Evolutionary Ecology Research, 2008, **10**: 295–308

Floral scents repel potentially nectar-thieving ants

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ABSTRACT

Hypothesis: Repellent floral volatiles are responsible for ants' conspicuous absence from flowers.

Organisms: A broad spectrum of flowers (32 species for volatiles, 72 for nectar tests) was screened for their effect on different ant species.

Methods: The ants' responses to floral scent bouquets and to individual floral volatiles were tested in a modified Pettersson four-arm olfactometer. Flower nectar was extracted with micro-capillaries and offered to ants to observe whether they accepted or rejected the nectar.

Results: While ants readily consumed almost all nectar offered to them, they were significantly repelled by a high proportion of floral scent bouquets. Repellent effects were confirmed for individual terpenoids commonly found in floral scents. Repellent floral scents provided an unequivocal explanation for the distribution of *Formica rufibarbis* ants among nectar-bearing flowers *in situ*.

Conclusions: This is the first clear demonstration that ant repellence from flowers is triggered by naturally emitted floral volatiles. This suggests that floral scents may function as allomones against enemies and not solely as synomones to attract mutualists.

Keywords: allomones, antagonists, floral traits, mutualism exploitation, nectar, olfactometer, repellence, terpenoids.

INTRODUCTION

While flower–pollinator interactions have been described extensively for centuries (Sprengel, 1793; Darwin, 1862; Waser, 2006), floral defence mechanisms have received much less attention. Suggested mechanisms for protecting flowers against antagonists include physical barriers, such as narrow corolla tubes that exclude larger non-pollinating insects from nectar glands (Pijl, 1955) or sticky stems of inflorescences (Harley, 1991), and chemical defences such as toxic nectar (Adler, 2000) and deterrent pollen (Dobson and Bergström, 2000). Some case studies have shown that defensive substances are emitted by flowers to deter unwanted flower visitors (e.g. Euler and Baldwin, 1996).

Protection of flowers against antagonists may be just as important for the reproductive success of plants as effective pollination, since individual enemies can affect plant

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performance to a greater extent than mutualists (Morris *et al.*, 2007). Animal-pollinated flowers thus face a dilemma: they need to attract pollinators, but they simultaneously profit from repelling antagonists (Irwin *et al.*, 2004) such as ants. Ants are often detrimental for pollination. On myrmecophilous plants, ants may not only deter herbivores and thus function as bodyguards for the plant, but also deter pollinators (Ness, 2006). However, this negative effect on pollination is not restricted to myrmecophilous plants: facultative flower-visiting and nectar-thieving ants decrease pollinator visitation frequency and duration either by attacking approaching pollinators (Tsuji *et al.*, 2004; Junker *et al.*, 2007) or due to exploitative consumption of nectar (Galen and Geib, 2007). These disturbances of pollinator visits may translate into reduced seed set (Galen, 1983). Ants are often unable to pollinate flowers due to their small-scale foraging (Hölldobler and Wilson, 1990), their smooth body surface or their glandular secretions that can severely reduce pollen fertility (Beattie *et al.*, 1984, 1985). Floral traits may therefore be selected for both their attractive and repellent functions, especially during anthesis, when pollinators are required but antagonists decrease reproductive success. In contrast, some former antagonists such as ants may be beneficial for pollinated flowers or unripe fruits, where they may protect seeds (Willmer and Stone, 1997).

In general, ant deterrence from flowers is unlikely to be due to unpalatable or even toxic floral nectar, a hypothesis proposed 30 years ago (Janzen, 1977) and repeatedly tested since then (Feinsinger and Swarm, 1978; Guerrant and Fiedler, 1981; Haber *et al.*, 1981; Koptur and Truong, 1998; Blüthgen *et al.*, 2004). However, the nectar of some plants contains substances that deter ants and other flower visitors (Adler, 2000; Kessler and Baldwin, 2006). Some studies have suggested that flower scent may repel ants, but their methodological procedures have not allowed them to demonstrate whether naturally emitted floral scents trigger this response. Studies did not discriminate between responses resulting from volatile and non-volatile substances, and/or did not establish whether effective substances represent natural signals or are merely wound saps or products of macerated floral tissue (Guerrant and Fiedler, 1981; Willmer and Stone, 1997; Ghazoul, 2001; Junker *et al.*, 2007).

In this study, we compared ants' responses to scents of regularly visited flowers with those of unvisited flowers, using a modified four-arm olfactometer. We also screened the potential ant-repellent function of floral scents from 30 additional, haphazardly chosen plant species. The olfactometer allowed us to clearly isolate effects triggered by naturally emitted floral volatiles, and to exclude responses that require contact chemoreception. Additionally, we offered floral nectars from 72 plant species to ants to test the general assumption that floral nectar is readily consumed by ants.

METHODS

Olfactory tests

Olfactometer set-up

In the modified Pettersson olfactometer (Fig. 1) used in this study, four distinct odour fields can be created in a four-pointed star-shaped arena (Fig. 1, A) in which ants can move and respond to different odours. The shape and function of the four-armed arena was developed by Pettersson (1970), who studied the sex pheromones of aphids. The whole set-up was modified by Vet *et al.* (1983), and the design was again adjusted to meet the requirements for this study. The arena of the olfactometer was manufactured from a single aluminium plate

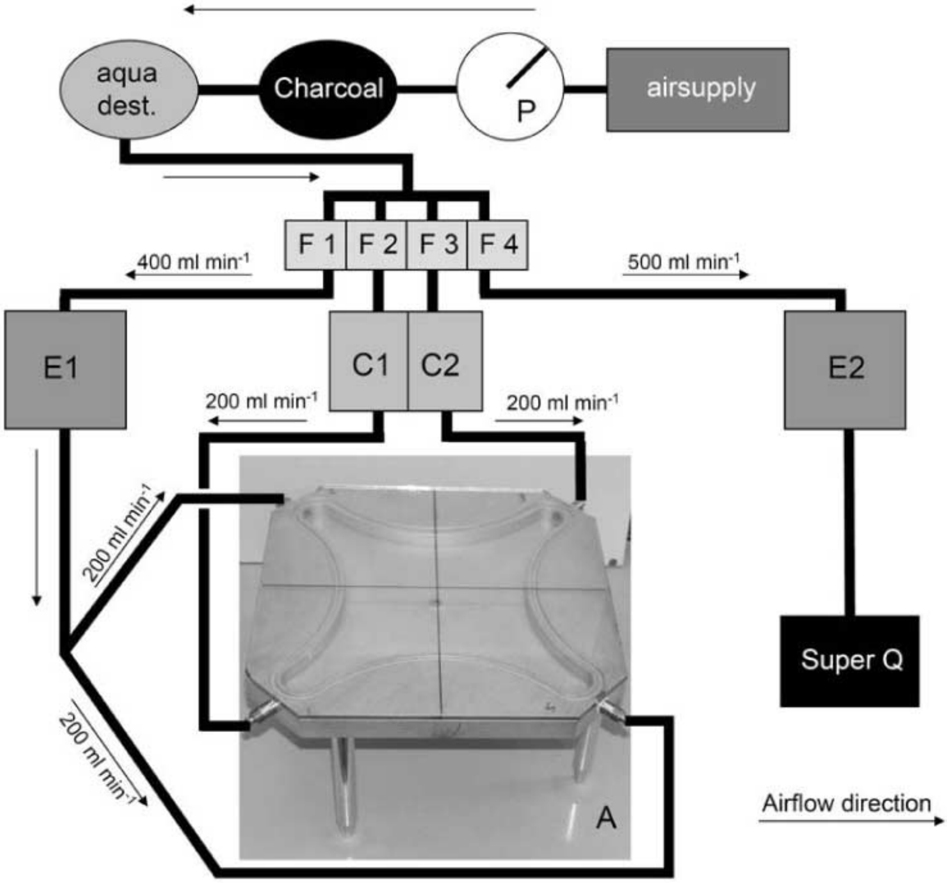


Fig. 1. Olfactometer set-up. Four distinct fields of odour were created in the arena (A), into which air is supplied from the four tips and drains off the central hole. P = pressure reducer, F = flowmeter, E = exsiccators for applying odour source, C = control chamber (washing flasks), A = arena, Super Q = volatile trap. Black lines are silicone tubes (prior to exsiccators and control chambers) or Teflon tubes (after exsiccators and control chambers).

(height 350 mm, length 350 mm, depth 20 mm) using a computer-controlled milling machine, resulting in a four-pointed, star-shaped (90° arc) flat chamber with a depth of 15 mm. A glass plate served as the top cover of the arena. The central hole allowed the air to drain off the arena. Each arm of the arena was connected to an air and odour supply. Holes for aerial inflow and outflow were obstructed with metal sieves to prevent ants from crawling out. The symmetry and the equal air flow into all corners of the arena maintained four distinct and homogenous odour fields with sharp boundaries (Vet *et al.*, 1983). Separate flow meters (Analyt, Müllheim, Germany) controlled the flow rate (Fig. 1, F1–F3) so that each field in the central arena received a flow of 200 ml $\cdot$ min^{-1}. Several flowers (Experiments a and b) in a vase, or pure substances (Experiment c), were placed into an exsiccator (Fig. 1, E1). Purified (Fig. 1, Charcoal) and moistened (Fig. 1, aqua dest.) air transported the emitted odours to two opposite arms of the arena. The remaining two arms were supplied by odourless air ($200\ \text{ml} \cdot \text{min}^{-1}$) to serve as a control (Fig. 1, C1 and C2). Injured plant parts (cut stalks) were placed under water to minimize contamination of floral scents with volatiles emitted from wounds. We minimized the vegetative plant parts involved. A strong neon lamp illuminated the arena equally. [Technical details are provided in Supplementary Appendix B: evolutionary-ecology.com/data/2248app2.pdf.]

Ten ant workers were simultaneously placed into the arena, and the number of ants in the odour and control fields was counted after 2, 3, 4, and 5 min. Subsequently, the treatments of the fields were switched (odour fields were transformed to control fields and vice versa) and the number of ants was counted again for a further 5 min. This procedure was repeated four to six times with different ant workers, producing a total of eight to twelve replicate trials per plant species. Each trial i was represented by a single value d_i, i.e. the mean difference in the number of ants for the 5-min period (d_i = mean number of ants in control fields – mean number in odour fields). Repellence of a floral scent was indicated by a significantly lower mean number of ants in odour fields than in control fields across trials. For chemical analysis of flower odours, an additional flow meter (Fig. 1, F4) was installed supplying a second exsiccator (Fig. 1, E2) with a flow of $500\ \text{ml} \cdot \text{min}^{-1}$. A volatile trap (Fig. 1, Alltech Super Q, Gainesville, USA) was attached to the second exsiccator.

Three experiments were carried out to determine whether floral scents are ant repellent:

(a) *In situ observations*. On a fallow field near the Biozentrum (University of Würzburg), we observed ants from a colony of *Formica rufibarbis* Fabricius (Formicinae) exploiting the nectar of *Daucus carota* and *Pastinaca sativa* (both Apiaceae). Flowers of other plant species (*Saponaria vulgaris* and *Silene alba*, both Caryophyllaceae) remained unvisited by these ants. This was despite the fact that they were growing near the ant nest, close to the visited *D. carota* and *P. sativa*, and also produced accessible nectar. Vases with a pair of visited and unvisited inflorescences (*D. carota* vs. *S. vulgaris*, *P. sativa* vs. *S. alba*) were placed in close proximity to the entrance to the ants' nest to determine whether *S. vulgaris* and *S. alba* still remained unvisited as compared to the other plants over several hours. Nectar was extracted from these two plant species using micro-capillaries and offered to the ants. Furthermore, olfactometer trials (see below) were conducted with workers from the same colony and flowers obtained from the field. All tests were started immediately after picking the flowers and ended 1½ h later.

(b) *Screening for ant-repellent floral bouquets*. To examine the distribution of ant-repellent floral volatiles, we tested a broad spectrum of plant species and reactions by three ant species that originated from different regions. Ant and plant species were thus unlikely to encounter each other *in situ* in contrast to the scenario described above (a). The ants' responses to floral scent from 30 plant species of 23 families were tested in the four-arm olfactometer in the same way as above. Three different colonies each of *Camponotus floridanus* Buckley and of *Lasius fuliginosus* Latreille (both Formicinae) ants were used for each test; the former were obtained from Florida and raised in the laboratory and the latter were collected in Würzburg prior to the study. Both ant species are known to consume large amounts of plant-based sugary solutions like honeydew and/or extrafloral nectar (Quinet and Pasteels, 1996; Dreisig, 2000). Some additional tests were conducted with *Monomorium floricola* Jerdon ants (Myrmicinae) collected in Borneo. The flowers used for the tests were picked in the Würzburg Botanical Garden or at the Biozentrum in the morning (usually 08:30 h). Native as well as exotic plants from nearly all continents and several climatic zones were included in the tests. Stalks with flowers and/or inflorescences from the botanical garden were transported in unused plastic bags and then stored in vases approximately 20 min later. All tests were started immediately after placing the flowers in vases and ended at the most 5 h after the flowers were picked. In addition to the tests with flowers, we examined the ants' responses to the scents of *Kolkwitzia amabilis* (Caprifoliaceae) fruits (75 fruits were placed in the exsiccator), the leaves of *Silene alba* (Caryophyllaceae) (10 stems), and the nectar of *Agave ferox* (Agavaceae) (8 ml).

(c) *Screening for ant-repellent floral scent compounds*. Eight commercially available pure substances were tested that commonly occurred in our samples of floral scents and elsewhere (Knudsen *et al.*, 2006). The substances were applied in the same concentration as they typically occurred in our flower samples that were used in the tests (b) and analysed by coupled gas chromatography and mass spectrometry (GC-MS), ranging between 15 and 5000 $\text{ng} \cdot \text{h}^{-1}$. The 0.75 quartile across different plant species was used if the substance occurred in more than one sample. The emission rates were obtained by diluting pure substances in liquid Paraffin (Uvasol®, Merck, Darmstadt, Germany; for further information see Appendix B: evolutionary-ecology.com/data/2248app2.pdf). To test potential additive effects, substances that were not repellent themselves for *C. floridanus* (benzaldehyde, β-caryophyllene, 1,8-cineol, limonene, and β-pinene) were mixed together in the same concentration as in previous individual tests.

Statistical analysis

To test the specific prediction regarding the response by *F. rufibarbis* towards each of four flower species (Experiment a), a one-sample *t*-test was performed for each plant species (null hypothesis: $\bar{d}_i = 0$, i.e. on average 5 : 5 ants in control vs. odour fields). Results from the four plant species were corrected for multiple tests by false discovery rate (Benjamini and Hochberg, 1995). For screening for potentially ant-repellent flower scents (Experiments b and c), a two-way analysis of variance (ANOVA) was carried out, with ant and plant species or substances as explanatory variables and $\bar{d}_i$ as the response variable. Subsequently, a one-way ANOVA for each ant species was performed with flowers as the explanatory variable and $\bar{d}_i$ as the response variable.

Foraging tests

Because in the above tests with the olfactometer no food was offered to the ants, it is questionable whether the results reflect foraging decisions of ants in a natural environment. To examine whether ants avoid resources that are surrounded by floral scents, the following test was conducted with two exemplary substances: two honey droplets were placed on a filter paper (diameter 9 mm). One droplet was surrounded by liquid paraffin (25 μl, Uvasol®) and the other by the same amount of paraffin containing linalool (1 : 257 v/v) or β-caryophyllene (1 : 31 v/v), matching the emissions of flower samples in the olfactometer. The filter paper was positioned on top of a column which was then placed inside a nest of *C. floridanus*. In the following 10 min, the number of ants feeding on the honey was counted every minute. A paired *t*-test was performed to determine whether choices are consistent across six different colonies of *C. floridanus*; for each colony, the mean number of ants sucking honey and those in the control over the 10-min period was used.

Acceptance of floral nectar

Nectar was extracted with micro-capillaries in the Würzburg Botanical Garden in the morning from flowers of 72 plant species comprising 37 families (including 11 species tested in the olfactometer) native to various continents. Droplets were offered immediately after extraction on object slides to one or two ant species (*C. floridanus*, *Lasius niger* or *Myrmica rubra*) [see Appendix A, Table A1: evolutionary-ecology.com/data/2248app1.pdf]. The ants' reactions when encountering the nectar droplets for the first time were observed and distinguished as: (a) *acceptance* – ants immediately consumed nectar; (b) *neutral* – ants consumed little amounts of nectar or ignored it; or (c) *deterrence* – ants abandoned nectar immediately after antennating it. For those plant species where nectar appeared to deter ants (c), this effect was examined in greater detail as a dual-choice test between the floral nectar and a sucrose solution of the same amount and concentration (measured with a hand-held refractometer). Both droplets were offered pair-wise on a glass slide, and five replicates were used for different ant colonies. Ants were counted five times in 2 min, and the mean value per trial was used for statistical analysis (paired *t*-tests).

RESULTS

Olfactory tests

(a) *In situ observations*

Field observations of *Formica rufibarbis* ants, their visits to flowers placed in vases, and olfactometer trials were all consistent: nectar from *Saponaria vulgaris* and *Silene alba* was neither exploited by ants on flowers in the field nor in the vase next to their nest. Extracted nectar from both species was readily consumed by the ants. The olfactometer trials confirmed the hypothesis that floral scents are responsible for the ants' avoidance of the flowers of these species: *F. rufibarbis* ants avoided odour fields in the olfactometer in both trials ($t_{11} \geq 3.2$, $P < 0.01$; Fig. 2). Nectar from *Daucus carota* and *Pastinaca sativa* was readily consumed by the ants in the field, usually by several workers per inflorescence at the same time, and flowers offered in the vase were also highly attended. Correspondingly, floral scents did not affect the ants in the olfactometer ($t_{11} \leq 1.2$, $P > 0.27$; Fig. 2).

(b) *Screening for ant-repellent floral bouquets*

Camponotus floridanus was repelled from 20 of 30 plant species, *L. fuliginosus* from 8 of 26; 95% confidence intervals (95% CI; Fig. 3) do not include zero in these cases. Responses of ant species on floral scents differed significantly (Table 1), a result that might partly be explained by the behaviour displayed by each species: *C. floridanus* ants moved slowly within the arena apparently perceiving the boundaries between the odour fields, whereas *L. fuliginosus* moved much faster. The total emissions of volatiles, which ranged from 4.4 $\text{ng} \cdot \text{h}^{-1}$ in *Catharanthus roseus* to 1.2×10^5 $\text{ng} \cdot \text{h}^{-1}$ in *Murraya paniculata*, did not correlate with $\bar{d}_i$ for *C. floridanus* (Spearman's $r_S = -0.01$, $P = 0.96$) and *L. fuliginosus* ($r_S = 0.23$, $P = 0.26$). This indicates that the specific bouquet rather than the concentration of volatiles is the important factor in the repellent effect. The fruits of *Kolkwitzia amabilis*, the leaves of *Silene alba*, and the nectar of *Agave ferox* were tested in the same way as flowers. Whereas flowers from all three species repelled *C. floridanus* (Fig. 3), the fruits, leaves, and nectar

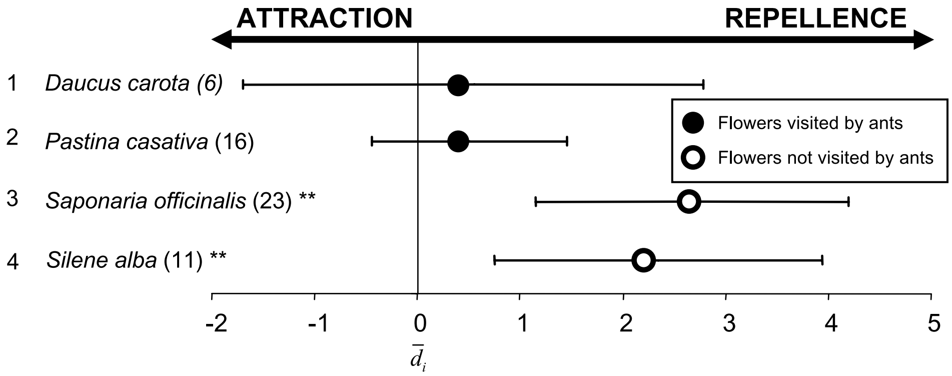
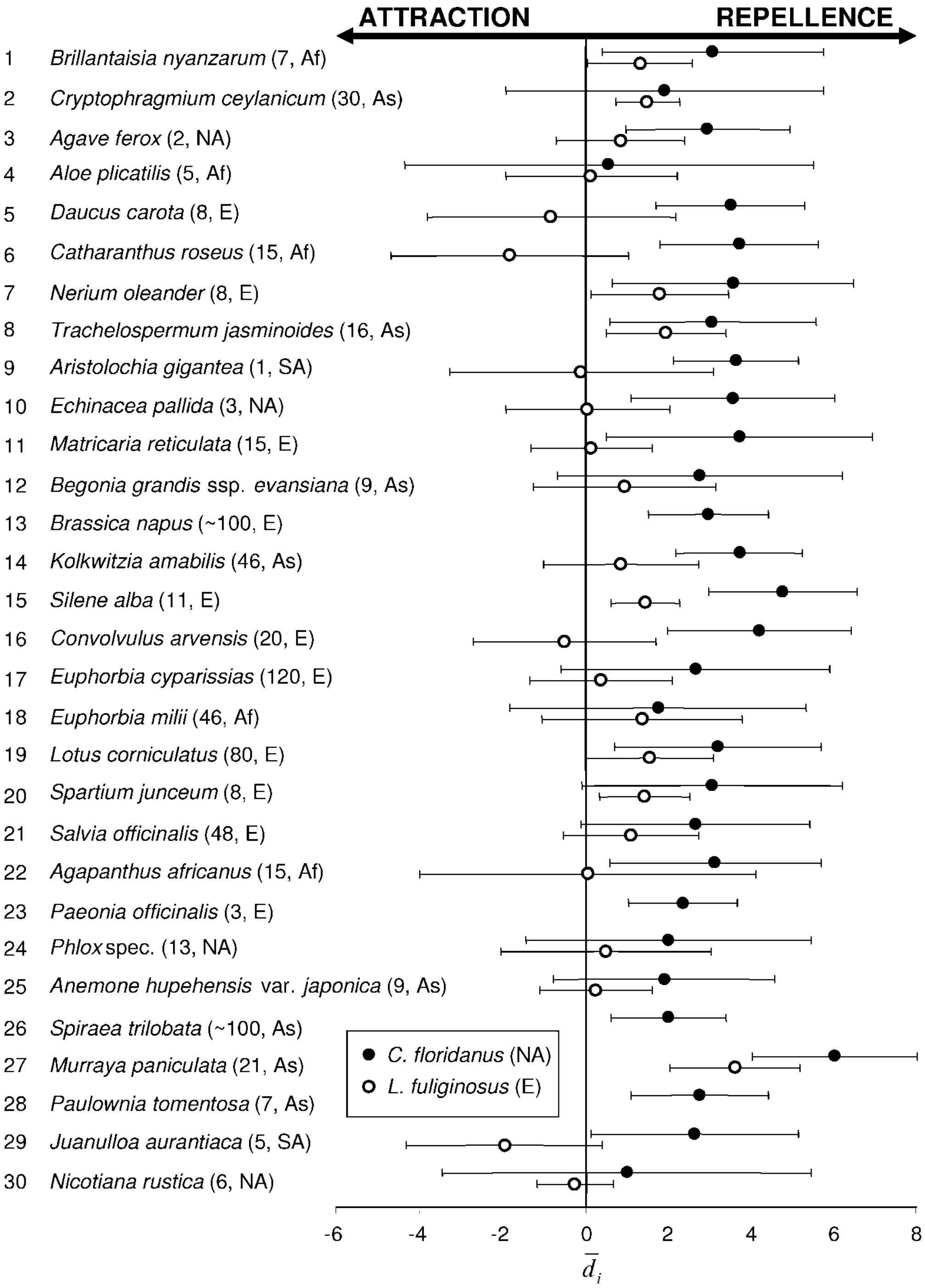


Fig. 2. Results of olfactometer tests with floral scent bouquets. Floral volatiles from four plant species were tested to establish whether they are repellent for the ant species *Formica rufibarbis*. Ants and plants co-occurred within a small area in a fallow field. Mean of differences ($\bar{d}_i$ = number of ants in control fields – number of ants in odour fields) and 95% confidence intervals are shown. Significant repellence marked with asterisks (**$P < 0.01$); significant results remained unaffected when corrected for false discovery rate. The numbers of flowers used for the tests are given in parentheses. Plants are from two plant families: Apiaceae (plant number: 1, 2); Caryophyllaceae (3, 4).

Table 1. Results of ANOVA for olfactometer tests with ant species and flowers or pure substances as explanatory variables and the mean difference (ants in control fields – ants in odour fields) as the response variable

	d.f.	*F*	*P*
Flower			
Ant	1	68.647	<0.001
Flower	29	1.336	0.117
Ant × flower	25	0.863	0.659
Camponotus floridanus			
Flower	29	0.772	0.795
Lasius fuliginosus			
Flower	25	1.833	0.012
Substance			
Ant	1	3.126	0.080
Substance	7	1.913	0.074
Ant × substance	7	1.080	0.381

were not ant-repellent ($t_9 \leq 1$, $P \geq 0.35$). The total number of substances and their overall concentration in the set-up was higher in *S. alba* leaves, but lower in *K. amabilis* fruits and *A. ferox* nectar, compared with the respective flowers. For two plant species, the repellent effect of floral scent was confirmed by a third ant species, *Monomorium floricola* (*Nerium*


ATTRACTION
REPELLENCE
1 *Brillantaisia nyanzarum* (7, Af)
2 *Cryptophragmium ceylanicum* (30, As)
3 *Agave ferox* (2, NA)
4 *Aloe plicatilis* (5, Af)
5 *Daucus carota* (8, E)
6 *Catharanthus roseus* (15, Af)
7 *Nerium oleander* (8, E)
8 *Trachelospermum jasminoides* (16, As)
9 *Aristolochia gigantea* (1, SA)
10 *Echinacea pallida* (3, NA)
11 *Matricaria reticulata* (15, E)
12 *Begonia grandis* ssp. *evansiana* (9, As)
13 *Brassica napus* (~100, E)
14 *Kolkwitzia amabilis* (46, As)
15 *Silene alba* (11, E)
16 *Convolvulus arvensis* (20, E)
17 *Euphorbia cyparissias* (120, E)
18 *Euphorbia milii* (46, Af)
19 *Lotus corniculatus* (80, E)
20 *Spartium junceum* (8, E)
21 *Salvia officinalis* (48, E)
22 *Agapanthus africanus* (15, Af)
23 *Paeonia officinalis* (3, E)
24 *Phlox* spec. (13, NA)
25 *Anemone hupehensis* var. *japonica* (9, As)
26 *Spiraea trilobata* (~100, As)
27 *Murraya paniculata* (21, As)
28 *Paulownia tomentosa* (7, As)
29 *Juanulloa aurantiaca* (5, SA)
30 *Nicotiana rustica* (6, NA)
● *C. floridanus* (NA)
○ *L. fuliginosus* (E)
-6
-4
-2
0
2
4
6
8
$\bar{d}_i$

oleander: mean of difference ($\bar{d}_i$) = 3.3, 95% CI = 1.22–5.47; *Trachelospermum jasminoides:* $\bar{d}_i = 2.1$, 95% CI = 0.03–4.07, both Apocynaceae).

(c) *Screening for ant-repellent floral scent compounds*

The ant-repellent effect was confirmed for some pure substances that commonly occur in floral scents including the plants in our study (Table 1); these were applied in the same concentrations found in flowers. According to the 95% confidence intervals (Fig. 4), linalool, geraniol, and α-pinene repelled *C. floridanus*, whereas linalool and limonene repelled *L. fuliginosus*. Linalool also repelled *Monomorium floricola* ($\bar{d}_i = 3.6$, 95% CI = 0.99–6.20). The additive mixture of five substances (each of which had a non-significant effect when applied alone) repelled *C. floridanus* (Fig. 4).

Foraging tests

In the foraging experiment, significantly fewer *C. floridanus* ants fed on honey baits that were surrounded by the scent of linalool compared with a scentless control ($t_5 = 3.1$, $P = 0.03$). As expected from the olfactometer bioassay, ants did not discriminate between baits surrounded by β-caryophyllene and odourless baits ($t_5 = 1.9$, $P = 0.11$).

Acceptance of floral nectar

Whereas ants were repelled from floral scent, they readily consumed nectar when it was extracted from a flower and offered to foraging ants. Of the 72 plant species examined, the flower nectar of most ($n = 64$) was readily taken up by the ants. In four of the five nectars that appeared to deter ants in the preliminary analysis, this effect was not found to be consistent in a more detailed examination (all $t_4 \leq 1.8$, $P \geq 0.13$). Only the nectar of *Nerium oleander* was consumed by significantly fewer ants than a sugar solution in the same amount and concentration ($t_4 = 3.9$, $P = 0.02$). The ants' acceptance of extracted flower nectar is shown in detail online (see Appendix A, Table A1: evolutionary-ecology.com/data/2248app1.pdf).

DISCUSSION

Our results confirm that nectar *per se* rarely deters ants (Feinsinger and Swarm, 1978; Guerrant and Fiedler, 1981; Haber *et al.*, 1981; Koptur and Truong, 1998; Blüthgen *et al.*, 2004) and although certain defensive substances may be dissolved in floral nectar (Stephenson, 1981; Koptur and Truong, 1998; Adler,

Fig. 3. Results of olfactometer tests with floral scent bouquets. Floral volatiles from 30 plant species were tested to establish whether they are repellent for the ant species *Camponotus floridanus* and *Lasius fuliginosus*. Mean of differences ($\bar{d}_i$ = number of ants in control fields – number of ants in odour fields) and 95% confidence intervals are shown. Number of flowers and native origin (**Af**rica, **As**ia, **E**urope, **N**orth **A**merica, and **S**outh-**A**merica) of plants and ants used for the tests are given in parentheses. Plants are from 22 plant families: Acanthaceae (plant number: 1, 2); Agavaceae (3); Aloaceae (4); Apiaceae (5); Apocynaceae (6–8); Aristolochiaceae (9); Asteraceae (10, 11); Begoniaceae (12); Brassicaceae (13); Caprifoliaceae (14); Caryophyllaceae (15); Convolvuaceae (16); Euphorbiaceae (17, 18); Fabaceae (19, 20); Lamiaceae (21); Liliaceae (22); Paeoniaceae (23); Polemoniaceae (24); Ranunculaceae (25); Rosaceae (26); Rutaceae (27); Scrophularniaceae (28); Solanaceae (29, 30).

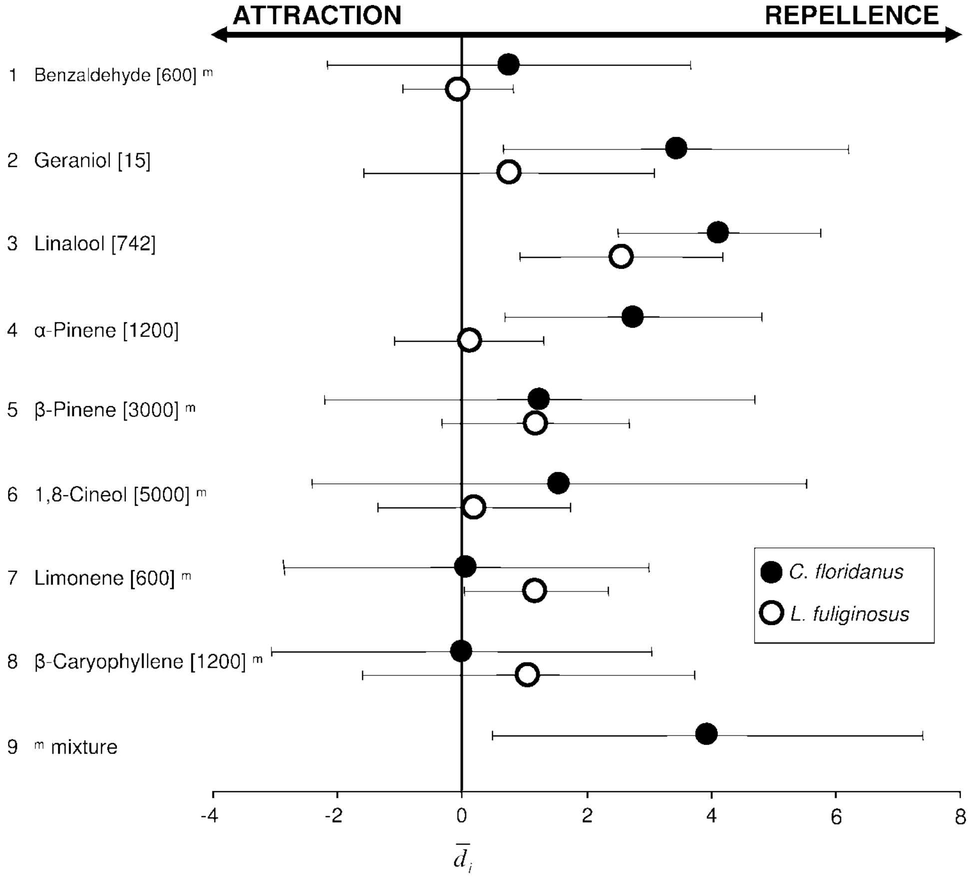


Fig. 4. Results of olfactometer tests with individual substances commonly occurring in floral scent compositions: Pure substances (benzenoides, number 1; monoterpenes, 2–7; sesquiterpenes, 8) dissolved in liquid paraffin were tested to establish whether they are repellent for the ant species *Camponotus floridanus* and *Lasius fuliginosus*. Mean of differences ($\bar{d}_i$ = number of ants in control fields – number of ants in odour fields) and 95% confidence intervals are shown. Emissions (ng·h^{-1}) are given in square brackets.

2000; Blüthgen *et al.*, 2004; Raguso, 2004; Kessler and Baldwin, 2006), they may either be rare or occur in concentrations that are insufficient to keep ants from consuming nectar.

The notion of floral defences against ants dates back to the work of Stäger (1931) and van der Pijl (1955). More recently, several bioassays were used to demonstrate the deterrent effect of floral tissues. This was done by smearing flowers on surfaces, ant trails or other plant parts (Willmer and Stone, 1997; Ghazoul, 2001; Raine *et al.*, 2002; Jaffé *et al.*, 2003) or directly observing the ants' reaction when encountering flowers or flower parts (van der Pijl, 1955; Junker *et al.*, 2007). Some of these studies suggested, but did not show empirically, that floral volatiles may be responsible for the repellent effect. Galen (1983) used an olfactory bioassay to examine choices of ants between two flower morphs of *Polemonium viscosum*, and ants were shown to have negative effects on plant reproduction.

Our finding that ant-repellent floral bouquets are widespread suggests that the composition of floral volatiles might not solely be due to their attractive function for pollinators, but also their repellent function against antagonists. The repellent effect of allomones, as demonstrated by olfactometer trials, provided an unequivocal explanation for the ants' distribution among plant species in the field: the odour of unvisited flowers repelled *Formica rufibarbis*, although their nectar was palatable to them, while the ants did not dicriminate between the scent of visited flowers and neutral air.

From the plant's perspective, ants are only detrimental to flowers during anthesis, while they may benefit plants by defending buds or unripe fruits from herbivores or parasites (Willmer and Stone, 1997). Consequently, some repellent functions may be specific to open flowers. Accordingly, while the odour of fruits of *K. amabilis* did not evoke a repellent effect in *C. floridanus* ants, the flowers were significantly repellent. It is known that scent compositions vary during the developmental stages of flowers (Schiestl *et al.*, 1997; Irwin and Dorsett, 2002; Verdonk *et al.*, 2003). However, the differences of scent bouquets between flowers and fruits and the consequences for interactions with other organisms have not been previously examined. Ant-repellence is not universal among plants and may not affect all ants. In some cases, ants may not be detrimental, for example they may contribute to the pollination of *Euphorbia cyparissias* (Schürch *et al.*, 2000), which did not repel ants in our experiment. Moreover, not all substances [benzaldehyde, 1,8-cineol, β-pinene, and β-caryophyllene (see also Kessler and Baldwin, 2006)] and not all plant parts were ant-repellent in our bioassay, indicating that 'neophobia' against all unfamiliar volatiles is unlikely to explain the ants' responses.

Some compounds both attract pollinators and repel ants. For instance, linalool in concentrations as they occurred in our samples was highly repellent to all three ant species tested, but clearly attracts several pollinators such as butterflies (Andersson *et al.*, 2002) and honeybees (Laloi *et al.*, 2000). When honeybees were placed in the same olfactometer as used for the ants, they did not discriminate between odour and neutral fields in experiments with several floral scent bouquets and pure substances (R.R. Junker, unpublished data). However, note that this olfactometer may be largely unsuitable for flying insects. We never observed a putative attraction to a given odour, either in trials with ants or with honeybees. Since we used unrewarded stimuli in the olfactometer trials, our set-up may in general be inappropriate for detecting attraction.

In contexts other than flower ecology, several mono- and sesquiterpenoids are known to repel a broad spectrum of insects from different orders, for some of which they have toxic effects (Harrewijn *et al.*, 1995; Gershenzon and Dudareva, 2007). The fact that terpenoids and other compounds are repellent against non-adapted animals suggests an interesting analogy to the theory of plant defences against herbivores: specialized herbivores are typically better adapted than generalists to overcome the defensive chemistry of their host plants, and secondarily utilize these as signals for host plant detection (Jaenike, 1990). Pollinators such as bees usually represent obligate, and often more specialized, consumers of floral resources, and could be better adapted to floral volatiles that may have evolved as a defence against more generalized antagonists. In fact, associative learning is important in the foraging decisions of various insects and is easily triggered by any volatile substance across a broad chemical spectrum (e.g. Laloi *et al.*, 2000). Since omnivorous ants do not depend on floral resources, their ability to overcome the initial repellence of floral volatiles or even to utilize them as signals may be less pronounced. In conjunction with recent case studies (Irwin *et al.*, 2004; Kessler and Baldwin, 2006) and reviews (Raguso, 2004; Morris *et al.*, 2007), our finding suggests that enemies may be powerful selective agents for traits that were once thought to be shaped chiefly by co-evolution between mutualists.

ACKNOWLEDGEMENTS

We thank S. Dötterl, A. Endler, K.E. Linsenmair, C. Murray, T. Schmitt, and M. Wendt for helpful comments, H. Feldhaar, O. Frank, A. Hilpert, T. Meiners, M. Rostás, and N. Schneider for valuable hints or technical support. The Würzburg Botanical Garden kindly provided the variety of plants. The project was supported by the Sonderforschungsbereich 'Mechanisms and Evolution of Arthropod Behaviour' (SFB-554) of the German Research Foundation (DFG). R.R.J. was supported by a scholarship provided by the 'Evangelisches Studienwerk e.V. Villigst'.

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