

Avoidance responses of an aphidophagous ladybird, *Adalia bipunctata*, to aphid-tending ants

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Abstract. 1. Insect predators often aggregate to patches of high prey density and use prey chemicals as cues for oviposition. If prey have mutualistic guardians such as ants, however, then these patches may be less suitable for predators.

2. Ants often tend aphids and defend them against predators such as ladybirds. Here, we show that ants can reduce ladybird performance by destroying eggs and physically attacking larvae and adults.

3. Unless ladybirds are able to defend against ant attacks they are likely to have adaptations to avoid ants. We show that *Adalia bipunctata* ladybirds not only move away from patches with *Lasius niger* ants, but also avoid laying eggs in these patches. Furthermore, ladybirds not only respond to ant presence, but also detect ant semiochemicals and alter oviposition strategy accordingly.

4. Ant semiochemicals may signal the extent of ant territories allowing aphid predators to effectively navigate a mosaic landscape of sub-optimal patches in search of less well-defended prey. Such avoidance probably benefits both ants and ladybirds, and the semiochemicals could be regarded as a means of cooperative communication between enemies.

5. Overall, ladybirds respond to a wide range of positive and negative oviposition cues that may trade-off with each other and internal motivation to determine the overall oviposition strategy.

Key words. Avoidance, cuticular hydrocarbons, footprints, inter-specific communication, predator–prey spatial dynamics.

Introduction

Many ladybirds (Coleoptera: Coccinellidae) are predaceous, feeding primarily on Homoptera (e.g. aphids, scales and mealybugs) (Hodek, 1973; Majerus, 1994), which are agricultural pests that impose considerable economic losses to crops (Buckley, 1987). Ants (Hymenoptera: Formicidae) too, prey upon Homoptera, but also tend them to obtain sugary honeydew, which also contains some amino acids and vitamins (Stadler & Dixon, 2005). Approximately 45% of ant genera contain homopteran-tending species (T.H. Oliver, in prep.) and, correspondingly, about one-quarter of aphid species appear to be always tended by ants, with many more species tended facultatively (Bristow, 1991; Stadler & Dixon, 1998). Thus, there is

likely to be conflict between many ants and ladybirds for homopteran resources. Ants are able to effectively defend resources and often show increased aggression to intruders closer to food sources (Brian, 1955; Way, 1963; Offenberg, 2001; Dejean, 2002). Regardless of whether such defensive behaviour has evolved primarily in response to competition by other ants (e.g. Blüthgen *et al.*, 2004), or in response to aphidophagous predators, the defences appear effective at repelling ladybirds (e.g. Banks & Macaulay, 1967; Nault *et al.*, 1976; Harmon & Andow, 2007). Conversely, some species of ladybird can avoid or defend against ant attacks and are more able to exploit ant-attended resources than other less well-defended ladybird species (for a comprehensive review see Majerus *et al.*, 2007).

The two-spot ladybird *Adalia bipunctata* L. (Coleoptera: Coccinellidae) regularly feeds on aphids such as *Aphis fabae* Scopoli (Homoptera: Aphididae) that are facultatively tended by ants (Banks & Macaulay, 1967; Hodek, 1973). Given that this competitive use of shared resources has continued for

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sufficient evolutionary time, we may expect *A. bipunctata* to have either: (i) adaptations to defend against ants or (ii) mechanisms to avoid ants (see Majerus *et al.*, 2007 for a review of coccinellid defences). In this study, we investigate the mechanisms *A. bipunctata* uses to avoid costly conflict with ants. Ladybirds may avoid ants by evading direct encounters; they may also use chemical cues, however, as signals of ant presence. Ants actively lay semiochemical trails by touching exocrine glands onto a substrate surface. These actively laid chemical marks are often used to recruit nestmates to profitable food sources (Hölldobler & Wilson, 1990). Semiochemicals can also be applied to substrates passively through shedding of cuticular hydrocarbons (Yamoaka & Akino, 1994; Depickière *et al.*, 2004). These cuticular hydrocarbons are important in colony nestmate recognition and, when transferred onto the ground, may also mark out territories (Devigne & Detrain, 2002). We test whether *A. bipunctata* uses ant semiochemicals as an oviposition cue to assess patch quality, avoiding laying eggs where ants have been present.

Ladybirds respond to many different cues to select patches that will maximise their own and their offspring's fitness. Oviposition cues for coccinellids reported to date include prey species (Blackman, 1967) and abundance (Dixon, 1959; Wratten, 1973; Oliver *et al.*, 2006), presence of- and chemicals from- competing females (Mishra & Omkar, 2006) and conspecific larval tracks (Hemptinne *et al.*, 1992; Doumbia *et al.*, 1998; Ružicka, 1997, 2001; Yasuda *et al.*, 2000; Hemptinne *et al.*, 2001). There is some evidence that certain heterospecific larval tracks may also inhibit oviposition (Ružicka, 2001). Furthermore, oviposition responses to patch quality cues may not be fixed, but flexible, and may depend upon the individual experience of the gravid female, along with internal physiological state (Frechette *et al.*, 2004). There is growing evidence that ant semiochemicals are often used as information signalling patch quality to other insects. Ant-induced oviposition has been observed in membracids (Hemiptera) (Hölldobler & Wilson, 1990) and lycaenids (Lepidoptera) (Wagner, 1997). Chrysomelid beetles (Coleoptera), in contrast, have found to be deterred by ant semiochemicals (Offenberg, 2004). There are no current reports, however, of ant semiochemicals affecting the oviposition strategy of coccinellids.

Materials and methods

Insect culture

Two-spot ladybirds, *A. bipunctata*, were reared at 20 °C ± 1 °C and a photoperiod of LD 16:8 h, in 5 litre plastic containers which also included a piece of corrugated filter paper, on which the ladybirds often laid eggs. The ladybirds were fed daily an excess of pea aphids *Acyrtosiphon pisum* Harris (Homoptera: Aphididae), which were reared on broad beans, *Vicia faba* L (Leguminosae). Black bean aphids *Aphis fabae* (Homoptera: Aphididae) were also used for food, but only in a minor capacity to supplement the diet if *A. pisum* cultures declined. Once a week the adults were transferred to clean containers to stimulate egg laying.

Experiment 1: oviposition response to ant semiochemicals

Adalia bipunctata adults were offered a choice of four oviposition sites contaminated by different levels of ant semiochemicals. Inside four 4.5-cm Petri dishes pieces of 4.25-cm filter paper were placed. Two, five or ten *Lasius niger* L. ants were placed into each Petri dish, with one left empty as a control. After 3 h the filter papers were removed, folded to corrugate them, and a small piece of Blu-tac® attached to the centre of one side. The filter papers were then secured in a square formation onto the base of a sterile 15-cm Petri dish. To mitigate any bias from spatial positioning, the light source was directly above and filter papers were placed an equal distance from the edge of the Petri dish. Ten satiated *A. bipunctata* adults, previously fed an excess of aphids, were placed into the large Petri dish again with an excess of *A. pisum* aphids. The number of coccinellid eggs on each filter paper was counted at intervals of 5 h and 10 h and then again after 24 h. The test was replicated 24 times.

A preliminary investigation had been carried out to ensure that aphids did not aggregate disproportionately to ant-treatment or control filter papers. Forty *A. pisum* aphids were placed in a 15-cm Petri dish with a filter paper contaminated by semiochemicals from 10 ants for 3 h and a clean control filter paper. The number of aphids in each half of the Petri dish was counted after 5 h.

Experiment 2: avoidance and oviposition response to ant presence

Adalia bipunctata ladybirds were given a choice to lay eggs in one of two adjacent arenas: one empty, the other occupied by *L. niger* ants. Two 10 litre closed plastic boxes (30 × 15 × 22 cm) were placed together with an open window in the upper half of the adjoining wall. Fluon® was applied in a horizontal line, below the window, halfway up the inside of the boxes. This prevented ants crossing from one arena into the other, yet ladybirds could still access both arenas by flying over the wall. Occasionally, ladybirds were also able to climb across the Fluon® barrier and thus occupy the upper half of the box and cross the dividing wall in this way. Ants, however, were always unable to traverse the Fluon® line. Before the experiment, the arenas were cleaned with hot water and dried to remove any residual semiochemicals. In the centre of each arena a 9-cm Petri dish was placed, raised above the base of the arena with a plastic stopper. In the centre of each dish a corrugated 4.25-cm filter paper was attached using Blu-tac® and an excess of *A. pisum* aphids placed onto the paper. The inside and outside rim of the Petri dish was coated with Fluon® to prevent aphids escaping or ants climbing in. At the start of the experiment, 50 *L. niger* ants were placed into one randomly chosen arena and six *A. bipunctata* adults placed into each arena (12 in total). At 4-h intervals, over a period of 24 h, the number of eggs laid in the raised Petri dishes or on the base of the arenas was counted and also the number of *A. bipunctata* in each of the two arenas. The experiment was replicated 12 times.

Experiment 3: fitness effects of ants on coccinellid eggs

Batches of *A. bipunctata* eggs recently laid on filter papers were counted (range: 5–40 eggs) and then randomly allocated to

one of two treatments. For the first treatment, eggs were placed in plastic boxes containing unqueened colonies of *c. 200 L. niger* for 24 h. Eggs were then left in a 9-cm Petri dish with Fluon®-coated sides and upon hatching the number of larvae emerging were counted. The second treatment comprised a control with no ants present. Sixteen replicates were conducted in total.

Statistical analyses

Analysis of deviance, using the program *R* (Ihaka & Gentleman, 1996), was used to compare the number of eggs laid on filter papers contaminated by different levels of ant semiochemical. Eggs were counted after 24 h, but also twice during the experiment because eggs can be cannibalised by adults. For this reason, the maximum count of the three recordings was used for analysis. Egg cannibalism was very rare during the experiment, however, as an excess of aphid prey was provided. The Poisson family of error structures was used to deal with the count data and overdispersion was accounted for where necessary using the *quasipoisson* family of error structures (Crawley, 2005). Factor levels were collapsed to test for differences between them and obtain the minimum adequate model.

A standard *t*-test was used to compare mean times ladybirds remained on ant-covered versus control plants. As the sample variances were different (as determined using an *F*-test), the Welch approximation for degrees of freedom was used. A paired Student's *t*-test was used to test for differences in ladybird numbers and eggs in the two arenas of the ant presence experiment. Again, maximum egg counts were used for analysis. Similarly, a *t*-test was used to compare the hatch rate of ladybird eggs on control and ant-treatment filter papers. Because the starting number of eggs on each filter paper varied, however, the proportion of eggs that hatched was used for analysis, with appropriate square root-arcsine transformation. All mean values listed are followed by \pm one standard error.

Results

Experiment 1: oviposition response to ant semiochemicals

The preliminary investigation confirmed that aphids did not aggregate disproportionately to ant semiochemical-contaminated filter papers. After 20 replicates, there were 20.3 ± 1.4 aphids in the half of the Petri dish with the control filter paper, compared with 19.7 ± 1.4 aphids in the half with the ant semiochemical-contaminated filter paper ($n = 20$, $t = 0.22$, d.f. = 19, $P = 0.83$).

The main experiment found ladybirds avoided laying eggs in the presence of *L. niger* semiochemicals. Over the course of 24 h, 10.3 ± 3.6 , 10.1 ± 2.7 , 11.4 ± 3.2 and 3.1 ± 1.4 eggs were laid on the filter papers contaminated by 0, 2, 5 or 10 *L. niger* ants, respectively. Oviposition was significantly reduced on filter papers contaminated with the highest level of *L. niger* semiochemicals compared with lower concentrations and the control ($F_{1,94} = 6.88$, $P < 0.05$) (Fig. 1). Oviposition on filter papers with lower concentrations of semiochemical was not different to the control (*L. niger*: $F_{2,92} = 0.05$, $P = 0.95$).

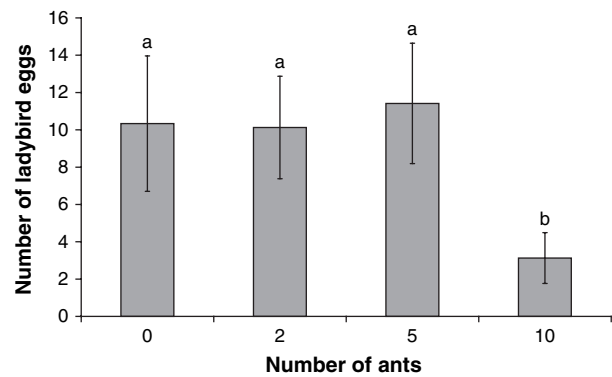


Fig. 1. The mean number of eggs laid by 10 *Adalia bipunctata* adults over 24 h on filter papers contaminated with semiochemicals from different numbers of *Lasius niger* ants. Different letters represent significantly different means ($P < 0.05$). Bars represent standard errors ($n = 24$).

Experiment 2: avoidance and oviposition response to ant presence

Ladybirds avoided the presence of ants and, correspondingly, fewer eggs were laid in the arenas containing ants. Ants often attacked the *A. bipunctata* ladybirds by biting them and arching the abdomen underneath the thorax to face the gaster forwards, a behaviour typically associated with the spraying of formic acid (Sloggett *et al.*, 1998). Throughout the experiment, there were on average 5.02 ± 0.29 ladybirds in the arena with *L. niger* ants, compared with 6.98 ± 0.29 in the arena without ($t = 3.37$, d.f. = 11, $P < 0.01$). After 24 h, 21.92 ± 3.55 eggs were laid in arenas with *L. niger*, compared with 64.08 ± 9.42 eggs in arenas without ants ($t = 4.93$, d.f. = 11, $P < 0.001$). Thus, almost three-quarters of all ladybird eggs were laid in the arena without ants.

Experiment 3: fitness effects of ants on coccinellid eggs

Ant attacks reduced the viability of ladybird eggs (Fig. 2). *Lasius niger* were observed to occasionally bite and damage the

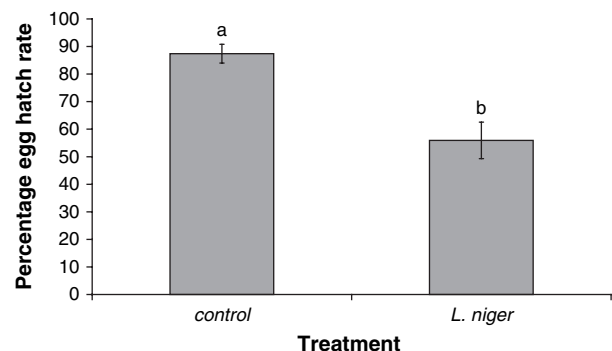


Fig. 2. Hatch rate of *Adalia bipunctata* eggs left with *c. 200 Lasius niger* for 24 h or a control without ants. Different letters represent significantly different means ($P < 0.05$). Bars represent standard errors about the means ($n = 16$).

eggs, although never to feed on the contents. As a result, significantly fewer eggs hatched successfully in the ant treatment ($t = 4.04$, d.f. = 30, $P < 0.001$).

Discussion

Adalia bipunctata ladybirds avoided *L. niger* ants, but also responded to ant semiochemicals alone, by reducing oviposition. A preliminary investigation confirmed aphids did not aggregate disproportionately to either the control or ant semiochemical-treated filter papers. Although aphids are known to reduce movement speed in response to ant chemicals (Oliver *et al.*, 2007), the effect is subtle and in the current experiment this did not result in significant differences in the numbers of aphids in either half of the Petri dishes. Thus, any differences in ladybird egg numbers on the filter papers can be attributed to direct effects of ant semiochemicals on ladybird oviposition, rather than an indirect effect of ants on ladybird prey.

Lasius niger effectively repelled *A. bipunctata* from homopteran resources, as in other studies (e.g. Banks & Macaulay, 1967; Harmon & Andow, 2007). There were some defences observed against ant attacks, including withdrawal of appendages and tilting of the carapace in adults (Bradley, 1973; Jiggins *et al.*, 1993; Majerus, 1994) and reflex bleeding in larvae (El Ziady & Kennedy, 1956; Happ & Eisner, 1961; Pasteels *et al.*, 1973; Bhatkar, 1982). Despite these stationary defences, however, ladybirds were often forced to evade ants. For example, in the arena experiments, ladybirds actively avoided the presence of the ants. More eggs were laid in the arena without ants and this could simply be as a result of interference of oviposition behaviour by ants and a consequence of greater ladybirds presence in the arena without ants. Semiochemicals, in the absence of ants, however, were also found to inhibit *A. bipunctata* oviposition. Thus, a combination of active avoidance of ants, oviposition interference by ants and oviposition inhibition by ant semiochemicals, may determine the overall oviposition strategy of ladybirds in patches with ants. Harmon & Andrew (2007) showed that ladybirds avoid foraging in patches with ants by assessing ant density relative to aphid abundance. This is the first demonstration, however, of coccinellids using ant semiochemicals as an oviposition cue. It is interesting to note that only the highest density of ant semiochemicals used in this study resulted in inhibition of egg laying. Thus, there may be a lower threshold of semiochemical concentration, indicative of a large number of ants in the local area, below which, oviposition levels remain normal, but above which, many fewer eggs are laid.

Laying fewer eggs near ants is likely to be adaptive: ants can sometimes damage ladybirds, especially larvae, during attacks (Bradley, 1973; Jiggins *et al.*, 1993; Sloggett & Majerus, 2003); time and energy are lost if ladybirds are forced to drop from plants and, finally, ants chew and destroy ladybird eggs. Indeed, we found that there was a greater than 35% reduction in egg hatch rate, as a result of ant attack (Fig. 2). Ants did not appear to feed on egg contents; indeed, they often contain toxic alkaloids (Daloze *et al.*, 1995; Pasteels, 2007). This attack of ladybird eggs could suggest specific adaptation in ants against

ladybird antagonists (e.g. Sloggett, 1998), rather than some extension of aggression towards moving insects close to food resources (e.g. Way, 1963).

The avoidance of ant-guarded prey patches is likely to benefit ants as well as ladybirds themselves. Defending aphids from natural enemies has time and energy costs for ants, and there may be also be increased risk of mortality (Stadler & Dixon, 2005). Ladybird responses to ant semiochemicals could thus be viewed as a form of cooperative communication between enemies, similar to the conspicuous communication between predators and prey, where both parties benefit from avoiding unsuccessful, and costly, chases of strong individuals (e.g. gazelle *flagging* signalling agile prey to predators) (Baalen & Jansen, 2001). In these cases, however, both predator and prey evolve to facilitate the communication: prey perform conspicuous signals, to which predators *tune in* and respond appropriately. Ant semiochemicals, in contrast, are likely to have multiple functions including intra-specific communication with nestmates and competitors (Hölldobler & Wilson, 1990; Devigne & Detrain, 2002), and mediation of aphid dispersal (Oliver *et al.*, 2007). Thus, the evolution of these chemicals may be somewhat constrained, and we propose it is more likely that ladybirds have evolved to receive and respond to the ant chemicals, rather than ant chemicals evolving to manipulate ladybird responses.

We have found that ladybirds avoid ovipositing in ant patches in search of better sites, yet the searching experience and internal physiology of ladybirds is also known to affect oviposition behaviour (Frechette *et al.*, 2004). Thus, it would be of interest to see whether internal factors, such as satiation, trade-off with external inhibitory cues from ants to determine the overall oviposition strategy. Such a trade-off with satiation has been found for inhibitory cues from conspecific larvae (Oliver *et al.*, 2006). The decision to lay eggs in a patch could be mediated by many different external cues interacting with internal motivation for oviposition.

In summary, ants that defend common resources such as Homoptera will frequently compete with predators such as ladybirds. Ant attacks have a negative impact on all ladybird developmental stages: eggs, larvae, and adults, and significantly reduce individual fitness. Ladybirds unable to tolerate ant presence are likely to have adaptations to avoid patches with ants. In addition to avoiding foraging on resources guarded by ants, we show that ladybirds use information from ant chemical cues and adjust their oviposition strategy accordingly. Ant semiochemicals signal the extent of ant territories allowing ladybirds to effectively navigate a mosaic landscape of sub-optimal patches. Thus, the spatial distribution of ants that share mutualisms with homopteran pests is likely to also shape the distribution of coccinellid predators.

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