Inclusive fitness benefits of scent-marking predators

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Because relatedness is high and dispersal is limited, one would expect to see higher levels of altruistic behaviour among clonal organisms than among animals of lesser relatedness. Enigmatically, however, parthenogenetic aphids do not emit alarm signals when a predator first enters a colony but only after being captured. Here, we report that an aphid smearing alarm pheromone directly onto a predator decreases the predation risk for clone-mates as the predator continues to search for additional prey. Adult multicoloured Asian ladybird beetles, *Harmonia axyridis*, daubed with alarm pheromone caused a greater proportion of aphids to drop off a plant and escape predation than did predators lacking pheromone droplets. Thus, along with direct fitness benefits of individual protection, aphid alarm signalling behaviour may have evolved through inclusive fitness benefits of protecting clone-mates by scent-marking predators.

Keywords: alarm pheromone; aphid; cornicle droplet; inclusive fitness; predation risk; scent mark

1. INTRODUCTION

Alarm signalling theory predicts that higher levels of altruistic behaviour will be observed between clonal organisms than among animals that are less closely related (Maynard Smith 1965) because clonal organisms' relatedness is extremely high, if not absolute (Lushai *et al.* 1997). Alarm signalling effectively reduces the risk of predation for nearby kin because it causes organisms to become more alert or disperse, thereby lowering their chance of being successfully attacked (Kats & Dill 1998). Thus, any alarm signal protecting clone-mates will have substantial inclusive fitness benefits for the signaller because the organism will be protecting exact copies of its genotype, i.e. its 'evolutionary self' (Janzen 1977).

Aphids have, however, remained enigmatic with respect to alarm signalling theory. Although aphids are parthenogenetic, individuals emit cornicle droplets containing the alarm pheromone E- β -farnesene (EBF) (Dahl 1971; Kislow & Edwards 1972) not when initially detecting a predator but only after being physically attacked (Nault & Phelan 1984). While cornicle droplets daubed directly onto predators results in the emitter's escape in *ca.* 10% of attacks (Dixon 1958; Edwards 1966), droplets are believed to function principally as alarm signals since they are emitted preferentially when an aphid individual is

surrounded by clone-mates as opposed to heteroclonal conspecifics or heterospecifics (Robertson *et al.* 1995). Alarm signal emission greatly facilitates aphid survival, causing clone-mates to stop feeding, walk and/or drop from their feeding sites to escape predation (Nault *et al.* 1973; Clegg & Barlow 1982). Therefore, why do aphids delay alarm signalling and seemingly put clone-mates at an increased risk of attack?

Because ladybird beetles consume multiple aphids in a feeding bout (Nakamura 1983), we integrate the two observations that (i) aphids frequently smear cornicle secretions onto predators (Dixon 1958; Edwards 1966; Mondor & Roitberg 2002); and (ii) cornicle droplets contain a volatile alarm pheromone that persists for several minutes (Nault *et al.* 1973) into a single hypothesis for aphid alarm signalling behaviour. Along with direct defensive benefits, we suggest that cornicle droplets may provide inclusive fitness benefits to the signaller of warning clone-mates of a predatory threat. A pheromone droplet smeared directly onto a predator would be carried throughout the colony as the natural enemy continues to search for additional prey, thereby increasing the likelihood that clone-mates escape predation. We addressed this hypothesis by assessing whether aphids are less likely to be captured by pheromone-marked versus unmarked ladybird beetles.

2. MATERIAL AND METHODS

(a) *Insects*

Pea aphids, *Acyrthosiphon pisum*, were reared in the laboratory at $23 \pm 2^\circ\text{C}$, $50 \pm 20\%$ relative humidity (rh), and on a 16 L : 8 D photoperiod (supplied by four 40 W cool fluorescent bulbs) on broad bean, *Vicia faba* c.v. 'Broad Windsor'. Multicoloured Asian ladybird beetles, *Harmonia axyridis*, were reared in the laboratory on pea aphids, banana and honey-water *ab libitum* at $21 \pm 2^\circ\text{C}$, $50 \pm 20\%$ rh, and on a 16 L : 8 D photoperiod. The adult beetles that were used for the experiment were less than four generations removed from wild stock.

(b) *Experimental set-up*

For bioassays, clonal groups of pea aphids were established on cut bean plants by allowing one mature apterous aphid to produce offspring over 48 h. Groups of aphids, on their own plants, were placed into water-filled 20 ml scintillation vials with rubber septa and situated in a water trap to prevent aphids from moving between plants. Aphids were allowed to mature to third and fourth instar on these plants over the following four days.

Beetles were placed in individual Petri dishes and deprived of food for at least 12 h to facilitate searching behaviour. Subsequently, one of the following treatments was applied to the elytra of a beetle using a 10 μl syringe: (i) no treatment; (ii) 1 μl of hexane; (iii) 1 μl of hexane containing 50 ng of EBF (a biologically relevant amount); (iv) 1 μl of hexane containing 500 ng of EBF; or (v) 1 μl of hexane containing 5000 ng of EBF ($n = 11, 11, 12, 12$ and 11, respectively). EBF (Bedoukian Research Inc.) was topically applied to beetles to quantify accurately the amount of pheromone carried by a predator. Aphid colonies respond similarly to both natural and synthetic EBF (Bowers *et al.* 1972; Montgomery & Nault 1978).

After application, the ladybird was placed at the bottom of a plant and allowed to search for prey for 5 min or until an aphid was captured. We recorded the number of aphids that dropped off the plant, both when approached and when physically contacted by a predator. The aphids remaining on the plant after 5 min were counted and the room exhausted of odours after each observation period. Aphid colonies and beetles were used only once during the experiment. Experiments were conducted at $20\text{--}22^\circ\text{C}$, $50\text{--}55\%$ rh, under two 40 W cool fluorescent bulbs.

(c) *Statistical analyses*

Plant heights, numbers of leaf pairs and numbers of aphids on the plant prior to predator searching were compared for each treatment using one-way ANOVA. The proportions of aphids dropping off the plant both before and after contact with a predator were transformed ($x' = \arcsin\sqrt{x}$) and compared with one-way ANCOVAs. The covariate (the time a predator spent searching a plant) was included to

Figure 1. Aphids dropping (a) before and (b) after contact with an adult ladybird beetle daubed with aphid alarm pheromone, EBF. Bars with the same letter do not differ significantly from one another; Tukey–Kramer Honestly Significant Difference, $p > 0.05$.

control for more or less aphids dropping off a plant during longer predator search times. Using logistic regression analyses we also determined whether the number of aphids dropping off a plant altered the chances of a clone-mate being captured.

3. RESULTS

Among treatments, plants did not differ (mean, 1 s.e.) significantly in height (7.86, 0.39 cm; $F_{4,52} = 0.96$, $p = 0.44$), number of bean leaf pairs (2.58, 0.08; $F_{4,52} = 1.13$, $p = 0.35$), or in the initial number of aphids that they sustained (14.75, 0.66; $F_{4,52} = 1.17$, $p = 0.34$). If a predator carried an alarm pheromone droplet on its elytra, however, aphids were more likely to drop from the plant than if an unmarked predator approached ($F_{4,51} = 11.76$, $p < 0.0001$; figure 1) irrespective of the length of time that predators spent searching plants ($F_{1,51} = 0.51$, $p = 0.48$). This pre-contact dropping behaviour directly altered the clone's predation rate; the chances of a clone-mate being captured significantly declined as more aphids dropped off the plant (logistic χ^2 approximation = 5.50, $p = 0.019$; figure 2).

Conversely, the number of aphids dropping when physically contacted by a predator did not differ among treatments ($F_{4,51} = 0.74$, $p = 0.57$; figure 1) and was

Figure 2. Number of aphids dropping off a plant prior to predator contact, and the likelihood of an individual being captured. Logistic regression χ^2 approximation = 5.50, $p = 0.019$.

independent of predator search time ($F_{1,51} = 0.26$, $p = 0.61$). Furthermore, the proportion of aphids that dropped when physically contacted did not alter predator success (logistic regression χ^2 approximation = 0.03, $p = 0.86$).

4. DISCUSSION

Animals use pheromones to scent-mark mates (Brown & Macdonald 1985), oviposition sites (Hoffmeister & Roitberg 1997; Yasuda *et al.* 2000) and territories (Brown & Macdonald 1985). Here, we show that an organism smearing alarm pheromone droplets directly onto its natural enemy provides colony members (i.e. clone-mates) with a dynamic alarm signal for several minutes after the predation event. In our experiment, we evaluated only dropping behaviour, the most extreme alarm response for a conservative test of the hypothesis, but even small changes in aphid behaviour may result in differential individual and clone survival (Montgomery & Nault 1978). For example, aphids not dispersing in response to alarm pheromone often become more sensitive to other stimuli such as vibrations from searching predators (Clegg & Barlow 1982).

Pheromone droplets associated with predators may also be adaptive by reducing false positive signalling errors (i.e. a signal to disperse when no predator is present). False positive responses would result in clone-mates unnecessarily abandoning their feeding sites, which can be fatal for young instars (Dill *et al.* 1990). A smaller active space of pheromone directly associated with the predator would be a more reliable cue of increased predation risk than would a large non-specific pheromone emission. As many aphid predators have poor vision and tortuous searching paths, clone-mates would then abandon feeding sites only when a predator approached.

False negative signalling errors (i.e. no alarm signal when a predator is present) may also be reduced by smearing a predator with a pheromone droplet. Aphids that have a diffuse colony structure over a host plant (Nault &

Phelan 1984) would still receive an alarm signal as the predator approached, even if not feeding at the initial predation site. The selective advantage of scent-marking predators may explain why aphid cornicles grow proportionally more than other appendages throughout development (Mondor & Roitberg 2002). Across species, aphids living in more scattered colonies tend to have longer cornicles than those living in close proximity (Mondor *et al.* 2002), perhaps to facilitate droplet transmission onto predators since many clone-mates may be feeding outside the active space of a simple pheromone emission.

Thus, along with any direct fitness benefits of cornicle droplets, the inclusive fitness benefits of scent-marking natural enemies could have been a substantial selective force for the evolution of cornicles and cornicle droplets in aphids. Because the substrate on which the compound is deposited, composition of the emission, environmental conditions (Regnier & Goodwin 1977) and the context under which the compound persists (Kats & Dill 1998) all affect pheromone transmission and organism responses, further investigation is required into the ecological and evolutionary significance of aphid alarm signalling behaviour among taxa.

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