

## Can floral repellents pre-empt potential ant–plant conflicts?

Jaboury Ghazoul  
TH Huxley School  
of Environment,  
Earth Sciences and Engineering,  
Imperial College of Science,  
Technology and Medicine,  
Silwood Park, Ascot,  
Berkshire SL5 7PY, U.K.  
E-mail: j.ghazoul@ic.ac.uk

### Abstract

Ants, by consuming floral nectar, are potential parasites of plant–pollinator mutualisms, the persistence of which depends on mechanisms preventing ants from visiting flowers. Here I report the existence of such a mechanism which, uniquely, appears general in its effects. I show that two acacia–ant mutualists are repelled by floral tissue chemicals from their own host-plants as well as those from 13 other plant genera, only one of which associates symbiotically with ants. Furthermore, 18 of 25 ant species, from several subfamilies representing degrees of ant–plant interaction, are repelled by acacia floral chemicals. Thus floral ant repellents are widespread among plants, repel most ant species, and can prevent ants from parasitizing plant–pollinator mutualisms.

### Keywords

Acacia, ant–plant interaction, coevolution, mutualism, pollination, *Pseudomyrmex*.

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### INTRODUCTION

Ants rarely collect floral nectar (Janzen 1977; Beattie *et al.* 1984), despite its apparent palatability (Haber *et al.* 1981; Ramsey 1995) and despite the fact that many plant species provide nectar to ants from extra-floral glands (Bentley 1977; Schemske 1980; de la Fuente & Marquis 1999). Why should ants relinquish one food resource but consume another that is both similar and accessible? For plants, the question is of supreme importance because ant consumption of floral nectars can dissuade visitation by pollinators (Janzen 1977; McDade & Kinsman 1980; Willmer & Stone 1997) and, despite reported cases of ant-pollination (Peakall *et al.* 1991; Gómez & Zamora 1992; García *et al.* 1995; Gómez *et al.* 1996; Gómez 2000; Schurch *et al.* 2000), ants themselves generally make poor pollinators (Buckley 1982; Beattie *et al.* 1984; Beattie *et al.* 1985; Hull & Beattie 1988; Peakall *et al.* 1991; Ramsey 1995; Puterbaugh 1998; Altshuler 1999). Thus, ants are potential parasites of plant–pollinator mutualisms, the persistence of which depends on mechanisms to prevent ants from visiting flowers, so-called Partner-Selection mechanisms (Bull & Rice 1991; Harley 1991). Physical and chemical ant-repellents have, indeed, been described previously (Harley 1991; Davidson & McKey 1993; Federle *et al.* 1997) though almost always as species-specific adaptations (Harley 1991; Federle *et al.* 1997). Floral ant-repellents, however, should be expected in most

plants that produce floral resources to attract insect pollinators.

Floral ant-repellents should, perhaps, be most expected among plants that form mutualistic interactions with ants. Therefore, this study investigates the behaviour of *Pseudomyrmex spinicola* and *P. flavicornis* ant mutualists on flowering *Acacia collinsii* trees in Costa Rica. *Acacia collinsii* host either of the two *Pseudomyrmex* ant species in enlarged hollow thorns (Janzen 1966, 1974). The ants attack vertebrates and most invertebrates they encounter, thereby reducing herbivory on the plant (Janzen 1966, 1974). Consequently, the *Pseudomyrmex* ants potentially deter pollinators from visiting *Acacia* inflorescences, especially since ant and pollinator activities coincide. In a similar African ant–*Acacia* system, this conflict is resolved by the presence of a floral ant repelling chemical that facilitates access to flowers by pollinators (Willmer & Stone 1997).

The aim of this study was to explore the generality of the ant–plant–pollinator interaction by observing ant–acacia mutualisms in Central America. The study further intended to test the alternative hypothesis that ants are not repelled by acacia flowers, but rather promote outcrossing by minimizing time spent at flowers by pollinators at any single tree (Altshuler 1999). Finally, the effect of flowers from a range of taxonomically diverse plants on acacia–ants, as well as the behavioural response of taxonomically diverse ant species to acacia flowers, was explored to determine the taxonomic generality of these responses.

## METHODS

The study was conducted at the height of the flowering period of *A. collinsii* (Mimosoideae) in February 2000 in the dry forests of Sector Santa Rosa, Area de Conservacion Guanacaste in north-west Costa Rica. *Acacia collinsii* is a small tree that bears inflorescences in clusters at the end of twigs. Anther dehiscence occurs in the mornings and pollen remains exposed for 48 h before the inflorescence begins to wither. No nectar is produced by the flowers throughout this period. Peak pollinator activity at inflorescences is between 08:00 and 11:00, and coincides with peak patrolling activity by *P. spinicola* and *P. flavicornis* ants.

### Acacia flower ant-repellent effect

Clusters consisting of two immature (unopened) inflorescences and one newly mature inflorescence were selected to estimate visitation frequency by *P. spinicola*. One unopened inflorescence was "wiped" with a mature inflorescence from a nearby cluster and the other was wiped with an old inflorescence. Ant responses to inflorescences were scored as "visited" when an ant walked onto an inflorescence, and "repelled" if an ant approached but turned away from an inflorescence before walking onto it. Each 5-min observation period (undertaken from 09:00 to 12:00) commenced after tapping the branch several times to encourage ant activity. Observation periods were repeated at 40-min intervals after renewed wiping of the treated inflorescences. Seven clusters were used with four sets of observations made at each one.

A series of Petri-dish experiments were undertaken to assess the response of a range of ant species to *Acacia* inflorescence chemicals. These consisted of wiping one half of a 9-cm diameter Petri-dish (lid and base) with a newly opened *Acacia* inflorescence, applied using forceps, and the other half with a 3–4-day-old withering inflorescence. The diameter of the Petri-dish on the external surface of lid and base was marked where the control and treatment sides met. The halves were aligned so that the appropriate sides matched. An ant placed in the centre of the Petri-dish was given a 20-s acclimatization period. The time spent in the control area by the ant was recorded over a 300-s observation period. Mid-way through the observation period (at 150 s) the Petri-dish was rotated through 180°. A new Petri-dish was used for each trial and trials were undertaken under shade. Results were analysed by deducting the time in seconds spent in the treated side from the time spent in the control and conducting a one-tailed *t*-test for departure from the null value of zero. These trials were preceded by a series of similar experiments to test for the response of *P. spinicola* ants to unfamiliar olfactory stimuli (neophobia). In these trials one half of the Petri-dishes were

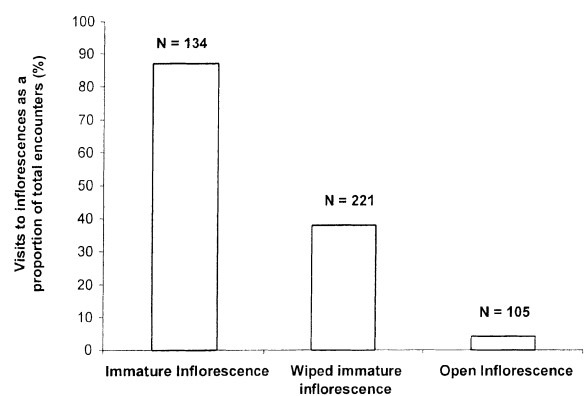
wiped with *A. collinsii* leaves, *Senna pallida* leaves, old *A. collinsii* inflorescences or bare finger tips. The other half remained untreated. In 5-min trials, ants displayed no significant preference to either half of the Petri-dish in terms of the time spent in each side except for the bare fingers treatment (mean  $\pm$  s.d., departure from expected value of zero =  $46.2 \pm 11.7$ ,  $t = 3.33$ , d.f. = 7,  $P = 0.05$ ). On the basis of these trials it was assumed that neophobia did not contribute a significantly to subsequent experimental trials as described above, and care was taken to avoid touching the inside surfaces of the Petri-dishes.

### Repellent effects of flowers of other plant species on acacia ants

The response of *P. spinicola* and *P. flavicornis* to floral chemicals of other plants, drawn from the available pool of flower-bearing plants at Santa Rosa during the period of study, was undertaken using Petri-dish trials as described above but substituting *Acacia* inflorescences with the appropriate flowers. Observation periods of 180 s were used.

## RESULTS

Visits to "wiped" buds by *P. spinicola* workers was significantly lower than to untreated floral buds ( $\chi^2 = 66.92$ , d.f. = 4,  $P < 0.001$ ), and higher than to open flowers ( $\chi^2 = 723.62$ , d.f. = 4,  $P < 0.001$ ; Fig. 1). On the rare occasions when *P. spinicola* ants did walk onto open inflorescences, their behaviour appeared aggressive, displaying increased antennation, cocked abdomen and repeated biting and stinging.



**Figure 1** Proportion of visits by *Pseudomyrmex spinicola* to *Acacia collinsii* inflorescences belonging to three experimental categories: (1) immature inflorescence wiped with an old inflorescence; (2) immature inflorescence wiped with a newly opened inflorescence and (3) open mature inflorescence wiped with an old inflorescence. *N* refers to the total number of encounters.

### Petri-dish trials: acacia ant responses to *Acacia* and non-*Acacia* flowers

In Petri-dishes *P. spinicola* and *P. flavicornis* avoided the area that had been wiped with an open *A. collinsii* inflorescence (Table 1). Moreover, ants in the treated half increased greatly the rate of antennation and speed of movement compared to the control side. Both *Pseudomyrmex* species showed similar avoidance and behavioural responses to floral extracts of 12 from 13 other plant genera (representing 12 families) tested in this manner (Table 1). Of these species, only *Cordia alliodora* harbours ant mutualists (*Azteca* spp.).

### Petri-dish trials: ant species responses to *Acacia* flowers

The responses of a further 25 ant species to *A. collinsii* floral extracts was highly nonrandom in that most species (sign test using a binomial proportion of 0.5 is significant at  $P < 0.01$ ) spent significantly more time in the control area (Table 2). Thus most ant species were clearly repelled by *Acacia* floral extract ( $n = 18$ ), others exhibited a nonsignificant tendency towards the control side of the Petri-dishes ( $n = 3$ ), and a few showed no preference at all ( $n = 4$ ). Of the species that did not significantly tend towards either side, *Crematogaster rochai*, *Azteca instabilis* and two *Cephalotes* spp., in common with all the other species for which a repellent response was recorded, displayed a heightened degree of typically aggressive behaviour in treated compared to untreated Petri-dishes, including biting, much antennation and rapid movement with cocked abdomen. Two other ants in this category, *Ectatomma ruidium* and *Atta*

*cephalotes*, that are known to regularly patrol or collect flowers (Wirth *et al.* 1997; Altshuler 1999), did not display aggressive behaviour.

### DISCUSSION

There is a potential conflict among plants and their mutualistic ant partners over access to flowers by pollinators. Among ant-acacia mutualisms so far studied, this potential conflict is rendered moot because none of the ant species patrols open flowers, although they will walk on floral buds (Fig. 1 and Willmer & Stone 1997). There is no evidence to support the promotion of outcrossing hypothesis among the ant-acacia mutualisms observed here.

However, and surprisingly, *P. spinicola* and *P. flavicornis* respond in a similar manner to floral chemicals that appear to be widely present among angiosperms, including among plant taxa that do not associate with ants. Chemicals from *A. collinsii* flowers also clearly repel many ant taxa, many of which form no symbiotic association with plants (but may consume extra-floral nectars). This suggests either that ant-repelling chemicals have evolved independently numerous times or, more parsimoniously, that they were derived early in angiosperm evolution, when ants were already present. In either case, floral ant-repellents, through the protection of floral resources from ants which are likely to contribute little to pollination (Janzen 1977; Beattie *et al.* 1985; Hull & Beattie 1988; Peakall *et al.* 1991; Altshuler 1999), appear adaptive.

Why have ants not evolved resistance to floral repellents, given that flowers represent a potentially rich resource? I

**Table 1** Repellent effect of floral extracts on *Pseudomyrmex spinicola* and *P. flavicornis* ants in Petri-dish trials. Values shown are time spent in the treatment side deducted from the time spent in the control during 300-s Petri-dish trials. The deviations of these values from 0, as predicted by the null hypothesis, were tested using one-tailed paired *t*-tests. Using the Dunn-Šidák method the experimentwise error rate of 0.05 is adjusted to a significance level of 0.0037 to minimize risk of Type II errors

Plant species (Family)	<i>Pseudomyrmex spinicola</i>			<i>Pseudomyrmex flavicornis</i>		
	<i>N</i>	Mean ± SE (s)	<i>P</i>	<i>N</i>	Mean ± SE (s)	<i>P</i>
<i>Acacia collinsii</i> (Mimosoideae)	16	184 ± 18	< 0.001	14	102 ± 18	< 0.001
<i>Ipomoea trifida</i> (Convolvulaceae)	12	171 ± 21	< 0.001	8	202 ± 13	< 0.001
<i>Cochlospermum vitifolium</i> (Cochlospermaceae)	15	163 ± 14	< 0.001	8	104 ± 10	< 0.001
<i>Senna palida</i> (Caesalpinioideae)	12	162 ± 19	< 0.001	8	123 ± 28	< 0.001
<i>Lantana camara</i> (Verbenaceae)	12	162 ± 27	< 0.001	8	161 ± 12	< 0.001
<i>Gliricidium sepium</i> (Papilionoidea)	11	159 ± 27	< 0.001	8	142 ± 28	< 0.001
<i>Trixis inula</i> (Asteraceae)	12	156 ± 33	< 0.001	8	199 ± 27	< 0.001
<i>Melanthera aspera</i> (Asteraceae)	12	148 ± 27	< 0.001	8	160 ± 18	< 0.001
<i>Cordia alliodora</i> (Boraginaceae)	12	121 ± 30	< 0.001	8	170 ± 29	< 0.001
<i>Oxalis frutescens</i> (Oxalidaceae)	8	109 ± 26	< 0.001	6	92 ± 21	0.01 (n.s.)
<i>Asclepias curassavica</i> (Asclepiadaceae)	9	109 ± 22	< 0.001	9	168 ± 25	< 0.001
<i>Ruellia inundata</i> (Acanthaceae)	8	109 ± 26	< 0.001	8	80 ± 27	< 0.001
<i>Psidium guajaba</i> (Myrtaceae)	12	92 ± 18	< 0.001	8	141 ± 29	< 0.001
<i>Hibiscus arborios</i> (Malvaceae)	12	34 ± 28	0.05 (n.s.)	8	120 ± 21	< 0.001

Species	<i>N</i>	Mean ± SE (s)	<i>P</i>
<i>Pseudomyrmex cretus</i>	17	39 ± 10	< 0.001
<i>Pseudomyrmex ita</i>	8	53 ± 8	< 0.001
<i>Pseudomyrmex gracilis</i>	13	33 ± 7	< 0.001
<i>Pseudomyrmex nigrocincta</i>	10	45 ± 11	< 0.001
<i>Camponotus mucronatus</i>	8	124 ± 10	< 0.001
<i>Camponotus sericeiventris</i>	9	85 ± 18	< 0.001
<i>Camponotus novogranadensis</i>	30	47 ± 5	< 0.001
<i>Camponotus JTL-001</i>	11	128 ± 7	< 0.001
<i>Camponotus rectangularis</i>	11	62 ± 12	< 0.001
<i>Camponotus canescens</i>	8	61 ± 11	< 0.001
<i>Camponotus atriceps</i>	8	50 ± 9	< 0.001
<i>Azteca coeruleipennis</i>	10	58 ± 12	< 0.001
<i>Azteca beltii</i>	9	50 ± 12	< 0.001
<i>Azteca instabilis</i>	9	20 ± 15	0.05 (n.s.)
<i>Cephalotes umbraculatus</i>	8	59 ± 9	< 0.001
<i>Cephalotes multispinosus</i>	8	24 ± 10	0.05 (n.s.)
<i>Cephalotes minutes</i>	8	8 ± 16	n.s.
<i>Pheidole fallax</i>	16	41 ± 11	< 0.001
<i>Pheidole</i> “ <i>kukrana</i> ” (E.O. Wilson m.s.)	8	29 ± 18	0.05 (n.s.)
<i>Pachycondyla foetida</i>	8	55 ± 15	< 0.001
<i>Ectatomma ruidum</i>	16	− 11 ± 16	n.s.
<i>Crematogaster rochai</i>	8	− 5 ± 19	n.s.
<i>Atta cephalotes</i>	16	13 ± 13	n.s.
<i>Eciton burchelli</i>	8	27 ± 7	< 0.001
<i>Dolichoderus multispinosus</i>	8	56 ± 9	< 0.001

hypothesize that these chemicals mimic ant alarm pheromones that are necessarily evolutionarily conserved and which are thought to be structurally similar across widely separated ant lineages (Blum 1982; Feener *et al.* 1996). Different concentrations of (and/or sensitivities to) alarm pheromones can produce divergent behaviours in ants, namely repulsion and aggression (Davidson *et al.* 1990) (note the aggressive behaviour of *Pseudomyrmex* on inflorescences and *Crematogaster rochai* and colleagues in Petri-dish trials). This suggests an interesting mechanistic explanation for the origin of castration behaviour in another ant–plant system (Yu & Pierce 1998), and the possible facilitation of the evolution of ant–plant interactions by pre-empting potential conflicts over pollinator access.

Given that floral chemicals are not universally repellent to ants, and that in some environments ants do frequently visit flowers for pollen and nectar resources (Gómez *et al.* 1996; Rico-Gray *et al.* 1998), further research on plant–ant interactions at flowers is needed to elucidate plant–ant–pollinator conflicts.

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**Table 2** Repellent effect of *Acacia collinsii* inflorescences on ants in Petri-dish trials. Values shown are time in the treatment side deducted from the time in the control (180-s observation period). Deviations of these values from 0, as predicted by the null hypothesis, were tested with one-tailed paired *t*-tests. Using the Dunn–Šidák method the experimentwise error rate of 0.05 is adjusted to a significance level of 0.002 to minimize the risk of Type II errors. *Camponotus* JTL-001 refers to an unidentified species in the collection of John T. Longino. *Pheidole* is under revision by E.O. Wilson and “*kukrana*” is an unpublished manuscript name

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#### BIOSKETCH

Jaboury Ghazoul is fascinated by plant–animal interactions, especially pollination. Further research interests include forest ecology and conservation, and the impacts of disturbance on ecological processes.

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