A mutualism's indirect costs: the most aggressive plant bodyguards also deter pollinators

Joshua H. Ness

Plant defenses against herbivores may be costly if they exclude mutualists. Here, I test the hypothesis that aggressive ant bodyguards of plants deter pollinators, and explore mechanisms by which *Ferocactus wislizeni*, an extrafloral nectary bearing cactus, limits conflicts between its pollinators and bodyguards. Flower visitation by ants and pollinating bees differed among plants tended by four different ant species. The ant species most rarely found in flowers showed the strongest aversion to *F. wislizeni* flower petals in laboratory assays, suggesting that those structures may include an ant-deterrent. Species-specific estimates of mean ant abundance within flowers and aggressiveness towards other arthropods were used to distinguish the relative threat of ant attack in flowers on plants tended by each ant species. Pollinator surveys in 2003 and 2004 demonstrated that bee visitation rates and the duration of flower occupation differed among plants with different ant associates, decreasing as the threat of ant attack increased. Flowers on plants tended by *Solenopsis xyloni*, the best ant bodyguard, were more dangerous than those on plants tended by three milder species, due to that ants' greater aggressiveness and abundance within flowers. These flowers were visited by pollinators least frequently and for less time per visit, and produced fruits with significantly lower total seed mass, fewer seeds, and lighter individual seeds, relative to fruits from similarly-sized plants tended by three other ant species. As a result, the best bodyguard may indirectly constrain plant reproduction in some settings. Conflicts between mutualistic guilds may be particularly common in generalized systems, where there is variation in partner quality and in the relative importance of the protection and pollination mutualisms.

J. H. Ness, Dept of Biology, Skidmore College, Saratoga Springs, NY 12866, and Dept of Ecology and Evolutionary Biology, Univ. of Arizona, Tucson, AZ 84721, USA (jness@skidmore.edu).

The costs of plant resistance to natural enemies can be manifested in plant–pollinator interactions. Tradeoffs between plant defense and reproduction can occur as a result of resource allocation (i.e. resources allocated to one function are not available for the other, Strauss et al. 1999). Costs may also be expressed within the context of a plant's interactions with other species. For example, foliar herbivory can influence floral traits (Strauss 1997, Mothershead and Marquis 2000), and natural enemies can exert selection pressures that run counter to that exerted by pollinators (Gomez 2003, Cariveau et al.

2004). Greater defense could also be accompanied by reduced competitive ability or deterrence of mutualists ('ecological costs' sensu Strauss et al. 2002). For example, the structures (Galen 1999, Agrawal et al. 2000), scents (Galen 1983), and phenologies (Brody 1997) that help plants deter or avoid natural enemies can also limit access to pollinators. Recent work even shows that indirect biotic defenses, such as ant 'bodyguards', may attack developing flowers (Yu and Pierce 1998, Stanton et al. 1999), and that specialized 'ant- plants' may be adapted to limit conflicts between their bodyguards and

pollinators (Willmer and Stone 1997, Raine et al. 2002). These bodyguard–pollinator interactions provide an opportunity to explore conflicts between defense and reproduction, and to explore a hidden cost that may be common within these widespread mutualisms between ants and plants.

Plants in >90 families engage in a food-for-protection mutualism by offering extrafloral nectar (hereafter, EFN) to attract ant bodyguards (Koptur 1992). Because EFN is both nutritious and highly predictable in time and space, many ants aggressively defend those plants from herbivores and other arthropods (Carroll and Janzen 1973). Ants may also collect floral nectar and pollen (Fowler and Whitford 1982, Galen 1983, 1999, Rico-Gray 1993, Visser et al. 1996, Puterbaugh 1998, Altshuler 1999). The presence of ants in or near flowers can decrease pollinator visitation, although that phenomenon has been demonstrated primarily with 'nuisance' ants that provide no benefit to the plant (Fritz and Morse 1981, O'Dowd and Catchpole 1983, Norment 1988, Visser et al. 1996, but see Altshuler 1999 and Tsuji et al. 2004 regarding ants that protect plant species that lack EFN). Whether flower visitation by ants is increased or decreased by the presence of alternative rewards such as EFN is controversial (Guerrant and Fiedler 1981, O'Dowd and Catchpole 1983, Wagner and Kay 2002, Raine et al. 2002, Galen 2005). The obligate ant–Acacia offer a variety of rewards to their coevolved ant bodyguards, but also use ant-deterrent compounds to them out of flowers (Willmer and Stone 1997, Ghazoul 2001, Raine et al. 2002). Unlike the ant–Acacia, most EFN-bearing plant species lack specialized partners, and instead interact with a suite of omnivorous ant species (an average of six to nine ant genera per plant species, Oliveira and Brandão 1991). Ant species differ in their responses to the same inflorescence volatiles (Ghazoul 2001), and if they also differ in their likelihood of foraging in flowers and/or attacking pollinators, the attractiveness of flowers to pollinators could vary with plant bodyguard identity.

This study explores costs incurred by a facultatively ant-tended plant as a result of antagonistic interactions between ant bodyguards and pollinators. First, I explore whether ant bodyguards differ in their likelihood of foraging in flowers, and whether interspecific differences are consistent with an aversion response to flower-derived cues. Second, I use interspecific differences in ant aggressiveness and abundance within flowers to contrast a pollinator's vulnerability when visiting plants tended by different ants. I then ask whether pollinator visitation and/or foraging duration per flower decrease as vulnerability increases. Third, I ask whether several components of plant fitness (total seed mass, individual seed mass, and seeds per fruit) are correlated with the variation I observed in the ant–pollinator interactions.

Study organism and site

The fishhook barrel cactus, Ferocactus wislizeni (Cactaceae), ranges from southern Arizona and southeastern California to northern Sonora, Mexico. A ring of areoles around the top of the cactus, the site of flowers and fruits, bears modified spines that exude EFN throughout the year. As a result, ants forage in close proximity to the buds, flowers and fruits that are attacked by orthopterans, hemipterans, and pyralid caterpillars (McIntosh 2002, J. H. Ness, pers. obs.). Plant protection by these ants is explored in a companion study (Ness et al. 2006); ant-tended plants host fewer herbivorous insects and mature a greater proportion of buds into flowers and flowers into fruits, relative to untended plants. Flowering typically occurs from late July to early October, and individual flowers last approximately three days (McGregor and Alcorn 1959). Solitary cactus bees (Ashmeadiella, Augochlorella, Diadasia, Idiomelissodes and Lithurge spp) are the most important pollinators at the one site where pollination has been studied, and outcrossing increases fruit set and the number of seeds per fruit (McGregor and Alcorn 1959, McIntosh 2002).

This study focused on 259 plants at the Tumamoc Hill Desert Research Laboratory in Tucson, Arizona, USA (32°13′N, 111°05′W). The site has been protected from livestock grazing since 1907. Average rainfall (1904-1980) is ∼300 mm yr^{-1}, and the dominant plants include Larrea tridentata, Cercidium microphyllum, Carnegia gigantea, Opuntia and Acacia spp (Goldberg and Turner 1986). Study plots ranged from 750–900 m elevation. The ants most frequently observed at EFN on these 259 plants are Crematogaster opuntiae, Solenopsis aurea, S. xyloni and Forelius sp. These ants are opportunistic foragers, collecting floral and extrafloral nectar from various desert plants (Pickett and Clark 1979, Fowler and Whitford 1982), and were found on F. wislizeni on 94% of the occasions when ants were observed in a year-long, bi-weekly survey of the plants. Co-occupation of plants by different ant species at a given time is rare, although a single plant is often visited by multiple ant species over a year (Morris et al. 2005). Plants predominantly tended by S. xyloni are occupied by fewer herbivorous insects and mature a greater proportion of reproductive units into fruits, relative to plants tended by other ant species (Ness et al. 2006).

Methods

Floral aversion experiment

I used a behavioral assay similar to that employed by Ghazoul (2001) and Raine et al. (2002) to test whether cues associated with F. wislizeni flowers influence ant foraging. I tested the effects of flower petals on foraging

because buds always have EFN within 1 cm, and thus these petals are the only structures between the EFN and the floral nectaries, stamen, stigma, etc. Half of an 8-cm petri dish (bottom and lid) was wiped with a *F. wislizeni* petal, handled with forceps. One ant was added to the closed dish, and provided 30 sec for acclimation and exploration. Ant location in the 'petal' versus 'control' hemispheres was then monitored for five minutes. The dishes were shaded throughout the experiment, and were rotated 180 degrees after 2.5 minutes. Trials were repeated with workers of each of the four ant species. Individual workers and dishes were only used once. All trials were performed outdoors, using ants collected from plants that lacked flowers at that time. Two-tailed paired t-tests compared the occupation time in each hemisphere for each ant species, and an ANOVA model tested whether the species differed in the proportion of time spent occupying the floral hemisphere.

Plant, ant, and pollinator surveys

The 259 plants were surveyed for ants and pollinators approximately once every week during the 2003 and 2004 flowering seasons (July to mid-October). During each survey, the species of EFN-collecting ant was noted, and each open flower was designated as occupied by ants, bees, both, or neither. Occupation was defined as the presence of ants and/or bees in the inner floral cup (the site of anthers, stigma and floral nectaries). Flowers were inspected as thoroughly as possible for approximately 30 seconds. The number of ants in each occupied flower was recorded in a random subset of flowers ($n_{2003} = 17$; $n_{2004} = 168$). Bees were not identified. Inspections were performed from early morning hours to midday. Flower visitation by cactus bees is greatest at that time (Mandujano et al. 1996), although ants collect EFN all day during this time of year. Individual flowers were inspected only once, and I pooled the observations over time to estimate the percentage of flowers visited for each plant.

I tested four hypotheses with these data. 1) A simple linear regression tested the hypothesis that mean ant abundance in a flower decreased as the ant species' avoidance of petal-derived cues increased. Mean ant abundance was estimated as the product of mean number of ants per occupied flower and the proportion of flowers occupied on plants tended by that ant. 2) An ANOVA model tested the hypothesis that ant species occupy different proportions of available flowers. The original model included ant species, plant identity, year, and a year × ant species interaction term as categorical variables. Non-significant terms were successively removed in a stepwise fashion. All proportions were arcsin-transformed. An identical analysis tested the

hypothesis that the proportion of flowers occupied by pollinators differs among plants tended by different ant species. 3) A simple linear regression tested the hypothesis that differences in pollinator visitation could be predicted from the relative threat of attack by ants among plants with different bodyguards. An earlier study (Ness et al. 2006) demonstrated that the fraction (F) of 10 *Manduca sexta* caterpillars killed by ants could be predicted using the Michaelis-Menton equation $F = A/(A+b)$, where A is ant abundance per plant and b is a species-specific constant describing the number of ants necessary to kill five caterpillars in 30 min ('per capita-effectiveness', Table 1). Here, I predict that relative aggressiveness of the ant species toward caterpillars is a useful predictor of their responses when confronting pollinators, and I use F to contrast the danger to bees of visiting flowers on plants occupied by different ants. To compute F for each plant, I estimated A as the product of the proportion of flowers occupied by ants and the average number of ants per occupied flower, and used species-specific values of b. The means from plants tended by each ant species were used as independent data points (n = 4), and separate analyses were performed for each year. 4) I used a one-tailed paired t-test to evaluate the hypothesis that pollinator visitation to individual plants would be greater when they were tended by an ant species less likely to attack pollinators. I used plants that were tended by more than one ant species over the flowering season as the shared unit, and distinguished the relative threat of ant attack in the manner described above.

Pollinator observations

To test the hypothesis that two components of pollination, visitation rate and foraging duration per visit, are influenced by ant occupation of flowers, inflorescences were observed in the field for 10-min intervals. Observations occurred between 06:00 and 12:00 h, the period during which desert bees are most active (Mandujano et al. 1996). The number of pollinator visits to the plant, total time each pollinator spent inside each flower, ant species tending the plant, ant abundance in each flower at the beginning and end of the observation period, and ambient temperature were recorded. Most bees (87%) were distinguished as either large (e.g. *Diadasia, Lithurge, Ashmeadiella, Augochlorella* and *Idiomelissodes* spp.) or small (halictids), and the former are particularly effective pollinators (McGregor and Alcorn 1959). Because a) bees can quickly transfer pollen among neighboring plants, and b) distinguishing new visitors from those returning to the plant is difficult, visits by similar-looking bees separated by >30 s were identified as distinct visits. Threat of attack in those flowers was estimated as above. Approximately 20%

Table 1. Summary data describing ant responses to flower petals, per-capita effectiveness of ants as bodyguards, occupation of *Ferocactus wislizeni* flowers by ants versus bees, duration of bee foraging bouts per flower, and three fitness components of plants tended by different ants. Means $\pm$SE shown. The number of replicates is given in parentheses, and indicate individual plants unless otherwise noted. Note that ant species with the lowest per-capita ant effectiveness value (b) are the most aggressive.

		C. opuntiae	*Forelius* sp	*S. aurea*	*S. xyloni*
% time in floral hemisphere (n =workers)		33$\pm$5 (15)	45$\pm$4 (18)	49$\pm$6 (22)	56$\pm$6 (18)
No workers per occupied flower (n =flowers)		1.3$\pm$0.2 (19)	2.5$\pm$0.3 (55)	4.0$\pm$0.5 (75)	3.8$\pm$0.3 (36)
Per capita-effectiveness (b)		18.8	31.7	14.2	9
% flowers occupied by ants	2003	14.5$\pm$3.4 (61)	38.4$\pm$7.4 (25)	16.2$\pm$3.1 (72)	27.3$\pm$4.1 (65)
	2004	10.8$\pm$3.1 (62)	52.0$\pm$6.8 (40)	30.9$\pm$4.1 (59)	44.2$\pm$4.3 (84)
% flowers occupied by pollinators	2003	14.2$\pm$3.1 (61)	7.6$\pm$3.5 (25)	10.5$\pm$2.7 (72)	4.6$\pm$2.3 (65)
	2004	23.3$\pm$4.5 (62)	14.2$\pm$4.9 (40)	13.6$\pm$3.1 (59)	4.2$\pm$1.2 (84)
Bee foraging during (s) per flower (n =bees)		43$\pm$12 (87)	44$\pm$18 (36)	76$\pm$19 (50)	15$\pm$3 (40)
Mass (mg) per seed		237$\pm$6 (23)	253$\pm$15 (4)	232$\pm$8 (14)	203$\pm$8 (16)
Seed number per fruit		1017$\pm$88 (23)	1037$\pm$206 (4)	1239$\pm$107 (15)	871$\pm$107 (16)
Seed mass (g) per fruit		2.32$\pm$0.16 (23)	2.43$\pm$0.38 (4)	2.82$\pm$0.21 (14)	1.77$\pm$0.20 (16)

of the focal plants were inadvertently observed more than once, and these multiple observations were averaged. Each plant was treated as an independent replicate.

I tested three hypotheses with this data. 1) A two-tailed t-test evaluated the hypothesis that ant abundance in an inflorescence changes after pollinator visitation (as might happen if, for instance, ants recruit to or avoid pollinators). I compared the change in abundance over the 10-minute interval among visited and non-visited inflorescences, performing separate tests for each ant species. 2) A simple linear regression tested the hypothesis that differences in pollinator visitation rate could be predicted from the relative threat of attack by ants among plants with different bodyguards. The means from plants tended by each ant species were used as independent data points (n =4). Separate analyses were performed for all visitors (pooled) and for large bees only. 3) The hypothesis that a bee's foraging time within a flower decreases as the threat of attack by ants increases was evaluated in two ways. First, I compared the foraging duration in flowers with and without ants. Second, I compared mean foraging duration among ant-occupied flowers that differed in the threat of attack. I estimated threat of attack (F) for each visited flower, and pooled flowers into groups by rounding that F to the nearest decimal point. Although statistical analyses are precluded by pseudo-replication at the plant scale (i.e. the presence of multiple flowers on a plant), these two comparisons are informative because they describe the ant-pollinator interaction at the most basic unit, the individual flower.

Ant species and plant reproduction

Three randomly selected mature fruits were collected from all plants tended by only one ant species throughout the flowering period in 2003 (n =59 plants). A sub-sample of seeds from each fruit (mean$\pm$SE =35$\pm$ 1.4 seeds) was counted and weighed in bulk. Seed number per fruit was subsequently estimated by dividing total seed biomass by the average mass of an individual seed. Thus, average seed mass (per fruit and per seed) and number of seeds per fruit were estimated for each plant. Three ANOVA models tested for differences in seed mass per fruit, mass of individual seeds, and number of seeds per fruit among plants tended by different ants, using bodyguard identity as the independent variable. Averages across the three fruits from individual plants were used as replicates.

To explicitly test the hypothesis that components of fitness differed among plants tended by the most aggressive ant (*S. xyloni*) and other species, I pooled results from the latter group and compared those three fitness components using two-sided t-tests. Two alternative explanations for inter-plant differences in these fitness components are that the different ant species tend different-sized plants, or that well-protected plants allocate less resource per fruit because they produce more fruits. A two-sided t-test compared the volume of plants tended by *S. xyloni* versus the other three ants. Volume was estimated using above ground biomass, treating plants as cylinders. A simple linear regression tested whether seed mass per fruit changed with number of fruit produced per plant.

Results

Floral aversion experiment

The four ant species responded to *F. wislizeni* flower petals differently (ANOVA, F =2.754, df =3,69, p = 0.049, Table 1). Only one species, *C. opuntiae*, spent significantly less time in the petal-treated hemisphere than in the control hemisphere (t =3.50, df =14, P = 0.004). Differences in foraging duration among the hemispheres for the other three species were not significant (Table 1; P >0.2 in all cases).

Ants and pollinators in flowers

Ants were found in 24% of the inspected flowers and bees were observed visiting 20% (n = 956 flowers inspected in 2003, n = 1045 in 2004). Both ants and bees collected floral nectar and pollen, although ants were typically observed merely walking in the flowers (as they do on sites lacking resources, such as cactus thorns). Bees and ants co-occupied 49 inspected flowers, half the number expected if the groups were distributed independently. Bees were often observed leaving flowers after being startled by the ants, and several individuals caught on the wing had detached ant heads clamped to their legs.

Ant species differed in their visitation of flowers, whether compared by 'occupancy' (= proportion of flowers occupied by ants) or 'abundance' (the number of ants observed in occupied flowers, Table 1). Fewer workers of *C. opuntiae*, the lone species that exhibited significant aversion to cues associated with flowers, were found in occupied flowers, relative to the other ants (one tailed t-test, t = 8.85, df = 131, p < 0.0001). Among-species differences in the mean number of ants per flower corresponded to the different responses to the flower petal assay (r^2 = 0.98; simple linear regression, F = 115.4, df = 3, p = 0.009, Fig. 1). Equally strong correlations ($r^2 \geq 0.98$) were observed with any combination of three of the four species, indicating that this relationship was not driven by the responses of any single ant species. The ANOVA model demonstrated that several variables were significant predictors of flower occupation by ants, including ant species (F = 10.58, df = 3, p < .0001), year (F = 8.43, df = 1, p = 0.004), and plant identity (F = 1.29, df = 222, p = 0.026). The year × ant interactions were also significant (F = 2.66, df = 3, p = 0.049), as three of the four species were more commonly found in flowers in 2004 than 2003.

Ant species was a useful predictor of the proportion of flowers occupied by pollinators (ANOVA, F = 6.88, df = 3, p = 0.0002), and the effects of year, plant identity, and year × ant interactions were non-significant. Bees were particularly rare in flowers on plants tended by *S. xyloni*, a difference predicted reasonably well by the greater danger of ant attack in those flowers in both 2003 (r^2 = 0.73; simple linear regression, F = 5.28, df = 3, p = 0.15) and 2004 (r^2 = 0.92; F = 22.69, df = 3, p = 0.04, Fig. 2).

In 78 instances (n_{2003} = 20, n_{2004} = 58), plants were tended by multiple ant species during the flowering season. Pollinators visited a greater proportion of the available flowers when a plant was tended by the ant bodyguard less likely to pose a threat within flowers (one-tailed paired t test, t = 1.63, df = 77, p = 0.053).

Pollinator observations

Flowering plants were observed for 154 10-minute intervals between July and Sept 2004. All observations were performed between 06:00 and 12:00 h, when ambient temperatures ranged from 20–34°C. Ninety-eight percent of the visitors were bees, although no
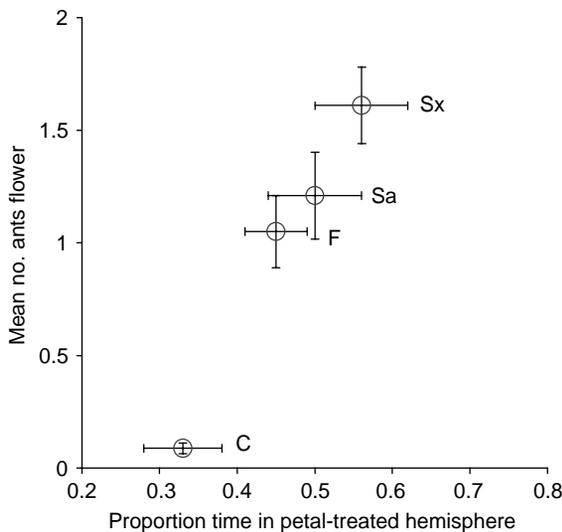

Fig. 1. The relationship between ant response to chemicals on *Ferocactus wislizeni* flower petals and the number of ants per flower. Proportions <0.5 indicate ant avoidance of chemical cues associated with flower petals. The latter value is the product of the mean number of ants per occupied flower and the proportion of flowers occupied. Figure code: C = *C. opuntiae*, F = *Forelius* sp., Sa = *S. aurea*, Sx = *S. xyloni*. Means ±SE shown.
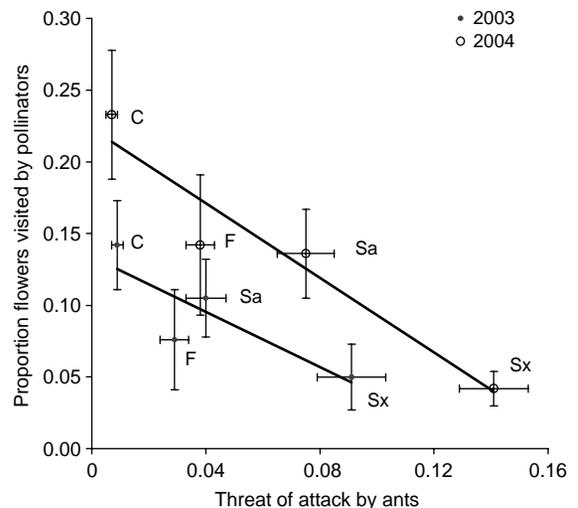

Fig. 2. The relationship between pollinator visitation and the danger associated with those flowers on plants tended by different ants. Danger is a function of the proportion of flowers occupied by ants, the mean number of ants in occupied flowers, and the per-capita effectiveness of those ants at deterring foreign arthropods. 2003 data shown in empty circles, 2004 in filled circles. Best-fit lines for each year are shown. Figure code as in Fig. 1. Means ±SE shown.

bees were observed visiting flowers at temperatures <23°C. Ant occupation of flowers was similar across the full temperature range, but I limited analyses to 139 observations at temperatures >23°C. The average flowering plant bore 2.3 flowers (SD = 1.3), and the number of open flowers was similar among plants tended by different ants (ANOVA, F = 1.46, df = 3, 132, p = 0.23).

Bee visitation did not alter ant abundance in flowers, relative to non-visited flowers, for any of the four species (t-tests, p > 0.2). Thus, I averaged the number of ants per flower at the start and conclusion of the 10-minute interval to compute the threat of ant attack in each inflorescence. The differences in visitation rate among plants tended by the four ant species were predicted reasonably well by the danger of attack in flowers (simple linear regression for all visitors pooled: $r^2 =$ 0.95, F = 39.6, df = 3, p = 0.02; for large bees only: $r^2 =$ 0.52; F = 2.14, df = 3, p = 0.28). Flowers on plants tended by *C. opuntiae*, *Forelius* and *S. aurea* were visited more frequently by all pollinators, and by large bees in particular, than were flowers on *S. xyloni*-tended plants (Fig. 3a).

The average foraging bout in flowers without ants (mean ± SE = 56 ± 12 s, n = 115 flowers) was longer than in ant-occupied flowers (36 ± 7 s, n = 123). The threat of attack in ant-occupied flowers varied >10-fold among flowers, due to differences in ant abundance and identity, and bee foraging times in flowers decreased as the threat of attack increased (Fig. 3b). Foraging bouts in flowers on plants tended by *S. xyloni* were shorter than those in plants tended by the other three ant species (Table 1).

Ant species and plant reproduction

Ant identity was significantly correlated with variation in total seed mass per fruit (F = 4.55, df = 3,52, p = 0.007) and the mass of individual seeds (F = 5.26, df = 3,52, p = 0.003). The correlation between ant identity and seed number per fruit was weaker (F = 2.03, df = 3,52, p = 0.12). The average seed mass per fruit, mass of individual seeds, and seed number per fruit were lowest on plants tended by *S. xyloni* (Table 1).

In comparisons between plants tended by *S. xyloni* versus the other three ant species, fruits on *S. xyloni*-tended plants had lower seed biomass (one sided-t = 4.26, df = 49, p = 0.0004), fewer seeds per fruit (t = 2.57, df = 53, p = 0.007) and lighter individual seeds (t = 5.03, df = 44, p = 0.00005, Fig. 4). The volume of the focal plants tended by *S. xyloni* and the other three ant species did not differ significantly (two-sided-t = 1.16, df = 46, p = 0.25), and seed mass per fruit did not vary significantly with the number of fruit produced (simple linear regression, F = 0.81, df = 1,19, p = 0.38)
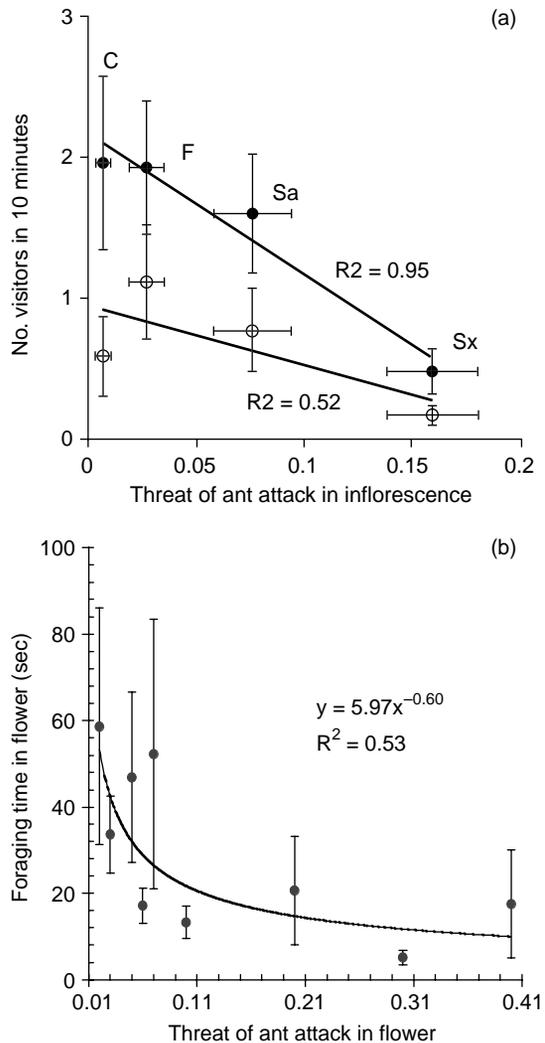

Fig. 3. Pollinator observations on plants tended by different ant bodyguards. (a) Number of pollinator visits observed in 10-minute observations. Results for all pollinators and large bees only are distinguished by filled and empty circles, respectively. Best-fit lines are shown. Figure code as in Fig. 1. (b) Bee foraging duration in flowers that differ in the threat of attack by ants. Means ± SE shown.

Discussion

Ecologists are becoming increasingly aware of the links between pollination and herbivory (Mothershead and Marquis 2000, Strauss et al. 2002, Strauss and Murch 2004) and between pollination and predation (Dukas and Morse 2003, Suttle 2003, Dukas 2005). This study focuses on interactions between pollinators and predaceous ants that act as alternatives for direct plant chemical and physical defenses against herbivory. I have contrasted the interactions of different ant bodyguard species with pollinators in several ways. First, differences among ant species in floral occupation correspond to their relative aversion to chemicals on
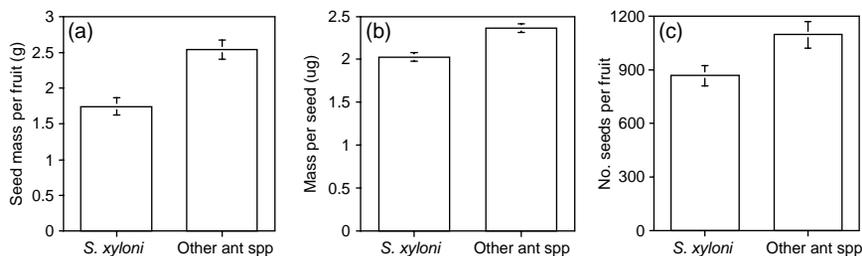
Fig. 4. Comparisons of fruits from plants tended by *Solenopsis xyloni* versus other ant species during flowering. (a) Seed mass per fruit. (b) Mass of an individual seed. (c) Number of seeds per fruit. Plants were used as replicates, and means ±SE are shown.

F. wislizeni flowers. Second, among-plant differences in the danger associated with visiting flowers, estimated as a function of the abundance of ants within flowers and their aggressiveness towards other arthropods, corresponded with among-plant differences in pollinator visitation

rate and foraging duration within flowers. Third, plants with the most aggressive ant bodyguards produced fruits with fewer and smaller seeds, a difference I attribute to ants deterring the pollinators. I discuss these points below.

Plants can exclude ants from flowers by several means, including slippery stalks (Harley 1991), modified flower architecture (Galen 1999), distasteful petals (Guerrant and Fiedler 1981), and volatile ant-repellent compounds (Galen 1983, Willmer and Stone 1997, Ghazoul 2001, Raine et al. 2002). In this study, only a small fraction of the ant workforce on the plant was typically found in the flowers (mean ±SD = 5 ± 3%, estimated using average abundances 'in flower' and 'on plant' for each species in the present study and Ness et al. 2006, respectively), indicating that most foraging effort was allocated towards activities associated with the ant-plant mutualism (i.e. patrolling the host plant and collecting EFN). Flower-borne deterrents can affect different ant species differently (Guerrant and Fiedler 1981, Ghazoul 2001), although those differences have never been linked to variation in flower visitation by ants, pollinators, or plant fitness. The four ant species in this study responded to *F. wislizeni* flower petals differently, and their mean abundance within flowers was correlated with the responses to the petals. Among-species differences in flower occupation were not merely a result of different numbers of workers foraging on plants, as worker abundance is similar on plants tended by *C. opuntiae*, *S. aurea* and *S. xyloni* from July–September (and less on plants tended by *Forelius*; Ness et al. 2006). Interestingly, the species with the greatest aversion to *F. wislizeni* flowers is also the attendant most frequently observed at *F. wislizeni* EFN in this population (48% of the occasions in which ants were observed on plants in 2003). Earlier selection on plants to lower the costs of this mutualism could have been driven by frequent interactions with this partner.

Occupation of flowers by bees also differed among plants tended by different ant bodyguards. Pollinators were most commonly observed at plants with

bodyguards less likely to attack them in flowers, such as *C. opuntiae*, *S. aurea* and *Forelius* sp. Bees were rarely observed visiting plants tended by *S. xyloni*, where flowers were typically occupied by several aggressive ants. Bees also spent less time foraging in flowers occupied by ants, relative to unoccupied flowers, and spent the least time per visit in *S. xyloni*-occupied flowers. I attribute this non-random foraging by the bees to their maximizing the net benefit per foraging trip. This benefit may be maximized if foragers avoid flower patches (individual plants) where predation risk is high, or has been high in the past (Dukas 2001, Dukas and Morse 2003). Solitary cactus bees should be particularly danger-averse, as a female's death has greater detrimental effect on her fitness than does the death of a social bee whose colony survives (Clark and Dukas 1994). Ants not only harass pollinators but may also consume shared resources such as *F. wislizeni* pollen and floral nectar. A bee's benefits can also be maximized by avoiding low quality patches (i.e. where ants have already removed some portion of the nectar or pollen), and pollinators may avoid flower patches or plants where robbing is common (Roubik 1982, Irwin et al. 2001). Because the threats of both predation and/or competition can increase with ant abundance in flowers, changes in pollinator foraging may be due to a combination of risk-aversion and exploitation competition. Further, the techniques many ant species use to practice interference competition, including attacking rivals and/or chemically modifying valued microsites, could alter a vistor's perception of the quality or danger associated with a flower. This protection of valued resources against perceived competitors has been interpreted as the ant's source of motivation to guard EFN-bearing plants (Carroll and Janzen 1973, Koptur 1992). Estimates of predation risk accurately predicted variation in pollinator foraging in this study, although the perceived importance of predation risk, exploitation competition, and interference competition likely vary among combinations of pollinator and ant species. It is unclear whether the distinction between those mechanisms is important to the plant.

Several measures of plant reproductive success, including seed mass per fruit, seed size, and seed number, were correlated with the identity of the plant-tending ant. All three measures were lowest on plants tended by *S. xyloni*, a pattern particularly striking because the

mass of individual *F. wislizeni* seeds typically increases as the number of seeds per fruit decreases (McIntosh 2002, J. H. Ness, unpubl.). I attribute this decrease in seed number and seed quality to the greater than threefold decreases in pollinator visitation rate and in-flower foraging times at *S. xyloni*-tended plants, relative to plants tended by other ant species. Whether that decrease significantly changes the quantity or quality of pollen (i.e. diversity, and parentage) delivered to the plant is unknown. Exposure to ants can decrease pollen viability (Hull and Beattie 1988, Wagner and Kay 2002, Galen and Butchart 2003), although whether the quality of delivered and/or donated *F. wislizeni* pollen is lessened is not yet known. I did not compare germina-tion rates of seeds derived from plants tended by different ants because most *F. wislizeni* seeds undergo a multi-year dormant stage (Bowers 2000). Ant identity could also influence male reproductive function of their host plants due to deterrence of pollen-collectors and pollen collecting by the ants (Galen and Butchart 2003).

I have used among-plant differences in ant foraging, pollinator visitation, and seed production as evidence that the costs of a biotic defense differ with partner identity. Because I used naturally occurring variation in ant bodyguard identity (as did Willmer and Stone 1997, Yu and Pierce 1998, Raine et al. 2002), rather than randomly assigning ant treatments to plants. I have not shown that ants (or particular ant species) influence seed production per fruit relative to the untended state. These ants are unlikely to act as outcrossing pollinators, due to the low population density of *F. wislizeni* (Hickman 1978), host plant fidelity by the ants, and the defense of neighboring plants by distinct colonies. Ants could nonetheless increase outcrossing rates if pollinators increase their among-plant movements in response to the threat of predation (Altshuler 1999) or nectar robbing (Maloof and Inouye 2000) without diminishing pollen transfer. This study does suggest that bees leave flowers occupied by ants more quickly, although any effects on pollen transfer rates have yet to be studied. Any such effect, however, would not change the conclu-sions regarding maternal plant function drawn from the comparisons among ant species. The effects I attribute to the ants may also be influenced by external variables. For example, ant populations are spatially heterogeneous and *S. xyloni* is disproportionately common on low-elevation plants. However, the low-elevation plants tended by *S. xyloni* have 30% fewer pollinator visits (2003-04 data pooled) and 30% less seed mass per fruit, relative to low elevation plants tended by the other three species. The similar size of focal plants and inflores-cences visited by different ants, and the significant within-plant changes in pollination rate when tended by different ant associates, implicate ant identity as an influential source of heterogeneity among plants.

Characterizing the ant attendants of *F. wislizeni* as purely beneficial or purely detrimental to the plant is misleading. Just as nominal plant 'parasites', such as floral larcenists or flower-dwelling predators of pollina-tors, can have indirect positive effects on plants (Maloof and Inouye 2000, Irwin et al. 2001, Romero and Vasconcellos-Neto 2004), the ant guards of *F. wislizeni* can have indirect costs to the plant (in addition to the direct cost of providing EFN). The benefits and indirect costs of aggressive bodyguards may occur at different stages, or affect different components of plant fitness (Altshuler 1999). For example, the most aggressive ants increase the survival rate of reproductive units relative to plants with milder bodyguards (Ness et al. 2006), whereas the costs incurred by pollinator deterrence may be manifested in decreased seed production per surviving fruit. The very characteristics that make *S. xyloni* the most effective bodyguard in one context (i.e. high abundance and aggressiveness) can increase its ecological costs in another. This differs from studies of partner conflict in other ant–plant mutualisms, wherein the 'parasitic' ants that attack flowers are also poorer bodyguards than are alternative partners (Yu and Pierce 1998, Stanton et al. 1999). Because total seed production is the product of fruit production, seeds per fruit, and fruit survival, gains in one could offset losses in another at the whole plant level (Louda 1982, Altshuler 1999). As the relative importance of protection versus pollination varies in time and space due to variation in plant vulnerability, pollen limitation, etc., the identity of the 'best' ant mutualist could also change.

References

Agrawal, A. A., Rudgers, J. A., Botsford, L. W. et al. 2000. Benefits and constraints on plant defense against herbivores: spines influence the legitimate and illegitimate flower visitors of yellow star thistle, *Centaurea solstitialis* L. (Astercaeae). – S. W. Nat. 45: 1–5.

Altshuler, D.L. 1999. Novel interactions of non-pollinating ants with pollinators and fruit consumers on a tropical forest. – Oecologia 119: 600–606.

Bowers, J. E. 2000. Does *Ferocactus wislizeni* (Cactaceae) have a between-year seed bank? – J. Arid. Environ. 45: 197–205.

Brody, A. K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. – Ecology 78: 1624–1631.

Cariveau, D., Irwin, R. E., Brody, A. K. et al. 2004. Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. – Oikos 104: 15–26.

Carroll, C. R. and Janzen, D. H. 1973. Ecology of foraging by ants. – Annu. Rev. Ecol. Syst. 4: 231–257.

Clark, C. W. and Dukas, R. 1994. Balancing foraging and antipredator demands: an advantage of sociality. – Am. Nat. 144: 542–548.

Dukas, R. 2001. Effects of perceived danger on flower choice by bees. – Ecol. Lett. 4: 327–333.

Dukas, R. 2005. Bumble bee predators reduce pollinator density and plant fitness. – Ecology 86: 1406–1410.

Dukas, R. and Morse, D. H. 2003. Crab spiders affect flower visitation by bees. – Oikos 101: 157–163.

Fowler, H. G. and Whitford, W. G. 1982. Floral visitation by Chihuahuan Desert ants. – J. Nat. Hist. 16: 879–881.

Fritz, R. S. and Morse, D. H. 1981. Nectar parasitism of *Asclepias syriaca* by ants: effects on nectar levels, pollonia insertion, pollinaria removal and pod production. – Oecologia 50: 316–319.

Galen, C. 1983. The effects of nectar thieving ants on seedset in floral scent morphs of *Polemonium viscosum*. – Oikos 41: 245–249.

Galen, C. 1999. Flowers and enemies: predation by nectar-thieving ants in relation to variation in floral form of an alpine wildflower, *Polemonium viscosum*. – Oikos 85: 426–434.

Galen, C. 2005. Catching flies with honey: an experimental test of distraction and satiation as alernative modes of escape from flower damaging ants. – Oecologia 114: 80–87.

Galen, C. and Butchart, B. 2003. Ants in your plants: effects of nectar-thieves on pollen fertility and seed-siring capacity in the alpine wildflower, *Polemonium viscosum*. – Oikos 101: 521–528.

Ghazoul, J. 2001. Can floral repellents pre-empt potential ant-plant conflicts? – Ecol. Lett. 4: 295–299.

Goldberg, D. E. and Turner, R. M. 1986. Vegetation change and plant demography in permanent plots in the Sonoran desert. – Ecology 67: 695–712.

Gomez, J. M. 2003. Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. – Am. Nat. 162: 242–256.

Guerrant, E. O. and Fiedler, P. L. 1981. Flower defenses against nectar-pilfering by ants. – Biotropica 13 (Reprod. Bot. Suppl.): 25-31.

Harley, R. 1991. The greasy pole syndrome. – In: Huxley, C. R. and Cutler, D. F. (eds), Ant-plant interactions. Oxford Univ. Press.

Hickman, J. C. 1978. Pollination by ants: a low-energy system. – Science 184: 1290–1292.

Hull, D. A. and Beattie, A. J. 1988. Adverse effects on pollen exposed to *Atta texana* and other North American ants: implications for ant pollination. – Oecologia 75: 153–155.

Irwin, R. E., Brody, A. K. and Waser, N. M. 2001. The impact of floral larceny on individuals, populations, and communities. – Oecologia 129: 161–168.

Koptur, S. 1992. Extrafloral nectary mediated interactions between ants and plants. – In: Bernays, E. A. (ed.), Insect-plant interactions. CRC Press.

Louda, S. M. 1982. Inflorescence spiders: a cost/benefit analysis for the host plant, *Haplopappus venetus* Blake (Asteraceae). – Oecologia 55: 185–191.

Maloof, J. E. and Inouye, D. W. 2000. Are nectar robbers cheaters or mutualists? – Ecology 81: 2651–2661.

Mandujano, M. D. C., Montana, C. and Eguiarte, L. E. 1996. Reproductive ecology and inbreeding depression in *Opuntiae rastrera* (Cactaceae): why are sexually derived recruitments so rare? – Am. J. Bot. 83: 63–70.

McGregor, S. E. and Alcorn, S. M. 1959. Partial self-sterility of the barrel cactus. – Cactus Succulent J. Am. 31: 88.

McIntosh, M. E. 2002. Plant size, breeding system, and limits to reproductive success in two sister species of *Ferocactus* (Cactaceae). – Plant Ecol. 162: 273–288.

Morris, W. F., Wilson, W. G., Bronstein, J. L. et al. 2005. Environmental forcing and the temporal dynamics of a competitive guild of cactus-tending ants. – Ecology 86: 3190–3199

Mothershead, K. and Marquis, R. J. 2000. Fitness impacts of herbivory through indirect effects on plant–pollinator interactions in *Oenothera macrocarpa*. – Ecology 81: 30–40.

Ness, J. H., Morris, W. F. and Bronstein, J. L. 2006. Variation in mutualistic potential among ant species tending extrafloral nectaries of *Ferocactus wislizeni*. – Ecology, in press

Norment, C. J. 1988. The effect of nectar-thieving ants on the reproductive success of *Frasera speciosa* (Gentianaceae). – Am. Midl. Nat. 120: 331–336.

O'Dowd, D. J. and Catchpole, E. A. 1983. Ants and extrafloral nectaries: no evidence for plant protection in *Helichrysum* spp. – ant interactions. – Oecologia 59: 191–200.

Oliveira, P. S. and Brandão, C. R. F. 1991. The ant community associated with extrafloral nectaries in the Brazilian cerrados. – In: Huxley, C. R. and Cutler, D. F. (eds), Ant–plant interactions. Oxford Univ. Press.

Pickett, C. H. and Clark, W. D. 1979. The function of extrafloral nectaries in *Opuntia acanthocarpa* (Cactaceae). – Am. J. Bot. 66: 618–625.

Puterbaugh, M. N. 1998. The roles of ants as flower visitors: experimental analysis in three alpine plant species. – Oikos 83: 36–46.

Raine, N. E., Willmer, P. and Stone, G. N. 2002. Spatial structuring and floral avoidance behavior prevent ant-pollinator conflict in a mexican ant-acacia. – Ecology 83: 3086–3096.

Rico-Gray, V. 1993. Use of plant-derived food resources by ants in the dry tropical lowlands of Veracruz, Mexico. – Biotropica 25: 301–315.

Romero, G. Q. and Vasconcellos-Neto, J. 2004. Beneficial effects of flower-dwelling predators on their host plant. – Ecology 85: 446–457.

Roubik, D. W. 1982. The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. – Ecology 63: 354–360.

Stanton, M. L., Palmer, T. M., Young, T. P. et al. 1999. Sterilizations and canopy modification of a swollen thorn acacia by a plant-ant. – Nature 401: 578–581.

Strauss, S. Y. 1997. Floral characteristics link herbivores, pollinators and plant fitness. – Ecology 78: 1640–1645.

Strauss, S. Y. and Murch, P. 2004. Towards an understanding of the mechanisms of tolerance: compensating for herbivore damage by enhancing a mutualism. – Ecol. Entomol. 29: 234–239.

Strauss, S. Y., Siemanns, D. H., Decher, M. B. et al. 1999. Ecological costs of plant resistance to herbivores in the currency of pollination. – Evolution 53: 1105–1113.

Strauss, S. Y., Rudgers, J. A., Lau, J. A. et al. 2002. Direct and ecological costs of resistance to herbivory. – Trends Ecol. Evol. 17: 278–285.

Suttle, K. B. 2003. Pollinators as mediators of top-down effects on plants. – Ecol. Lett. 6: 688–694.

Tsuji, K., Hasyim, A., Nakamura, H. et al. 2004. Asian weaver ants, *Oecophylla Smaragdina*, and their repelling of pollinators. – Ecol. Res. 19: 673–699.

Visser, D., Wright, M. G. and Giliomee, J. H. 1996. The effect of the argentine ant, *Linepithema humile* (Mayr) (Formicidae), on flower-visiting insects of *Protea nitida* Mill. (Proteaceae). – Afr. Entomol. 285–287.

Wagner, D. and Kay, A. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. – Evol. Ecol. Res. 4: 293–305.

Willmer, P. G. and Stone, G. N. 1997. How aggressive ant-guards assist seed-set in Acacia flowers. – Nature 388: 165–167.

Yu, D. L. and Pierce, N. E. 1998. A castration parasite of an ant-plant mutualism. – Proc. R. Soc. 265: 375–382.