Evidence that insect herbivores are deterred by ant pheromones

Joachim Offenberg[1][*]**, Mogens Gissel Nielsen**[1]**, Donald J. MacIntosh**[2]**, Sopon Havanon**[3] **and Sanit Aksornkoae**[4]

[1]*The Institute of Biological Sciences, University of Aarhus, Universitetsparken, DK-8000 Aarhus C, Denmark*
[2]*Centre for Tropical Ecosystems Research, The Institute of Biological Sciences, University of Aarhus, Universitetsparken, DK-8000 Aarhus C, Denmark*
[3]*Royal Forest Department of Thailand, Ranong Mangrove Forest Research Centre, Tumpon Ngao, Muang District, Ranong 85000, Thailand*
[4]*Faculty of Forestry, Kasetsart University, Phahonyothin Road, Chatuchak, Bangkok 10900, Thailand*
[*]*Author for correspondence (offenberg@biology.au.dk).*

It is well documented that ants can protect plants against insect herbivores, but the underlying mechanisms remain almost undocumented. We propose and test the pheromone avoidance hypothesis—an indirect mechanism where insect herbivores are repelled not only by ants but also by ant pheromones. Herbivores subjected to ant predation will experience a selective advantage if they evolve mechanisms enabling them to avoid feeding within ant territories. Such a mechanism could be based on the ability to detect and evade ant pheromones. Field observations and data from the literature showed that the ant *Oecophylla smaragdina* distributes persistent pheromones throughout its territory. In addition, a laboratory test showed that the beetle *Rhyparida wallacei*, which this ant preys on, was reluctant to feed on leaves sampled within ant territories compared with leaves sampled outside territories. Thus, this study provides an example of an ant–herbivore system conforming to the pheromone avoidance hypothesis.

Keywords: ant–plant protection; herbivore deterrence; ant pheromones; mangrove trees; *Oecophylla smaragdina*; *Rhyparida wallacei*

1. INTRODUCTION

Ecological research during the past three decades has recognized that ants protect their host plants against a multitude of herbivores (reviewed by Way & Khoo 1992; Agrawal & Rutter 1998; Bronstein 1998). Nonetheless, the mechanisms behind ant protection have been studied only rarely (Rudgers *et al.* 2003), probably because of the general assumption that ants prey on or deter herbivores only during direct encounters. However, indirect mechanisms may operate at the same time if herbivores are able to detect the territories of enemy ants. In such cases, herbivores should avoid plants where they detect the former presence of ants (i.e. within an ant territory) since the predation risk here will remain higher than on plants without ants (assuming a positive correlation between the cue

that identifies the presence of ants and the actual predation risk exerted by the ants). Such a mechanism would make ant protection more efficient than protection based exclusively on direct encounters between ants and their prey, and it would also explain why ants, despite their protective effect, are only rarely observed attacking herbivores (Bronstein 1998).

Most ants produce ephemeral trail pheromones (lasting from a few seconds to several days). Some species also produce more persistent faecal deposit pheromones (lasting from weeks to months) to mark and navigate within their territories (reviewed by Dejean & Beugnon 1991). *Oecophylla* ants produce some of the most persistent ant pheromones recorded so far. Under laboratory conditions, it has been shown that these ants mark their entire territories and trails with visible pheromones produced in the rectal sac (anal spots) and with invisible trail pheromones produced in the rectal gland (Hölldobler & Wilson 1977, 1978; Hölldobler 1983; Dejean & Beugnon 1991; Beugnon & Dejean 1992). Anal spots and trail pheromones can be identified by the ants after at least 10 months and nine weeks after their production, respectively, even if they have been washed by rain water (Beugnon & Dejean 1992). Thus, *Oecophylla* pheromones may serve as a long-lasting warning signal to herbivores if the herbivores are able to detect the pheromones. Furthermore, *Oecophylla* ants have been acknowledged as one of the most efficient groups of ants in controlling plant pests (Way & Khoo 1992), which may indicate a positive correlation between persistency of ant pheromones and plant protection efficiency.

Based on these published findings, we propose the pheromone avoidance hypothesis arguing that ant prey may have evolved traits enabling them to detect and avoid areas contaminated by ant pheromones; a mechanism leading to increased benefit to ant-protected plants compared with a mechanism relying solely on direct encounters between ants and herbivores. The weaver ant *Oecophylla smaragdina* preys on the chrysomelid beetle *Rhyparida wallacei* and protects the mangrove tree *Rhizophora mucronata* against these beetles (Offenberg *et al.* 2004). To test the pheromone avoidance hypothesis, we observed whether *O. smaragdina* marked *R. mucronata* host trees with pheromones in the field, and whether *R. wallacei* showed a feeding preference between leaves sampled inside versus outside ant territories.

2. METHODS

Field observations were conducted within the Ranong Biosphere Reserve in southern Thailand, in mangrove forest along the Ngao Estuary (9°50′ N, 98°35′ E). In an area with *O. smaragdina* colonies, we haphazardly selected nine trees with ants and nine control trees without ants. Shoots on ant-trees were divided into categories with high, medium or low ant activity. Shoots with ant nests, or with colonies of ant-attended high activity, nearest neighbouring shoot medium activity and shoots 2 m away (randomly selected at an equal height above ground) low activity. Each time a high-activity shoot was found, we selected one medium- and one low-activity shoot. From each control tree, we randomly selected four shoots at heights equal to the heights of high-activity shoots on ant-trees. All leaves from the selected shoots were collected and the number of anal spots on each leaf was counted.

Twenty-seven chrysomelid beetles (*R. wallacei*) were collected in the field and kept in the laboratory in separate plastic boxes (18 cm × 12 cm × 6 cm) at ambient temperature (27–32 °C) and humidity on a 12 L : 12 D cycle. Each beetle was offered two newly flushed *R. mucronata* leaves: one sampled next to an ant nest (ant leaf) and one sampled from a neighbouring tree without ants (control leaf). All leaves were taken from different trees but at similar heights above the ground. After one night (12 h) the feeding rate (number

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Table 1. Distribution of *Oecophylla smaragdina* ant pheromones (anal spots) in relation to ant activity within ant-colonized trees (*Rhizophora mucronata*).
(The mean numbers of spots per leaf include leaves without spots.)

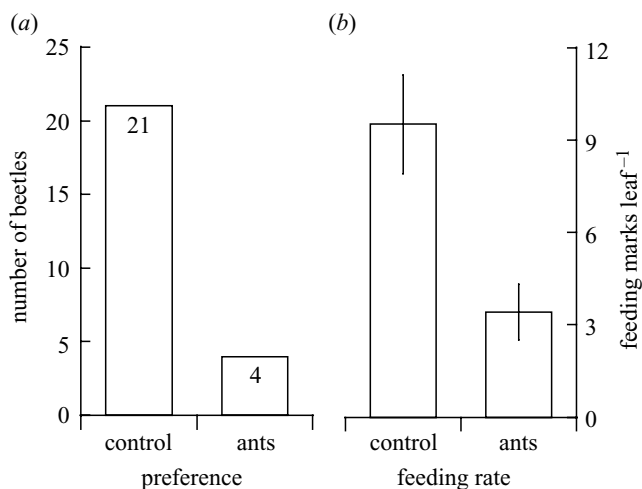| ant activity	fraction of leaves with anal spots ($p = 0.005$)	mean (s.e.) number of anal spots per leaf
high ($n = 150$)	0.38	1.03 (0.16)
medium ($n = 191$)	0.29	0.47 (0.07)
low ($n = 188$)	0.19	0.25 (0.05)
average	0.28	0.55 (0.06)

Figure 1. Leaf beetles (*Rhyparida wallacei*) feeding on leaves from trees without ants (control), and leaves sampled next to ant nests (ants). (*a*) The number of beetles preferring control leaves versus leaves from ant-trees ($p < 0.0001$). (*b*) The mean ($\pm$ s.e.) number of feeding marks on control leaves versus leaves from ant-trees ($n = 25$ leaf pairs; $p = 0.001$).

of feeding marks) on each leaf was registered and for each beetle the preferred leaf was recorded as the leaf with the most feeding marks. After excluding non-feeding beetles, feeding preference was analysed with a G-test and differences in feeding rate were analysed with Wilcoxon's signed-rank test (JMP 3.2.2) where the two leaves offered to each beetle formed a pair.

3. RESULTS

On average, 28% of the leaves on ant-trees were marked with anal spots with an overall mean of 0.55 spots per leaf (table 1). Between low- and high-activity ant areas, the fraction of marked leaves doubled (G-test: $p = 0.005$) and spot density increased fourfold (table 1), but even in the low-activity areas, almost every fifth leaf was marked with anal spots (table 1). On the control trees without ants, we found no spots ($n = 341$ leaves). Thus, ants place their pheromone markings on leaves throughout their host trees, but more intensively in areas with high ant activity.

The beetles in the feeding experiment showed a significant preference (G-test: $p < 0.0001$) for leaves collected from trees without ants compared with leaves collected from ant-trees next to ant nests. Two beetles were excluded from the analysis since they did not feed. Out of the remaining 25 beetles, 21 preferred the control leaf and only four preferred the ant leaf (figure 1*a*). Also, the beetles made almost three times more feeding marks (Wilcoxon signed-rank test: $p = 0.001$) on control leaves (mean number of holes per leaf $\pm$ s.e. = 9.52 $\pm$ 1.61) than

on leaves from ant-trees (mean number of holes per leaf $\pm$ s.e. = 3.41 $\pm$ 0.9; figure 1*b*).

4. DISCUSSION

A certain degree of predation pressure is required to cause insect herbivores to evolve defence mechanisms against ants. During previous field and laboratory studies we found that *O. smaragdina* ants preyed on *R. wallacei*; the density of beetles and the feeding damage they caused were significantly lower on ant-trees than on trees without ants (Offenberg *et al.* 2004). Furthermore, trees connected to ant-trees, but only rarely visited by ants, also showed low damage levels—an observation indicating that other mechanisms beside direct encounters between ants and beetles could be operating in the system. Therefore, in the present ant–plant protection system, it is probable that *R. wallacei* have evolved a mechanism enabling them to avoid *O. smaragdina* territories.

Observations from the current study showed that *O. smaragdina* ants marked their host trees in the field with anal spot pheromones (table 1). These field observations correspond to previous laboratory studies showing that the other *Oecophylla* species, *O. longinoda*, distribute anal spots throughout their territory, and produce higher spot densities around high-activity areas such as nests and trails (Hölldobler & Wilson 1977, 1978; Dejean & Beugnon 1991). The distribution of pheromone marks in the ant territory can therefore signal increased ant-predation risk to potential prey, and trigger the prey to flee. The persistent nature of *Oecophylla* pheromones lasting weeks in the field (J. Offenberg, unpublished data) and even months in the laboratory (Dejean & Beugnon 1991; Beugnon & Dejean 1992) further strengthens the warning potential of these chemicals. The warning effect of pheromones was also supported by the beetle-feeding experiment, since most of the beetles avoided leaves from ant-trees (G-test: $p < 0.0001$) when compared with control leaves (figure 1*a*). Eighty-four per cent of the beetles preferred to feed on, and thus stay on, leaves collected from trees without ants as an alternative to leaves sampled next to ant nests (figure 1*a*).

These results support the idea that ant pheromones can deter insect herbivores. However, this is a preliminary study and it does not show definitively that ant pheromones are the cause of beetle deterrence since other factors correlated with the presence of ants could be responsible. Further steps need to be taken to resolve whether or not ant pheromones are causative in this case and if ant pheromones deter herbivores in ant–plant protections in general. However, we find ample evidence to propose the pheromone avoidance hypothesis.

(i) Theoretical considerations speak in favour of the hypothesis. When ants exert a significant predation pressure on herbivores, those individuals that evolve mechanisms to detect and avoid ant territories will gain a selective advantage (other things being equal, i.e. if the selection pressure to avoid ant predation is not counteracted by different selection pressures) and the trait should evolve.

(ii) In the present system, the ants did indeed exert a predation pressure on the beetles. They reduced their numbers and the damage they inflicted on their host trees.

(iii) Even trees only rarely visited by ants showed low beetle damage.

(iv) Ants distributed long-lasting pheromones on their host trees.

(v) The beetles were reluctant to feed on leaves sampled from ant-trees.

If ant pheromones can indeed deter insect herbivores, an important next step would be to identify the chemical substance(s) responsible. Such identification, and the possible synthesis of these or related compounds, artificially may have huge implications in pest management research since such products could be used as 'natural' and environmentally inert alternatives to existing insecticides. However, these implications must remain speculative until further research is forthcoming on the chemical basis for the present results.

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