PLANT ANIMAL INTERACTIONS

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Conflict resolution in an ant–plant interaction: *Acacia constricta* traits reduce ant costs to reproduction

Abstract Many plant species attract ants onto their foliage with food rewards or nesting space. However, ants can interfere with plant reproduction when they visit flowers. This study tests whether *Acacia constricta* separates visiting ant species temporally or spatially from newly opened inflorescences and pollinators. The diurnal activity patterns of ants and *A. constricta* pollinators peaked at different times of day, and the activity of pollinators followed the daily dehiscence of *A. constricta* inflorescences. In addition to being largely temporally separated, ants rarely visited open inflorescences. A floral ant repellent contributes to the spatial separation of ants and inflorescences. In a field experiment, ants of four species were given equal access to inflorescences in different developmental stages. On average, the frequency with which ants made initial, antennal contact with the floral stages did not differ, but ants significantly avoided secondary contact with newly opened inflorescences relative to buds and old inflorescences, and old inflorescences relative to buds. Ants also avoided contact with pollen alone, indicating that pollen is at least one source of the repellent. The results suggest *A. constricta* has effectively resolved the potential conflict between visiting ants and plant reproduction.

Keywords *Acacia* · Ant repellent · Conflicts · Mutualism · Pollination

Introduction

All interspecific interactions involve conflicts of interest between the species involved. The resolution of the conflict defines whether the relationship is mutualistic or

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antagonistic (Bronstein 2001). Ant-associated plants are in such a conflict with their visiting ants. Ants can increase plant fitness by defending against herbivores, pruning encroaching vegetation, reducing fungal and bacterial growth, and increasing soil nutrients (Janzen 1966; Bentley 1977; Beattie et al. 1985, 1986; Madden and Young 1992; Davidson and McKey 1993; Wagner 1997; Letourneau 1998; Stapley 1998; Sagers et al. 2000; Fischer et al. 2003). Yet, ants can also have negative impacts on components of plant fitness by both reducing pollinator visitation and rendering pollen inviable. Ants can discourage pollinator visitation indirectly, by robbing nectar or simply being on the plant, and directly, by chasing or attacking pollinators (McDade and Kinsman 1980; Normant 1988; Buys 1990; Galen 1999). Furthermore, most ant species secrete an antibiotic substance from the metapleural gland onto the integument that reduces pollen viability (Beattie et al. 1984, 1985, 1986). Plant species in which ant visitation is common possess a variety of mechanisms, often morphological or chemical, that reduce the cost of ants to plant reproduction (Feinsinger and Swarm 1978; Guerrant and Fiedler 1981; Harley 1991; Federle et al 1997; Willmer and Stone 1997; Galen 1999; Ghazoul 2001; Raine et al. 2002; Wagner and Kay 2002).

Much of our understanding of ant–plant-pollinator interactions comes from the genus *Acacia* (subfamily, Mimosideae; family, Fabaceae). Acacias attract ants onto the foliage with extrafloral nectaries (EFNs), nectar-secreting glands found on leaves. In some species swollen thorns (nesting space) and protein-rich Beltian bodies further attract ants. Many acacias require outcrossing by pollen vectors (Kenrick and Knox 1989; Kenrick 2003), setting up a potential conflict between the ants and pollen vectors. Additionally, *Acacia* species are pollinator generalists (Bernhardt 1989); their stamens and stigmas are exposed to anything that lands or crawls over the flowers, making acacia pollen particularly susceptible to the metapleural secretions of ants. Acacias, as well as other plants with exposed stamens, have likely evolved non-morphological mechanisms to

resolve the potential conflict among ants, flower visitors, and pollen.

In this study we investigate mechanisms that may reduce conflicts between *Acacia constricta* and visiting ants, which can have harmful effects on plant reproduction. *A. constricta* plants associated with the ant, *Formica perpilosa*, set more seeds than plants not associated with ants (Wagner 1997). However, *F. perpilosa* ants reduce pollen viability when they contact flowers (Wagner 2000). This study tests the hypothesis that *A. constricta* segregates four common ant species from flowers and pollinators by presenting pollen at periods of low ant activity and by producing a floral ant repellent. We also test the hypothesis that pollen is the source of the ant repellent.

Materials and methods

Study system and species

The study was conducted 5 km northeast of Portal, Arizona, at an intersection of the Chihuahuan and Sonoran deserts (31°54′01″N, 109°05′26″W). Vegetation at the site was dominated by *Prosopis juliflora* (mesquite) and *A. constricta*. Tests were conducted from mid July to mid September 2004.

A. constricta is a deciduous shrub that produces leaves and inflorescences following heavy rains that typically occur in July and August. Flowering typically ceases in late September or October, and seeds ripen by late October or November. Inflorescences are yellow, spherical, largely self-incompatible (Wagner 2000), about 10 mm in diameter, and contain 25–80 flowers each. Flowers within an inflorescence open virtually simultaneously and produce no detectable nectar. Pollen is presented in polyads, with 16 grains per polyad. Leaves bear EFNs along the rachis.

A. constricta is associated with the ant, *F. perpilosa*. *F. perpilosa* feeds on nectar secreted by *A. constricta*'s EFNs as well as tended caterpillars and homopterans. At the study site, *F. perpilosa* colonies form permanent nests under *P. juliflora*. Colonies expand after the summer rains, forming satellite nests under *A. constricta*. Although there is no evidence that ants reduce herbivory, plants with basal ant nests have significantly higher soil nutrients at their base and produce about twice as many seeds as plants without basal nests (Wagner 1997). Bioassays have shown that *F. perpilosa* significantly reduces pollen viability of *A. constricta* upon contact (Wagner 2000). Three other ant species, *Myrmecocystus mimicus*, *Dorymyrmex* sp. (*smithi* complex), and *F. pruinosus*, commonly visit *A. constricta*. All of these species have metapleural glands and likely reduce pollen viability as well (Beattie et al. 1984, 1985).

An important herbivore on *A. constricta* at the study site is the lycaenid caterpillar *Hemiargus isola*. Eggs are laid singly on flower buds. Third and fourth (final) instar caterpillars shift from buds to open inflorescences, where they consume, and efficiently digest, pollen (Wagner and Martinez del Rio 1997). Caterpillars are tended by ants, to which they secrete food rewards upon demand. Ant tending increases *H. isola* survivorship and growth rates (Wagner 1993, 1995; Wagner and Kurina 1997).

Temporal separation

Dehiscence

To test the diurnal pattern of dehiscence, we sampled the pollen-to-anther ratio on *A. constricta* inflorescences. We obtained the pollen-to-anther ratio of an inflorescence by lightly dabbing the inflorescence on clear adhesive tape, placing the tape on a microscope slide, and counting the polyads and anthers (Stone et al. 1998). Before dehiscence only young anthers are removed on the tape. The pollen-to-anther ratio rises as pollen is released and decreases as pollinators remove pollen. We sampled six inflorescences (three unbagged and three bagged) on each of six plants approximately every 2 h from 0530 to 1730 hours on 19 August 2004. From 0600 to 1200 hours on 22 September and from 1300 to 1800 hours on 21 September 2004, we sampled three to five unbagged inflorescences hourly on each of four different plants. The difference in ratio between the bagged and unbagged flowers reflects pollen removal by insects.

Ant–pollinator observation

We conducted two studies to determine the overlap in temporal activity patterns of ants and putative pollinators. The first involved direct observation of ants and flower visitors on *A. constricta*. For 5 min we watched *A. constricta* plants with at least two newly opened inflorescences and recorded the number and species of each flower visitor. Immediately after the 5-min observation period, we counted the number and species of ants on the entire plant. Ants were counted on the foliage nearest the observer while walking around the plant at a constant rate; a census of a typically sized shrub lasted about 1 min. Observations were conducted continuously, moving from plant to plant, from 0600 to 1800 hours. Over the course of 6 days (three mornings and three afternoons), 170 observations were conducted on 84 flowering plants. Air temperature during the study was recorded with a Hobo data logger.

Pan traps

In addition to observations, we monitored the diurnal activity patterns of putative pollinators and ants by trapping insects throughout the day in bowls filled with soapy water (pan traps). Although pan traps are typically used for catching flying insects and estimating pollinator abundance (LeBuhn et al. 2003), ants also

commonly appeared in our traps as well. The number of ants and bees collected during a time interval presumably reflects the activity of ants and bees at that time. Fifteen bowls, some of which were white and some yellow, were set approximately 3 m apart on open ground along a single transect near large flowering *A. constricta*. Contents of bowls were collected hourly from 0600 to 1800 hours for three mornings and three afternoons. Bees were identified to genus and ants to species. Bees caught in the bowls were examined under a microscope and scored for the presence or absence of *A. constricta* pollen.

Spatial separation

In order to determine if, and how often, ants visit inflorescences, we quantified the numbers of ants visiting different plant tissues. While counting the number of ants per plant as described above, we also tallied the number of ants on branches, leaves, new inflorescences, old inflorescences, and buds. New inflorescences had opened within 24 h and were bright yellow. Old inflorescences were 2–3 days old, dark yellow to brown, and losing flowers from the inflorescence. To investigate finer-scale patterns of ant visitation, we focused more intensively on a set of 12 plants, chosen because they had relatively high ant visitation. Three times during the morning (0700–1000 hours) for 3 days, we counted the number of ants on stems, leaves, buds, new inflorescences and old inflorescences of each plant. For the latter data set, we compared the average number of ants per plant visiting the three floral stages using ANOVA, with plant as a blocking factor.

Floral ant-repellent

We tested three predictions about floral repellence in *A. constricta*.

1. Newly dehisced inflorescences (hereafter "new inflorescences") repel ants.
2. The repellent is detected before contact rather than upon contact.
3. The repellent is effective against a wide range of ant species in the community.

To test these predictions, we measured the rate at which ants of four species contacted buds, new inflorescences, and old inflorescences. We confirmed the presence of pollen on new inflorescences before including them in the experiment. We placed one of each of the three floral stages in a semi-circle approximately 4 cm from a central source of sugar water, used to attract ants to the area of the experiment. For *F. perpilosa*, who make their permanent nests under *P. juliflora*, we put the floral stages and sugar water on 4×6-inch platforms mounted in *P. juliflora* branches ($n = 14$ colonies). For

M. mimicus, *Dorymyrmex* sp., and *F. pruinosus*, who have entrances on open ground, the floral stages were placed around the nest entrance ($n = 20$ colonies for each species). We situated the sugar water between the nest entrance and the floral stages. For *F. perpilosa* colonies, we placed sugar water next to the main entrance onto the tree platform (the branch to which the platform was attached), such that the sugar water was between the inflorescences and the main entrance. We conducted these experiments between 0730 and 1100 hours for each ant species.

We recorded the number and type of ant contacts to each floral stage during a 10-min observation period. After touching an inflorescence with the antennae, ants either moved away or extended contact by moving onto the inflorescences. We categorized ant contacts as exploratory, involving contact with antennae only, or protracted, involving contact with one or more legs.

If new inflorescences emit a repellent that ants detect before contact, then ants should make fewer mean total contacts (explorative + protracted) to new inflorescences than to buds or old inflorescences. If the repellent is detected upon contact, then the mean proportion of all contacts that are protracted should be lower for new inflorescences than for buds or old inflorescences. We examined the effect of floral stage on total number of contacts and proportion of contacts that were protracted using separate linear mixed model ANOVAs (PROC MIXED; SAS institute 2004). Floral stage, ant species, and the interaction between floral stage and species were treated as fixed effects and ant colony by species was included as a random factor. If floral stages explained a significant amount of variation in the models, we conducted pairwise comparisons of means using Tukey–Kramer tests.

Pollen repellent

To test whether pollen itself is repellent to ants, we placed agar on opposing ends of a microscope slide and tapped newly opened inflorescences above the agar on one side so that pollen dusted the agar. If anthers, as well as pollen, stuck to the agar, the slide was not used. We placed slides approximately 5 cm from nest entrances. If ant activity was low, we placed sugar water baits equidistant from the two ends of the microscope slide. We counted the number of times an ant crossed the control and pollen agar within a 5-min period. Slides were not reused. We tested 14 colonies of *F. perpilosa* and 20 colonies each of *M. mimicus*, *Dorymyrmex* sp., and *F. pruinosus* (one trial per colony). We compared the number of contacts to the control and pollen agar using a linear mixed model (PROC MIXED) with fixed effects of treatment, species, and their interaction, and a random factor of colony by species (SAS Institute 2004).

For all analyses, data were tested for equality of variances and model residuals were examined for normality.

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Data were log-transformed when necessary to meet parametric assumptions.

Results

Temporal separation

Dehiscence

Pollen dehiscence began around 0800 hours and peaked between 1100 and 1200 hours on 19 August and between 1000 and 1100 hours on 21 and 22 September (Fig. 1a). After 1200 hours, the pollen-to-anther ratio of un-bagged inflorescences declined more rapidly than bagged inflorescences, likely due to pollen removal by insects.

Ant–pollinator observation

Bee and ant activity peaked at different times of day (Fig. 1b). Bee activity on *A. constricta* plants followed a similar pattern to dehiscence, beginning between 0800 and 0900 hours, increasing to its peak between 1100 and 1200 hours, and ending between 1500 and 1600 hours (Fig. 1b). On the other hand, ants were active mainly in the mornings and evenings, with peak activity between 0800 and 0900 hours and between 1600 and 1700 hours (Fig. 1b). A period of overlap of bee and ant activity occurred between 0800 and 1000 hours (Fig. 1b). The time of overlap between ant activity and pollen availability extended from 0600 to 1500 hours, with the most extensive overlap between 0700 and 1000 hours (Fig. 1b).

Over the course of observations, we counted 66 flower visitors, (73% of which were bees). Most visiting bees carried visible pollen loads on arrival and crawled in circles over the inflorescence collecting pollen. The only other visitor that appeared to carry pollen in its hairs was a beetle (*Acmaeodera* sp.) (16.7% of visitors). These beetles consumed flowers, likely making them poor pollinators. Visiting bee taxa included *Dialictus* spp. (Halictidae) (50% of visiting bees), *Exomalopsis* spp. (Anthophorinae) (12.5%), an unknown genus (Anthophorinae) (12.5%), *Megachile* spp. (Megachilidae) (10.4%), *Halictid* spp. (Halictidae) (8.3%), and *Dianthidium* spp. (Anthidiini) (4.2%). Of the bee visitors, 2.1% could not be identified.

Pan traps

Bees and ants collected in pan traps followed a pattern similar to those observed on plants. Bees were trapped from around 0800 until 1500 hours, with peak abundance between 1000 and 1100 hours (Fig. 1c). Ants were most abundant in pans traps from 0600 to 1000 hours and from 1500 to after 1800 hours (Fig. 1c). Again, an overlap in ant and pollinator diurnal activity occurred between 0800 and 1000 hours (Fig. 1c). The contents of

pan traps included 68 bees representing nine species and 91 ants in four species (95% *Dorymyrmex* sp). Eight of the bee species collected had at least one representative carrying *A. constricta* pollen, suggesting these species at least occasionally visit *A. constricta*.

Temperatures during observations and pan-trapping ranged from 16 to 52°C. The average maximum temperature of 40°C occurred between 1100 and 1300 hours (Fig. 1d).

Spatial separation

In the broad survey of ants on plants, ants visited inflorescences infrequently unless an ant-tended herbivore
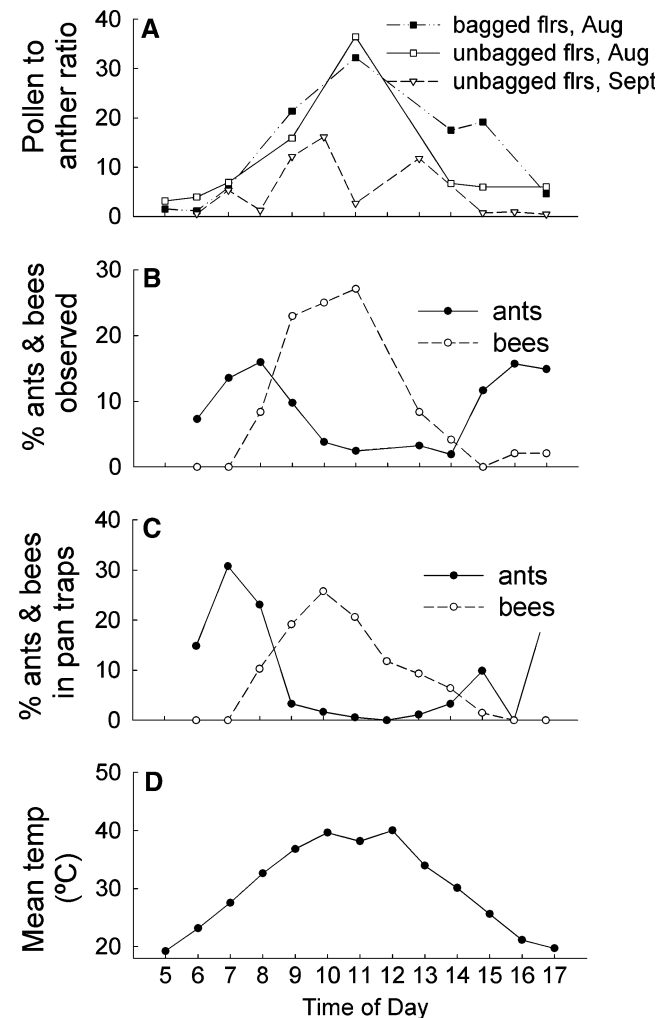
Fig. 1a–d Diurnal trends. **a** Daily pattern of dehiscence for bagged and unbagged inflorescences (*flrs*). Bagged ratios are averages for 19 August (*Aug*), and unbagged ratios are averages for 19 August and 21–22 September (*Sept*). **b** Diurnal activity pattern of ants and bees observed on *Acacia constricta*. Data points represent the percentage of the total ants (n=370) and bees (n=48) observed at each time interval. **c** Percentage of ants and bees caught in pan traps at hourly intervals. A total of 91 ants and 68 bees were collected. **d** Average temperatures (*temp*) during ant–pollinator observations and pan-trapping (15, 16, 18, 22, 21, 23 September 2004). *Time of day* represents hourly intervals

was present. We observed a total of 370 ants on 108 plants, an average of 3.1 (± 3.5 SD) ants per plant during the ant's active periods. On average, 11.4% of total ants were observed on inflorescences (1.4% on buds, 8.1% on new, and 1.9% on old inflorescences), 21.3% were visiting leaves, and 67.3% were moving along branches. The majority of ants visiting new inflorescences were tending *H. isola* caterpillars (7.0% of total visitation). A similar distribution across plant tissues held true between 0800 and 1000 hours, when ants and bees overlap in time. In all, we observed ants visiting a total of only 20 inflorescences on 16 of the 108 plants. Averaging across plants, we observed ants on 1% (± 0.7 SE) of all buds, 3.5% (± 1.7) of new inflorescences and 0.7% (± 0.1) of old inflorescences.

More intensive sampling of individual plants indicated that, unless a tended caterpillar was present, ant visitation to inflorescences fell after flower buds opened. These plants were visited by an average of 6.1 ants each and contained at least 40 inflorescences in each developmental stage. Averaging across plants, 11.9% (± 1.6 SE) of ant visits were observed on inflorescences: 4.1% (± 0.6) on buds, 5.4% (± 1.3) on new inflorescences, and 2.4% (± 0.5) on old inflorescences. Of the ants visiting new inflorescences, 65% were tending *H. isola* caterpillars, 20% were prying into flowers, and 15% were walking over inflorescences. Overall, ants were slightly more likely to be observed on new flowers than buds or old inflorescences, but there was no statistically significant difference among floral stages (Fig. 2; log-transformed data, $F_{2,22} = 3.1$, $P = 0.07$). However, when inflorescences with *H. isola* larvae were disregarded, ants were significantly more likely to visit buds than either new or old inflorescences (Fig. 2; $F_{2,22} = 6.1$, $P = 0.008$).

Plant (block) was not significant in either analysis ($F_{11,22} < 1.6$, $P > 0.2$).

Floral ant-repellent

In experimental tests, ants did not preferentially contact or avoid any of the floral stages (Fig. 3a; $F_{2,140} = 2.0$, $P = 0.1$), suggesting ants do not detect a repellent before contact. Species differed in their overall tendency to contact flowers ($F_{3,70} = 4.7$, $P < 0.005$), and varied in their response to floral stages (interaction $F_{6,140} = 3.3$, $P < 0.005$). Inspection of Fig. 3a suggests that *F. pruinosus* responded as predicted and avoided new inflorescences more than buds and old inflorescences. *Dorymyrmex* sp. and *F. perpilosa* contacted new inflorescences as much or more than other treatments and *M. mimicus* appeared to favor contact with buds over new and old inflorescences (Fig. 3a).

The proportion of ant contacts that were protracted varied significantly among floral stages ($F_{2,137} = 34.9$, $P < 0.001$). Ants strongly avoided protracted contact with new inflorescences relative to buds and old inflorescences (Tukey–Kramer $P < 0.001$), and avoided old inflorescences slightly, but significantly, more than buds (Tukey–Kramer $P < 0.05$). This result suggests there is a floral repellent and it is detected upon contact. Ant
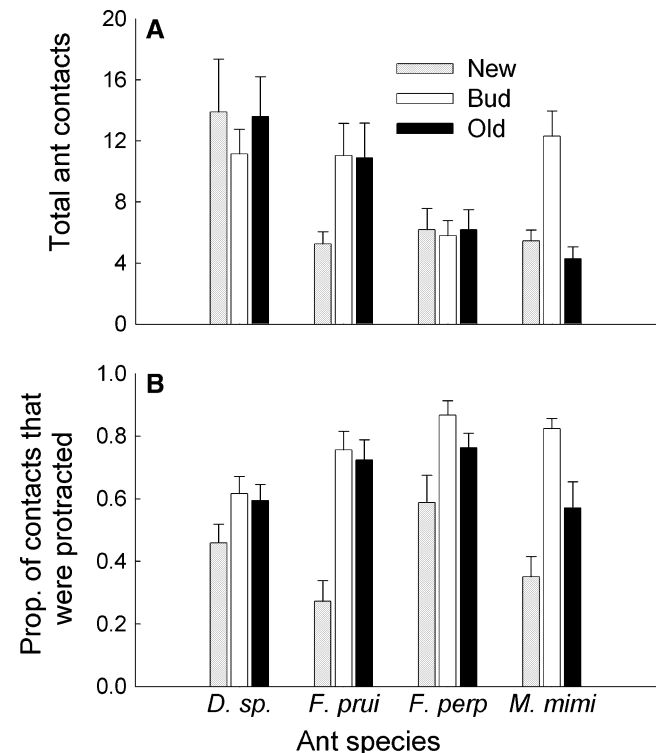

Fig. 2 Mean ($\pm$ SE) number of ants visiting *A. constricta* flowers in three developmental stages. Bars drawn in *solid lines* represent the number of ants visiting inflorescences, excluding those tending lycaenid caterpillars. Bars annotated with *different lower case letters* are significantly different using Tukey–Kramer honestly significant difference ($P < 0.05$). The *dashed bar* is the overall mean number of ants visiting new inflorescences, and includes those tending caterpillars

Fig. 3 a Mean number of ant contacts to floral stages (including explorative and protracted contacts) for each ant species. **b** Mean proportion of contacts to floral stages for each ant species that were protracted. *Error bars* are SEs. *D. sp. Dorymyrmex* sp., *F. prui Formica pruinosus*, *F. perp F. perpilosa*, *M. mimi Myrmecocystus mimicus*

species varied significantly in their response to floral stage (Fig. 3b; $F_{3,70} = 3.9$, $P = 0.013$). Although there was a significant interaction between ant species and floral stage ($F_{6,137} = 2.8$, $P = 0.013$), the rank order of mean responses to the three floral stages was identical for all ant species.

Pollen repellent

Ants significantly avoided agar dusted with pollen relative to controls (Fig. 4; $F_{1,70} = 23.8$, $P < 0.001$). The number of contacts to agar did not differ among species (Fig. 4; $F_{3,70} = 0.8$, $P < 0.5$) and there was no significant interaction between ant species and treatment (Fig. 4; $F_{3,70} = 0.8$, $P = 0.5$).

Discussion

In this study, we established that *A. constricta* separates ants from new inflorescences and pollinators in a least two ways. First, *A. constricta* inflorescences dehisce when visiting ants are least active, temporally separating ants from pollen and pollinators. *Acacia zanzibarica* also dehisces when ants are least active (Willmer and Stone 1997). However, temporal separation of ants from new inflorescences and pollinators is not found in all *Acacia* species. For instance, *A. hindsii* shows less temporal separation (Raine et al. 2002), and ants and pollinators are active at the same time of day on *A. collinsii* (Ghazoul 2001). Second, *A. constricta* has a floral ant repellent, which may be a trait common to *Acacia* species. Including this study, six *Acacia* species, comprising species with ant association (*A. collinsii, A. constricta, A. hindsii, A. zanzibarica*), species without ant association (*A. angustissima, A. macracantha*), species from Africa (*A. zanzibarica*), and species from the neotropics, have been tested and all have displayed a floral ant repellent (Willmer and Stone 1997; Ghazoul 2001; Raine et al. 2002).
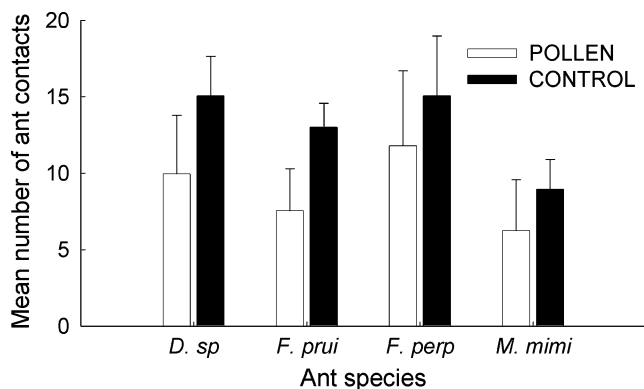

Fig. 4 Pollen as an effective ant repellent represented by the average number of ant contacts to agar with pollen and control agar. *Error bars* are SEs. For abbreviations, see Fig. 3

Most ant species in this study detected *A. constricta* floral repellent upon contact rather than before contact. Our findings suggest that the repellent is not particularly volatile. A highly volatile substance would likely have affected the overall frequency with which ants contacted inflorescences, rather than the frequency of protracted contacts alone. This appears consistent with the response of ants to floral repellents in other plant species where ants spend less time on newly opened flowers (Willmer and Stone 1997) and on petri dishes wiped with newly opened flowers (Ghazoul 2001). Similarly, Raine et al. (2002) found ants to either "pass through" areas of stem wiped with newly opened flowers or to halt at the wiped boundary, suggesting ants must closely approach the area of repellence to detect it.

In particular, we found that ants avoided contact with *A. constricta* pollen (Fig. 4). This finding is the first to support the hypothesis that pollen is a component, if not the sole source, of the floral ant repellent. It is also possible that anther glands are repellent. Anther glands are globular structures attached to the top of an anther by a stalk and are found in many *Acacia* spp. (Kenrick 2003). These glands may play a role in producing floral scents and function as pseudo-pollen or a true food reward to attract pollinators when stigmas are receptive (Stone et al. 2003). In our tests, along with the pollen, a few anther glands were dusted onto the agar. Since anther glands are only slightly larger than pollen and fall from anthers like pollen, it was not feasible to exclude them from the agar. Thus, anther glands cannot be eliminated as a potential repellent source.

A. constricta pollen repelled all four visiting ant species, representing two subfamilies, suggesting the repellent is effective on a broad range of ant species. Since multiple ant species often visit plants (Koptur 1992; McKey and Davidson 1993), the ability of a plant to repel a range of ant species from flowers is an important adaptation. Yet, the repellent appears to be specific enough to ants that their hymenopteran relatives, the bees, are not also repelled.

The majority of ants observed on new inflorescences were tending caterpillars of the lycaenid butterfly species *H. isola*. Because *H. isola* caterpillars consume pollen, they typically occupy new inflorescences most repellent to ants. Although it is clear that the attractiveness of the lycaenid caterpillars often overrides the repellency of pollen, it is possible that the floral repellent affects such factors as the number of ants tending caterpillars or the constancy of attendance. Ant tending increases *H. isola* larval growth rates and survivorship (Wagner 1993; Wagner and Kurina 1997), so if the floral repellent discourages ants from tending, it could also act to reduce florivory.

In field surveys and experiments, ants avoided open flowers, both new and old, relative to buds. Avoidance of new inflorescences is consistent with our experimental evidence that pollen is repellent. Residual pollen may account for ant avoidance of old inflorescences. Ants might avoid open inflorescences for other reasons as

well, such as poor footing. Clusters of protruding stamens found on open inflorescences may be more difficult to walk over than the more compact buds.

Prior to this study, the effect of ants on male and female function in *A. constricta* appeared to conflict. Positive effects of *F. perpilosa* on seed set (Wagner 1997) appeared to be accompanied by reduced pollen viability when ants contacted flowers (Wagner 2000). Here we show *A. constricta* reduces potential costs to male function using mechanisms that limit contact between ants and flowers, while still maintaining the benefits of ants. Investigation of the conflicts inherent in interspecific interactions and how those conflicts are resolved contributes to our understanding of how mutualisms, such as those between ants and plants, persist.

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