Ladybird-induced life-history changes in aphids

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Predator-mediated plasticity in the morphology, life history and behaviour of prey organisms has been widely reported in freshwater ecosystems. Although clearly adaptive, similar responses have only recently been reported for terrestrial organisms. This is surprising as aphids are polyphenic and develop very rapidly compared with their predators and often produce very large colonies, which are attractive to predators. Therefore, one might expect terrestrial organisms like aphids to show a facultative change in their development in response to the presence of predators and other results have confirmed this. The results presented below indicate that the pea aphid responded to the tracks left by ladybird larvae by producing a greater proportion of winged offspring, which avoid the impending increased risk of predation by dispersing. Associated with this was a short-term increase in activity and reduction in fecundity. The black bean and vetch aphids, which are afforded some protection from ladybirds because they are ant attended and/or unpalatable, did not respond in this way to the presence of ladybird larvae.

Keywords: predation; ladybirds; aphids; life history

1. INTRODUCTION

If animals living in risky habitats are able to assess local levels of predation risk, then facultative changes in their life-history characteristics may allow them to reduce the probability of predation. For example, the offspring of the rotifer *Keratella cochlearis* and the water flea *Daphnia pulex* are induced to develop defensive spines and crests, respectively, in the presence of, or in environments conditioned by, their predators (Krueger & Dodson 1981; Stemberger & Gilbert 1984). Others reduce the risk of predation by shortening their developmental time as in the case of the snail *Physella virgata virgata* and the cladoceran *Daphnia hyalina* (Crowl & Covich 1990; Stibor 1992). Some, like *Daphnia magna*, respond to fish by changes in their morphology, life history and behaviour. However, clones differ widely in their responses, with those from fish habitats more responsive than those from fishless habitats (Boersma *et al.* 1998). These responses are induced by chemicals secreted by predators (kairomones) and, in aquatic environments, have been shown to affect the development and behaviour of a wide range of organisms from algae to fish (Tollrian & Harvell 1998). That is, a given genotype produces adaptive phenotypes in response to environmental changes.

Aphids are parthenogenetic and many are polyphenic in that they show facultative changes in life-history characteristics, being unwinged and having a high reproductive rate when conditions are favourable and switching to producing wingless fecund individuals when conditions become unfavourable. That is, they can facultatively switch their development from a mainly reproductive mode to a dispersal mode. This switch is usually attributed to overcrowding and/or poor host quality (Dixon 1998). In addition, aphids are patchily distributed in space. Even in the absence of natural enemies, patches of aphids characteristically show dramatic increases and decreases in abundance with time, with the decreases mainly due to the exodus of winged aphids. The period for which each patch of aphids remains suitable for ladybird larvae is similar to the time it takes larvae to complete their development. Therefore, it would be advantageous if ladybirds laid their eggs early in the development of an aphid colony or patch (Kindlmann & Dixon 1993) and empirical data tend to support this prediction (Hemptinne *et al.* 1992; Dixon 1997; Doumbia *et al.* 1998). Weisser *et al.* (1999) showed that colonies of the pea aphid that develop in the presence of an adult two- or seven-spot ladybird for 27 days produce more winged individuals than control colonies and that this is probably not a consequence of the ladybird disturbing the aphids. However, adult ladybirds are unlikely to stay with an aphid colony for this length of time. It is more probable to be a few days, sufficient time for an adult beetle to mature and lay a cluster of eggs, as its fitness is dependent on locating and ovipositing in high-quality patches of prey (Dixon 1997). Ladybird larvae become more voracious and successful at capturing aphids as they increase in size, with the fourth-instar larvae taking 40% of the total developmental time and consuming 65% of the total prey required for development (Dixon 2000). Thus, the predation pressure exerted by ladybird larvae on aphid colonies is greatest towards the end of the existence of an aphid patch, i.e. approximately three aphid generations after ladybirds lay their eggs (Dixon *et al.* 1997). Therefore, it would be advantageous if polyphenic aphids could respond to the presence of ladybird larvae

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and produce more winged aphids earlier, which then disperse before predation pressure becomes severe. The tracks of ladybird larvae contain a pheromone that deters conspecific adults from ovipositing (Doumbia *et al.* 1998). Thus, it is possible that some aphids might switch to producing winged offspring in response to chemicals in the tracks of ladybird larvae (kairomones). We tested this hypothesis by exposing the polyphenic pea aphid *Acyrtho-siphon pisum* (Harris), in which morph determination is maternally controlled (Sutherland 1969), to environments contaminated by larvae of one of its ladybird predators, *Adalia bipunctata* L. By way of a control the responses of two other polyphenic species of aphid to the same lady-bird were also determined. These aphids were the vetch aphid *Megoura viciae* Buckton, which is toxic to *A. bipunctata* and the black bean aphid *Aphis fabae fabae* Scopoli, which compared to the pea aphid is a poor quality prey for *A. bipunctata* and frequently also protected by ants (Dixon 1958; Blackman 1967). The cost of this protection to the aphid, at least in the case of ant attendance, is significant (Stadler & Dixon 1998). The prediction was that the more vulnerable pea aphid should show a stronger response to the presence of ladybird predators than either the black bean or vetch aphid.

2. MATERIAL AND METHODS

(a) *Ladybirds*

A stock culture of the two-spot ladybird *A. bipunctata* L. was kept in plastic containers (6 cm × 17.5 cm × 11.6 cm), which each contained damp tissue, a piece of corrugated paper (10 cm × 15 cm) and live pea aphids, which were replaced daily. The pea aphids were reared on broad bean plants (*Vicia faba* L. cultivar 'Bourdon') grown in compost in a greenhouse and kept asexual at 20 °C and an 18 h photoperiod. The stock culture of ladybirds was kept at 21 °C and an 18 h photoperiod, where the ladybirds readily laid clusters of eggs on the corrugated paper and tissue. Clusters of eggs were transferred to 5 cm diameter Petri dishes, one cluster per dish and the larvae fed a mixture of first- and second-instar pea aphids until the larvae reached the third instar when three of them were transferred to a 9 cm diameter Petri dish and fed on pea aphids. Fresh aphids were supplied every 24 h when the dead aphids, their cast skins and ladybird faeces were removed. The adult ladybirds were kept in groups of ten males and ten females.

(b) *Aphids*

The aphids used in the experiments were standardized. Fourth-instar individuals were taken from monoclonal stock cultures of green and red pea aphids, black bean aphid and vetch aphid, which were established specifically for this study. These were reared individually to adulthood and their offspring were similarly reared individually from birth. This was done at 20 °C and a 16 h photoperiod on bean plants that were initially at the six-leaf stage and individually planted in 10 cm diameter pots. These adult aphids were used on reaching maturity.

(c) *Experiments*

One-day-old, fourth-instar ladybird larvae and one-day-old, adult apterous aphids were used in the experiments, which were either done in plastic tubes or on potted plants. All tubes (8 cm long and 3.5 cm diameter) were washed and dried before use and a piece of gauze held in place by a rubber band covered the open end of each tube. The potted broad bean plants were 12 days old, 11–12 cm in height, bearing six leaves and grown individually in 10 cm diameter pots. A 10 cm diameter filter paper was placed on the soil around the base of each bean plant. A band of 'Fruit Tree Grease' (Synchemicals), was applied around the base of the stem of each plant to prevent larvae or aphids from leaving. These plants were isolated from one another by placing a ventilated plastic cylinder over each plant.

(d) *Aphid response to the tracts of ladybird larvae*
(i) *In tubes with aphids*

A ladybird larva (fourth instar) was placed in each plastic tube and supplied with 15 aphids of different instars of either the green or red morph of the pea aphid, black bean aphid or vetch aphid. The control consisted of tubes each containing 15 aphids of the same species, colour morph and instars to those used in the experimental tubes but lacking ladybird larvae. After 15 h the ladybird larvae and all aphid material were carefully removed from the tubes using a fine 'O' paintbrush. A one-day-old, 'standardized' adult apterous aphid was carefully placed on the apical portion of a young (nine-day-old) broad bean plant. After the aphid had settled, the apical portion bearing four leaves was cut off and carefully placed in a 'control' or 'experimental' tube, the open end of which was covered with gauze. The numbers of nymphs that were born to the aphid in the first and second 24 h were recorded and then the adult aphids were removed. The plant material was replaced with fresh material every 24 h. This was repeated ten times in both the experimental and control tubes. The development of all the offspring was monitored daily until they matured, when their morph was recorded.

(ii) *In tubes without aphids*

This experiment was similar to the one above but ladybird larvae were each placed in an empty tube for 15 h. After 15 h the larvae were removed. The control consisted of similar tubes that had had no ladybird larvae in them for 15 h. A one-day-old, adult red pea aphid on the apical portion of a young bean plant was placed in each of these tubes, as described above.

(iii) *On plants*

A ladybird larva (fourth instar) was placed 1 cm above the grease band on a potted plant infested with 15 aphids of different sizes of either the green or red morph of the pea aphid, black bean aphid or vetch aphid. The control consisted of ten potted plants, each infested with only 15 aphids. After 15 h all ladybird larvae and aphid material were removed from the plants and a standardized adult aptera of either the green or red morph of the pea aphid, black bean aphid or vetch aphid placed on the uppermost leaf of each plant. The number of offspring born to these apterae was recorded after 48 h and then the adult aphids were removed. The development of the offspring was monitored daily until they had matured, after which time their morph (apterae or alatae) was recorded. Tubes and plants that had had ladybird larvae in or on them are referred to as contaminated.

(e) *Effect of the tracts of ladybird larvae on the activity of aphids*

To determine whether the activity of aphids in tubes previously occupied by larvae differed, adult apterous green pea aphids were subjected to the same treatment as previously and

Table 1. *Mean percentages of winged offspring produced by pea aphids in environments conditioned by larval ladybirds compared to clean environments*

(In tube experiments offspring were reared to maturity within tubes and new bean shoots were provided every day. Fourth-instar ladybird larvae were used in the experiments. Figures in parentheses are standard errors and the number of replicates $n = 10$.)

experiment	clone used	treatment (contaminated tube or plant)	control (clean tube or plant)	significance (*t*-test on arcsine-transformed data)[a]
ladybird larvae + aphids in tube	green	12.7 (2.4)	2.1 (1.1)	$t = 3.5$, d.f. $= 18$[*]
ladybird larvae + aphids in tube	red	21.5 (2.2)	5.4 (1.6)	$t = 5.1$, d.f. $= 14$[**]
ladybird larvae only in tube	red	20.6 (2.3)	6.0 (1.8)	$t = 4.5$, d.f. $= 14$[**]
ladybird larvae on plant	green	12.3 (1.2)	1.7 (0.6)	$t = 7.2$, d.f. $= 15$[**]
ladybird larvae on plant	red	17.1 (1.5)	3.4 (0.7)	$t = 7.4$, d.f. $= 18$[**]

[a]Reduced degrees of freedom when variances were not equal.
[*]$p < 0.01$ and [**]$p < 0.001$.

Table 2. *Mean percentage of winged offspring produced by black bean and vetch aphids in tubes and on plants contaminated and uncontaminated by larval ladybird tracks*

(Figures in parentheses are standard errors and the number of replicates $n = 10$.)

experiment	aphid	contaminated	uncontaminated	significance (*t*-test on arcsine-transformed data)
ladybird larvae + aphids in tube	black bean	2.43 (1.65)	2.55 (2.50)	$t = 0.024$, d.f. $= 14$[n.s.]
ladybird larvae + aphids in tube	vetch	1.62 (0.83)	0.48 (0.48)	$t = 1.180$, d.f. $= 14$[n.s.]
ladybird larvae + aphids on plant	black bean	2.53 (1.65)	2.50 (2.50)	$t = 0.180$, d.f. $= 18$[n.s.]
ladybird larvae + aphids on plant	vetch	1.83 (0.86)	0.63 (0.48)	$t = 0.140$, d.f. $= 18$[n.s.]

n.s., not significant.

whether they were feeding or walking was recorded at 1, 3, 6, 18 and 24 h.

3. RESULTS

(a) *Do aphids switch to producing winged offspring in response to the tracts of ladybird larvae?*

(i) *In tubes with aphids*

Both the green and red morphs of the pea aphid produced significantly higher percentages of winged forms in the contaminated than in the control conditions (table 1). In contrast, in the same conditions black bean and vetch aphids failed to produce proportionally more winged offspring in response to the tracts of ladybird larvae (table 2).

(ii) *In tubes without aphids*

The red pea aphid clone used in this experiment produced a significantly higher percentage of alate offspring in the tubes contaminated by ladybird larvae than in the control tubes (table 1).

(iii) *On plants*

The green and red morphs of the pea aphid kept on contaminated plants produced significantly higher percentages of winged individuals than those on uncontaminated plants (table 1). As in the above experiment the black bean and vetch aphids did not produce significantly more winged offspring in response to the tracts of ladybird larvae (table 2).

Thus, the previous presence of ladybird larvae induced the pea aphid to produce a greater percentage of winged offspring, whereas this did not occur with the black bean and vetch aphids. The similar level of response shown by the red pea aphid to both tubes contaminated with ladybird larvae plus aphids and only ladybird larvae supports the contention that the aphids are responding to a product of the predator rather than that of attacked aphids.

(b) *Effect of the tracts of ladybird larvae*

(i) *The fecundity of aphids*

Adult apterae of both the green and red morphs of the pea aphid produced fewer offspring on plants contaminated with the tracts of ladybird larvae than on uncontaminated plants. This was most marked and significant in the first 24 h although there was no difference in the following 24 h. In contrast, there was no significant difference in the fecundity of the black bean and vetch aphids on contaminated and uncontaminated plants in either the first or second 24 h (table 3).

(ii) *The activity of aphids*

In tubes contaminated with the tracts of ladybird larvae, a higher proportion of the green pea aphids were active in the first, third and sixth hours (table 4).

That is, the previous presence of ladybird larvae had a short-term effect of reducing the fecundity and increasing the activity of the pea aphid, whereas it did not affect the fecundity of the black bean and vetch aphids.

Table 3. *Mean fecundity of pea aphids on the first and second day on plants contaminated by larval ladybirds compared to uncontaminated plants*

(Figures in parentheses are standard errors and the number of replicates $n = 10$.)

	day 1			day 2		
aphid and clone used	contaminated	un-contaminated	t-test significance	contaminated	un-contaminated	t-test significance
pea aphid						
green	11.1 (0.59)	17.2 (1.24)	3.14^{**}	10.8 (0.55)	11.8 (0.39)	$1.05^{n.s.}$
red	9.6 (0.72)	16.2 (0.63)	4.88^{***}	10.4 (0.79)	11.3 (0.40)	$0.72^{n.s.}$
black bean aphid	5.2 (0.47)	4.9 (0.69)	$0.25^{n.s.}$	4.7 (0.52)	5.7 (0.47)	$1.01^{n.s.}$
vetch aphid	8.2 (0.80)	8.9 (0.62)	$0.49^{n.s.}$	7.7 (0.70)	7.6 (0.72)	$0.07^{n.s.}$

$^{**}p < 0.01$, $^{***}p < 0.001$ and n.s., not significant.

Table 4. *The number of pea aphids feeding and not feeding on pieces of bean plant in uncontaminated tubes and tubes contaminated with the tracks of ladybird larvae 1, 3, 6, 18 and 24 h after the start of the experiment*

	contaminated		uncontaminated		Fisher's exact test
time (h)	not feeding	feeding	not feeding	feeding	χ^2
1	9	1	4	6	0.030^{*}
3	6	4	1	9	0.030^{*}
6	5	5	0	10	0.016^{*}
18	2	8	0	10	$0.470^{n.s.}$
24	0	10	0	10	$1.000^{n.s.}$

$^{*}p < 0.05$.
n.s., not significant.

4. DISCUSSION

Both the green and red clones of the pea aphid consistently responded to ladybird kairomones by producing proportionally more winged offspring. This aphid appears to be able to perceive and respond to cues associated with predators and escape the increasing threat they pose by producing more winged forms and dispersing. That this aphid was initially also more active and produced fewer offspring in the presence of ladybird kairomones also indicates the presence of such a cue. Interestingly, offspring of another member of the tribe Macrosiphini, *Sitobion avenae* (F.), which are destined to develop into winged individuals, are larger at birth than those that develop into unwinged individuals (Newton & Dixon 1990). Therefore, at least part of the reduction in fecundity may have been a consequence of producing more of the large offspring destined to become winged adults.

In response to crowding a pink clone of the pea aphid has been observed to produce proportionally more winged offspring than a green clone (Sutherland 1969). The pink clone used in this study was similarly very sensitive to crowding. There was a more marked response to ladybird kairomones associated with this sensitivity to crowding. It is possible that the coupling of these responses in this pink clone reflects its generally greater sensitivity to certain environmental cues. However, a more detailed analysis of the responses of a large number of green and pink clones and their hybrids to various environmental cues is needed to determine the degree and extent of the coupling between life-history traits. It is possible that some suites of life-history traits will be at a greater selective advantage in certain habitats.

As predicted, the two other aphids that are afforded some protection from natural enemies because they are ant attended and/or unpalatable did not respond as strongly to ladybird kairomones as the pea aphid. Thus, aphids appear to show a range of responses to the presence of ladybirds. It has been known for some time that some species of aphids are very adept at avoiding capture by insect predators. Depending on the relative size of the predator and aphid, the latter will attempt to escape by kicking, walking away, dropping to the ground or daubing wax onto the head of a predator (Dixon 1958). Other species either produce specialized morphs called soldiers, which attack and kill natural enemies (Aoki 1975, 1977) or are unpalatable or ant attended (Dixon 1958). Although based on few species, the results of this study indicate that those species that attempt to avoid capture by predators also respond to ladybird kairomones by switching to producing more winged forms, which can then avoid predation by dispersing.

Predator-mediated plasticity in morphology, life history and behaviour has been widely reported in aquatic organisms (Tollrian & Harvell 1998). Pea aphids similarly appear to be able to respond to chemical cues associated with their predators and escape the impending increased risk of predation by dispersing. The feature that aphids

and the prey organisms in aquatic systems have in common is a short generation time relative to that of their natural enemies. This may enable them to produce forms that can disperse before predation pressure becomes severe.

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