890 Series II gas chromatograph [split/splitless injector at 250°C; column: HP-5MS, 30 m × 0.25 mm, $d_f = 1$ μm; temperature programme: 40°C (1 min) to 200°C (6°C·min^{-1}) then to 280°C at 15°C·min^{-1}, final hold of 10 min at 280°C; carrier gas: helium at 1.0 $ml \cdot min^{-1}$ (linear velocity = 36.3 $cm \cdot s^{-1}$); electron impact mode at 70 eV; mass range scanned: 35–550 amu, interface at 280°C and source at 250°C]. The identification was performed by comparing the recorded mass spectra with those in the Wiley 138.L and NBS 75 K.L libraries.

Is the searching behaviour of first instar larvae of Adalia bipunctata *affected by β-farnesene?*

The chemical composition of the solution of farnesene in hexane, supplied by P. Harrewijn (University of Wageningen), was determined using the above analytical procedure. The effect of this solution on the searching behaviour of first-instar *A. bipunctata* was also studied in the olfactometer. This was done as described above but prior to placing a larva in the central chamber, farnesene dissolved in hexane was injected, using an Hamilton® microsyringe, into the glass tubes attached to the two opposite entry ports of the olfactometer. The hexane was left to evaporate for 3 min before the tubes were connected to the olfactometer. The first 20 larvae were exposed to the odour from 1 μl of the solution and the next three batches of 20 larvae were exposed to the odour from 3 μl of this solution. As the quantity of solution did not significantly affect the responses shown by the four batches of larvae ($\chi^2 = 0.168$, 3 d.f., $P > 0.05$), the results were pooled. The response of larvae to hexane was determined similarly.

Statistical analysis

The results of the olfactometer experiments were analysed using binomial tests (Siegel 1956; Zar 1996).

Results

Is the searching behaviour of first-instar larvae of Adalia bipunctata *affected by the presence of larvae eating aphids?*

Larvae searched the mesh covered hole in the centre of the lids of the dishes containing only aphids for an average of 96.5 s ($N = 10$; standard deviation = 93.8). When larvae eating aphids were present in the dishes, the larvae searched the mesh for an average of 371.4 s ($N = 10$; standard deviation = 240.2). This four-fold difference is significant ($F = 7.338$, 1 and 18 d.f., $P = 0.014$) and indicates that larvae searched the mesh for longer when conspecific larvae eating aphids were present in the dish. That is, larvae search an area more

Table 1 The number of first-instar larvae of *Adalia bipunctata* in the different odour fields (+) and their respective controls (−)

Odour field	Number of larvae		Binomial test	
	−	+	Value	Significance
Aphids	8	12	0.252	NS[a]
Aphids eaten by larvae	4	16	0.006	**
Crushed aphids	5	15	0.021	*
Larvae fed artificial diet	13	7	0.132	NS
Air passed first over:				
a) Larvae then aphids	12	8	0.252	NS
b) Aphids then larvae	8	12	0.252	NS

[a] NS: not significant, * $P<0.05$, ** $P<0.01$

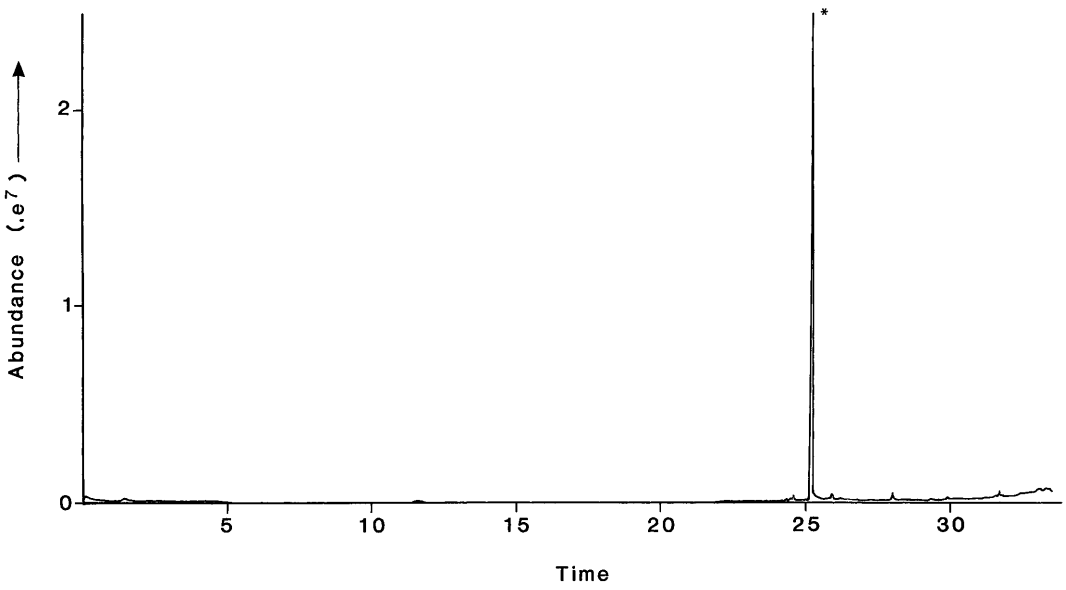

Fig. 1 Chromatogram of the Solid Phase Micro-extraction sampling of volatiles released by 40 mg of crushed *Acyrthosiphon pisum* (★ β-farnesene)

intensively in the presence than in the absence of volatiles from larvae eating aphids.

Do first-instar larvae of Adalia bipunctata *react to volatiles released by aphids or feeding larvae?*

First-instar larvae were not significantly more attracted to the odour of aphids than to clean air. In marked contrast, however, the odour from larvae eating aphids and that of crushed aphids was significantly more attractive to larvae. Air that had passed over larvae feeding on a cube of artificial diet, or first over aphids then larvae, and vice versa, was not attractive to first-instar larvae (Table 1). These results indicate that an aphid captured by a larva releases a volatile that is attractive to other larvae. A similar response was observed to the odour from crushed aphids.

Chemical analysis of the volatiles released by aphids

A single peak was obtained when the substances adsorbed by the SPME fibre from the vapour phase above crushed aphids were injected into the Gas Chromatography Mass Spectrum. It was eluted after 25.19 min (Fig. 1). The mass spectrum of that substance indicates that crushed aphids only released β-farnesene [(*E*)-7,11-dimethyl-3-methylene-1,6,10-dodecatriene], which is the aphid alarm pheromone (Bowers *et al.* 1972).

Is the searching behaviour of first-instar larvae of Adalia bipunctata *affected by β-farnesene?*

The searching behaviour of the larvae was not affected by hexane. The sample of farnesene contained 41.8% *β*-farnesene, 18.7% *α*-farnesene, and at least 12 other sesquiterpene hydrocarbons. Larvae spent significantly more time searching the farnesene sample odour field than that of the control (Table 2). This supports the previous experiment and increases the likelihood that it

Table 2 The number of first-instar larvae of *Adalia bipunctata* in the different odour fields (+) and their respective controls (−)[a]

Odour field	Number of larvae		Binomial test	
	−	+	Value	Significance
Hexane	10	10	0.588	NS
Alarm pheromone	27	53	2.8	**

[a] Test based on the normal approximation of the binomial test; NS: not significant, ** $P<0.01$

is the alarm pheromone released by an aphid being eaten by a larva that is attractive to other larvae.

Discussion

The results presented indicate that, unlike adults (Nakamuta 1991), young ladybird larvae respond to aphid alarm pheromone by searching the immediate area intensively. This is likely to greatly enhance their chances of locating an aphid that has been caught and is being eaten by one of their sibs. That is, the alarm pheromone that serves to warn other aphids of the presence of natural enemies (Dahl 1971; Kislow & Edwards 1972) is also used by young ladybird larvae to locate aphid prey. As a larva's first meal is a critical determinant of its survival to the second instar (Dixon 1959), this social feeding on an aphid corpse is clearly adaptive. At this stage of the study one cannot rule out, however, the possibility that larvae just use the alarm pheromone as an orientating cue.

Several substances probably influence the foraging behaviour of ladybirds as adults searching for oviposition sites or food are sensitive to the presence of honeydew or wax or the odour of their prey (Colburn & Asquith 1970; Evans & Dixon 1986; Obata 1986; van der Meiracker *et al*. 1990; Sengonca & Liu 1994). The chemical nature of these attractants is unknown. This study indicates that β-farnesene may act as a kairomone for first-instar larvae.

Aphidophagous ladybirds tend to lay their eggs in clusters and their larvae pursue and subdue their prey. The probability of young larvae capturing another aphid greatly increases with each subsequent meal, with the first capture being particularly critical (Dixon 1959). In contrast, the coccidophagous ladybirds tend to lay eggs singly under the egg sacs or bodies of their prey (*e.g*. Merlin *et al*. 1996). That is, their young do not have to pursue and subdue their prey as they are literally surrounded by prey. Thus, it is tempting to suggest that an additional adaptive feature of egg clustering is that it facilitates social feeding by kin, which greatly increases the probability of the young larvae surviving to the next instar. This hypothesis needs to be tested. The prediction is that larvae of aphidophagous ladybirds that hatch from eggs laid in clusters have a greater probability of surviving to the second instar than larvae hatching from eggs laid singly.

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