

## INTEGRATING QUALITY AND QUANTITY OF MUTUALISTIC SERVICE TO CONTRAST ANT SPECIES PROTECTING *FEROCACTUS WISLIZENI*

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**Abstract.** Generalized, facultative mutualisms are often characterized by great variation in the benefits provided by different partner species. This variation may be due to differences among species in the quality and quantity of their interactions, as well as their phenology. Many plant species produce extrafloral nectar, a carbohydrate-rich resource, to attract ant species that can act as “bodyguards” against a plant’s natural enemies. Here, we explore differences in the quality and quantity of protective service that ants can provide a plant by contrasting the four most common ant visitors to *Ferocactus wislizeni*, an extrafloral nectary-bearing cactus in southern Arizona. The four species differ in abundance when tending plants, and in the frequency at which they visit plants. By adding surrogate herbivores (*Manduca sexta* caterpillars) to plants, we demonstrate that all four species recruit to and attack potential herbivores. However, their per capita effectiveness in deterring herbivores (measured as the inverse of the number of workers needed to remove half of the experimentally added caterpillars) differs. Using these among-species differences in quality (per capita effectiveness) and quantity (number of workers that visit a plant and frequency of visitation), we accurately predicted the variation in fruit production among plants with different histories of ant tending. We found that plant benefits (herbivore removal and maturation of buds and fruits) typically saturated at high levels of ant protection, although plants could be “well defended” via different combinations of interaction frequency, numbers of ant workers per interaction, and per capita effects. Our study documents variation among prospective mutualists, distinguishes the components of this variation, and integrates these components into a predictive measure of protection benefit to the plant. The method we used to average saturating benefits over time could prove useful for quantifying overall service in other mutualisms.

**Key words:** ant; *Ferocactus wislizeni*; maximum likelihood; Michaelis-Menten; mutualism; plant defense; protection; Sonoran Desert.

### INTRODUCTION

Mutualisms, interspecific interactions that benefit both participants, are typically open to participation by a variety of partner species. However, partner species may differ in the benefits they provide to a shared mutualist, as shown in mutualisms involving a trade of food for pollination (e.g., Schemske and Horvitz 1984, Herrera 1987, 1989, Morris 2003), for seed dispersal (e.g., Schupp 1993, Ness et al. 2004), and for protection against natural enemies (e.g., Inouye and Taylor 1979, Horvitz and Schemske 1984, Koptur 1984, Rico-Gray and Thien 1989, Cronin 1998, Del-Claro and Oliveira 2000, Stanton 2003, Djieto-Lordon et al. 2004). These differences may arise from variation in the quality and/or quantity of service provided by a partner (Herrera 1987, 1989). By “quality,” we mean the benefit received by a single individual when it interacts with a single partner individual. By “quantity,” we mean the frequency of

interaction between the two species or the number of individuals involved in an interaction when it occurs.

Determining the overall benefit that each partner species provides requires that we integrate the quality and quantity components of service. However, mutualist quality and quantity are typically not independent. As the quantity of service (i.e., the number or frequency of interactions) increases, quality (i.e., the benefit provided at each interaction) will often decline, usually in a nonlinear fashion. Declining per interaction benefits arise simply because there is a maximum benefit that a focal individual can receive (Holland et al. 2002); consequently, excess interactions will be redundant. For example, a pollinator’s visit will not increase seed production if previous visitors have already fertilized all the ovules. Similarly, excess bodyguards beyond those needed to remove all natural enemies can be redundant, because each natural enemy can only be killed once. Excess interactions can even decrease net benefits (e.g., pollinating seed–parasite mutualisms [Holland et al. 2002] or mycorrhizal associations [Gange and Ayres 1999]). When the quality and quantity of service are not independent, we cannot determine the total service by

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multiplying a constant quality of service by quantity (as proposed by Herrera 1987, 1989). Instead, we must multiply the quantity of service by a quality of service that is a nonlinear function of quantity. Here, we quantify the nonlinear relationship between the quality and quantity of mutualistic service, and offer an integrated measure of total service provided by different partner species.

We expect differences among partners to be particularly pronounced for mutualists that offer rewards accessible to a variety of members of the surrounding community. For example, extrafloral nectar (hereafter, EFN) can be eaten by “practically any ant that encounters it” (Carroll and Janzen 1973), and the average EFN-bearing plant species is visited by six to nine ant genera (Oliveira and Brandão 1991). EFN-visiting ants often protect plants from herbivores, although interspecific differences among the ant partners can have significant effects on plant fitness (Horvitz and Schemske 1984, Rico-Gray and Thien 1989, Djieto-Lordon et al. 2004) and perhaps on selection pressures fostering the mutualism (Rudgers 2004). Here, we use our integrated measure of total service to assess how the quality and quantity of protection provided by different ant species contribute to their effectiveness as bodyguards.

We focus on the ant assemblage associated with *Ferocactus wislizeni* (Cactaceae), an EFN-bearing cactus of the Sonoran Desert. Although EFN-bearing cacti are common in desert ecosystems (Lloyd 1908, Pemberton 1988) and the diversity of ants attracted to individual species has been noted to be quite high (Blom and Clark 1980, Oliveira et al. 1999), little is known about the benefits that EFN visitors confer (but see Pickett and Clark 1979, Oliveira et al. 1999). The *F. wislizeni* system has several characteristics that make it amenable to studying the links between the quality and quantity of protection provided by different ant species. Extrafloral nectaries are spatially concentrated due to the plant's simple architecture (making it possible to observe ant behaviors and count every ant on the plant); ant species are readily distinguishable in the field; and ants are sufficiently competitive that generally only one ant species visits a plant at a time (although species turnovers occur frequently).

We sought to distinguish ant species tending barrel cactus EFN by the following features that may influence their value as plant bodyguards: (1) frequency of interaction with plants over time; (2) number of ants present during an interaction; (3) ability to recruit quickly to herbivores arriving on a plant; and (4) per capita effectiveness in removing herbivores as a function of the number of ants present. By incorporating species differences in protection that can occur as a result of differences in quantity and quality of service, we predicted the average protection each ant species confers to a cactus when it is present, and successfully linked these among-species differences to variation in plant reproduction.

## METHODS

### *Study system*

The fishhook barrel cactus, *Ferocactus wislizeni* (Cactaceae), ranges from southern Arizona and southeastern California to northern Sonora, Mexico. A ring of areoles on the crown of the plant, the site of flower and fruit production, bears modified spines that exude extrafloral nectar. Our study was conducted at the Desert Research Laboratory (Tumamoc Hill) in Tucson, Arizona, USA (32°13' N, 111°05' W). Our study plots ranged from 750 to 900 m elevation. Average rainfall (1904–1980) is <300 mm/yr, and midday temperatures >40°C are common from June to September. The site has been protected from livestock grazing since 1907. Dominant plants include *Larrea tridentata*, *Cercidium microphyllum*, *Carnegie gigantea*, *Opuntia*, and *Acacia* species (Goldberg and Turner 1986).

Four omnivorous ant species were frequently observed at *F. wislizeni* EFN: *Crematogaster opuntiae* (Myrmicinae; see Plate 1), *Solenopsis aurea*, *S. xyloni* (Myrmicinae), and *Forelius* sp. (Formicinae). The role of the individual species as plant protectors is not yet known, although the exclusion of all ants from plants increases bud mortality rates (J. H. Ness, unpublished data). *Crematogaster opuntiae* can decrease herbivore occupation on *Opuntia* cacti (Pickett and Clark 1979). To our knowledge, interactions between the other three species and EFN-bearing plants have not been studied.

### *Attendant ants: frequency and abundance*

We monitored ant visitors to 259 marked plants at the study site biweekly from January to December 2003. We used inspections during the diurnal hours with the mildest temperatures (mornings and/or evenings in the summer, midday during cooler months) to identify the ant species associated with individual plants. We chose these periods after an hourly census of 16 plants over 15 hours demonstrated that EFN-collecting ants are minimally active during the warmest portion of summer days in this site (see also Blom and Clark 1980, Oliveira et al. 1999), and that the ant identity on individual plants did not change over that duration. We recorded the identity of each ant on the top of the plant (i.e., in the vicinity of EFNs) during all inspections ( $n = 6661$  inspections), and recorded worker abundance on 4063 occasions (82% of the inspections when ants were found). We used two-sample Kolmogorov-Smirnov tests to contrast the distributions of ant numbers for the four species. We continued the biweekly survey of the marked plants throughout 2004 ( $n = 7084$  inspections). However, due to time limitations, we only recorded the identities of ants on each plant.

### *Contrasting the per capita effectiveness of ants as bodyguards*

We evaluated ant aggressiveness toward herbivores by adding laboratory-reared second and third instar *Manduca sexta* (Lepidoptera: Sphingidae) larvae to ant-



PLATE 1. *Crematogaster opuntiae* ants collecting *Ferocactus wislizeni* extrafloral nectar and attacking surrogate herbivores (*Manduca sexta* caterpillars). The photo is centered on two workers attacking a caterpillar, and single workers are visiting extrafloral nectaries at the left, bottom, and top of the photo. Surrogate herbivores were added to plants to contrast the per capita effectiveness of different ant species in their roles as plant protectors. Photo credit: W. G. Wilson.

tended plants. *Manduca sexta* does not feed on *F. wislizeni* in nature; however, these instars are approximately the same size ( $\sim 1.25$  cm) as the mid- to late-instar larvae of the pyralid moth *Pseudoschinia elautis* that attacks the immature fruit of *F. wislizeni* at other sites in southern Arizona (McIntosh 2002). The use of surrogate herbivores is well established in studies of protection mutualisms (see *Discussion*). We placed 10 *M. sexta* caterpillars that had been reared on a standard artificial diet in the laboratory on the top of each barrel cactus. The numbers of incapacitated caterpillars and ants on the plant were monitored every 5 minutes for 30 minutes. Incapacitated caterpillars were either killed by the ants or knocked off the plant (see Plate 1). The number of ants on the plants varied over the course of a trial (see *Results*), and we focus our analyses on two numbers: (1) the maximum number of ants on the plant prior to the incapacitation of the 10th caterpillar or, if some caterpillars survived, the maximum number observed during the trial; and (2) the number of ants on the plant prior to the addition of caterpillars. The former value provides an estimator of each ant's capacity to kill caterpillars; the latter takes into account any recruitment of nestmates that occurs, and how this recruitment may vary with pretreatment abundance.

We used the method of maximum likelihood, assuming binomially distributed errors, to fit the following Michaelis-Menten equation describing  $F$ , the fraction of

the 10 caterpillars that were incapacitated by ants during a trial, as a function of  $A$ , the number of ants observed on the cactus:

$$F = \frac{A}{b + A} \quad (1)$$

where  $b$  is the number of ants at which half of the caterpillars were incapacitated in 30 min. If  $A \ll b$ , then  $F \approx (1/b)A$ ; hence, the reciprocal of  $b$  measures the per capita ant effectiveness when ant numbers are low. As  $A$  increases,  $F$  approaches 1 (i.e., the protection benefit is predicted to saturate at high ant numbers). We first fitted Eq. 1 to the data for each ant species separately to estimate species-specific values of  $b$  (and their associated log likelihoods). We then used a likelihood ratio test to evaluate the null hypothesis that the policing abilities of the different ant species did not differ. Specifically, we computed the log likelihood of Eq. 1 fit to the data for all four species simultaneously, which assumes  $b$  does not differ among species. We then calculated twice the difference between this log likelihood and the sum of the log likelihoods over all ant species. If this value exceeds  $\chi^2_{0.05}(4)$ , the value of the chi-square distribution with  $P = 0.05$  and four degrees of freedom (equal to the number ant species), then  $b$  differs significantly among species (Hilborn and Mangel 1997).

We performed two smaller experiments to test whether changes in ant and caterpillar abundance were attribut-

able to the presence of one another. We monitored a subset of control plants without caterpillars added, to clarify that any change in ant abundance was due to the presence of caterpillars rather than observers. We also added 10 caterpillars to plants without ants, to estimate the rate that caterpillars might abandon the plants or be removed by other predators in the absence of ants.

*Integrating quantity and quality components of ant effectiveness to predict protection*

For each ant species, we computed a measure of total protection service that integrates the quantity and quality components of service. For each observation of a given ant species in the 2003 surveys, we substituted the observed number of workers and that ant's maximum likelihood estimate of  $b$  into Eq. 1 to obtain a measure of potential herbivore removal for that observation. We then averaged these values over all observations of that ant species. As Eq. 1 is a nonlinear (convex) function of the number of workers, the alternative procedure of substituting the average number of workers observed across surveys into Eq. 1 would overestimate the average protection a plant would receive when the ant species is present. Because the survey describes the number of ants present prior to any herbivore-induced response, we used the values of  $b$  derived from abundance data before caterpillars were added. To gauge the likely range of total protection, we also estimated average protection as described previously but using the upper and lower 95% confidence limits of  $b$  for each ant species. Because the observed number of workers on occupied plants was less in April–June than in July–September (see *Results*), estimates for those two periods were separated.

*Ant effectiveness and plant reproduction*

We counted the numbers of floral buds, flowers that failed to produce fruits, and mature fruits on each of the 259 plants on 1–3 October 2004. To measure plant reproductive success during the bud maturation phase (April–June), we computed the survival of buds to flowering as the ratio of the observed number of floral buds that flowered (i.e., the sum of senesced and/or abscised flowers and mature fruits on each plant in October) to the number of buds each plant was expected to produce. We estimated the expected number of buds for each plant based on a linear relationship with plant diameter (see also McIntosh 2002). At our site, this relationship was best described by the equation: number of buds produced =  $1.6(\text{diameter}) - 22.8$ , and we omitted plants too small to produce buds from subsequent analyses. We also counted the herbivorous insects on plants during the April–June surveys, to contrast ant-tended and untended plants. From July through October, flowers open and fruits develop. To measure reproductive success during this fruit maturation period, we computed the fraction of flowers that developed into mature fruits (i.e., the ratio of fruits to fruits plus flowers in October).

We computed a protection index for each plant that accounted for all ant species observed on that plant. Specifically, for each of the 2004 survey dates for each plant, we took the average service (as calculated in Methods: Integrating quantity and quality) provided by the ant species that was present at that survey (or zero if no ants were present) and averaged those values over all surveys. Although it would have been preferable to apply Eq. 1 to the actual number of workers at each survey, the fact that we only recorded presence/absence of ant species in 2004 forced us to assume that the average service was provided whenever a given ant species was present. In the rare cases when other ant species were found on plants, we substituted values for *Forelius* (the most common “subordinate” ant).

For statistical analyses, we organized the plants into groups in two ways. The first organized plants into: a group that included plants never tended by ants (where the Michaelis-Menten  $F=0$ ), a group of the best defended plants (i.e., plant always tended by the ant species expected to kill the greatest fraction,  $F$ , of herbivores), and groups based on 10% incremental differences in their protection index relative to the best-defended plant. The average protection and plant performance for each group were used in the analyses. Groups with fewer than three individual plants were pooled for analyses. The second approach organized plants by their most common ant attendant. We classified each plant as being tended by a particular ant species if that species was foraging on the plant on at least half of the surveys we performed during each period (bud maturation vs. flowering/fruitletting), and as untended if no ants were observed on the plant on at least half of those surveys. All plants were thus designated as tended by one of the four ant species, untended, or tended by a mixed assemblage (i.e., no one species tended the plant during greater than half of the surveys). Mean protection index and reproduction in each group were used in the analyses described in the following paragraph. Note that for a plant tended predominantly by, say, *C. opuntiae*, this second approach uses the protection provided by other ant species for those survey dates when ants other than *C. opuntiae* were present, and zeros when no ants were present.

We used linear regressions to test the hypotheses that bud production and the percentage of flowers that failed to mature into fruits were related to our indices of ant protection. When analyzing bud production, we used the protection index for April through June as the independent variable, and when analyzing the percentage of flowers setting fruit we used the index from July through September. We evaluated the hypothesis that fewer herbivorous insects occupied plants tended by ants, relative to untended plants, with a one-sided  $t$  test.

Last, we asked whether a plant's success over the entire reproductive period, including both the bud maturation and flowering/fruitletting phases, was influenced by which ant species was the predominant visitor to the plant over that period. We used the survey data from April to September to estimate as before the protection conferred

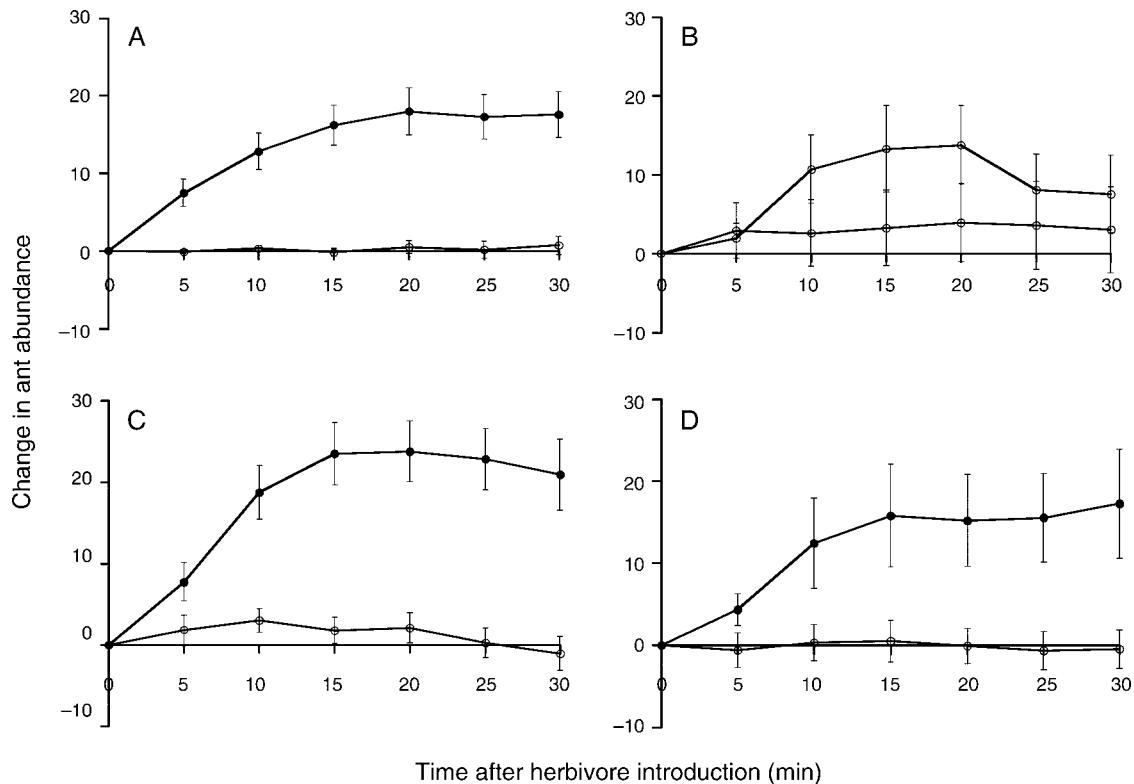


FIG. 1. The change in ant abundance on *Ferocactus wislizeni* plants after the addition of 10 *M. sexta* caterpillars (mean  $\pm$  SE). The difference between ant abundance at time  $X$  and time zero is shown. Solid circles indicate treatment plants; open circles indicate control plants without caterpillars added. The species of plant-occupying ant is: (A) *Crematogaster opuntiae*, (B) *Solenopsis xyloni*, (C) *S. aurea*, and (D) *Forelius* sp.

by the ants. We used the ratio of surviving fruits produced to the number of buds expected based on plant size as the dependent variable. As before, we used linear regressions to compare how mean reproductive success changed with mean protection by ants.

## RESULTS

### *Attendant ants: frequency and abundance*

Plants were occupied by only one ant species at a time in 94% and 95% of the inspections at which ants were observed in 2003 and 2004, respectively. Ant turnovers were common, and 92% of the plants hosted more than one ant species over the course of the 2003 survey ( $3.3 \pm 1.4$  species/plant, mean  $\pm$  SD; maximum = 8 species/plant). *Crematogaster opuntiae* was the ant most commonly observed at EFN (55% and 44% of the plant censuses in which ants were found in 2003 and 2004, respectively). *Solenopsis* spp. were the next most common (*S. aurea*, 22% and 16%; *S. xyloni*, 13% and 26%, for 2003 and 2004, respectively), and *Forelius* sp. was the rarest of the four common species (9% and 12%). Other ant species (*Camponotus ocreatus*, *C. festinatus*, *Dorymyrmex* sp., *Myrmecocystus* sp., and *Monomorium* sp.), accounted for <6% of all visits, as a group.

The distribution of ant numbers on occupied plants differed significantly among all four species during

April–June, with one exception (two-sample Kolmogorov–Smirnov tests,  $P < 0.01$  in all cases except *S. aurea* vs. *S. xyloni* where  $P = 0.36$ ). In June–September, the distribution of *Forelius* differed from the other three species ( $P < 0.0012$ ) although those three did not differ from each other ( $P > 0.12$ ). The mean, median, and maximum numbers of workers observed in July–September were greater than in April–June for each species (Appendix A).

### *Contrasting the per capita effectiveness of ants as bodyguards*

All four ant species increased in abundance after the addition of caterpillars to the plant, and the magnitude of the increase did not differ among species (repeated-measures ANOVA; species main effect,  $F = 0.98$ ,  $df = 3$ , 135,  $P = 0.4$ ; species  $\times$  time interaction,  $F = 0.69$ ,  $df = 15$ , 675,  $P = 0.8$ ; time main effect,  $F = 16.22$ ,  $df = 5$ , 675,  $P = 0.0001$ ). Increases were not observed on control plants without caterpillars (Fig. 1). The number of ants required to incapacitate five caterpillars within 30 min ( $b$ ) differed among the four species, whether that number was estimated using the initial number of ants on the plants (log likelihood ratio = 18.5,  $df = 4$ ,  $P < 10^{-5}$ ) or the maximum number of ants (log likelihood ratio = 30.7,  $df = 4$ ,  $P < 10^{-5}$ ). *Solenopsis xyloni* had the highest per

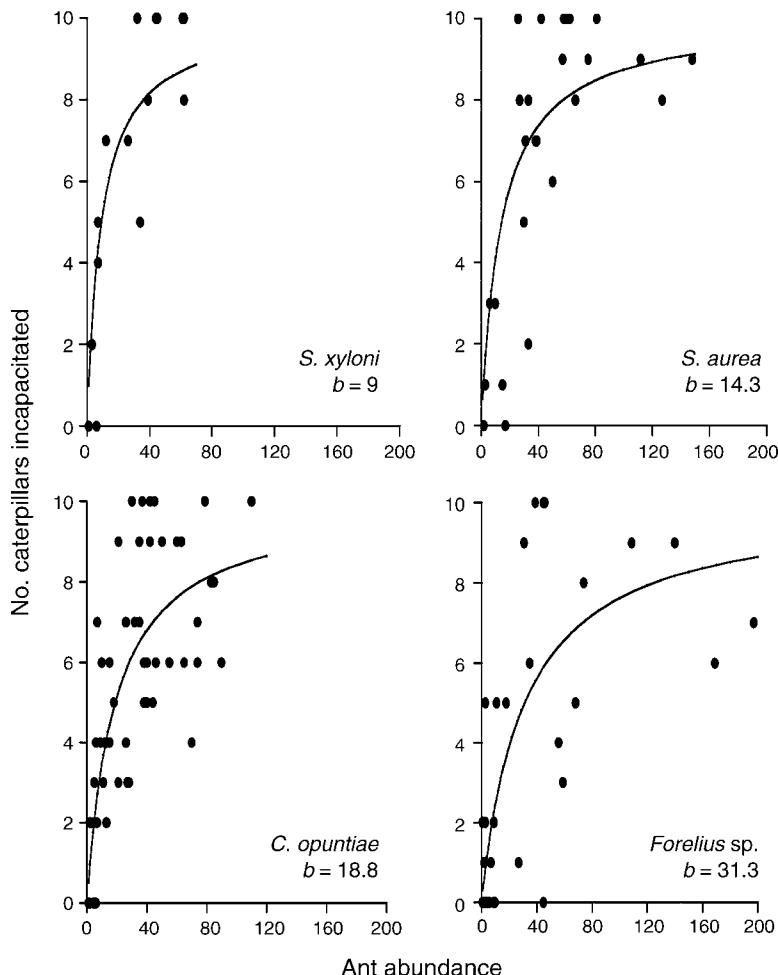


FIG. 2. The relationships between maximum ant abundance on a plant and the number of caterpillars incapacitated in 30 minutes for the four most common species of ant bodyguards. Separate Michaelis-Menten equations were fit for each of four species. The Michaelis-Menten constant,  $b$ , indicates the number of ants necessary to incapacitate half the caterpillars. Each point represents one plant. See Appendix B for summary statistics.

capita effectiveness at low numbers (i.e.,  $1/b$ ), followed by *S. aurea*, *C. opuntiae*, and *Forelius* sp. (Fig. 2; Appendix B). For any given ant species, the maximum likelihood estimate of  $b$  using the maximum number of ants observed lay outside of the 95% confidence interval of  $b$  for any other species (Appendix B); therefore, all species differed significantly in terms of per capita effectiveness. In contrast, the maximum likelihood estimates and 95% confidence intervals of  $b$  using the initial number of ants indicated that *Forelius* differed from *S. xyloni*, *S. aurea*, and *C. opuntiae* (Appendix B), but that the latter three ant species were not statistically distinguishable. Nearly all the 10 caterpillars added to plants without ants were still on the plants after 30 min ( $9.64 \pm 0.17$  caterpillars/plant, mean  $\pm$  SE,  $n = 14$  plants).

*Integrating quantity and quality components of ant effectiveness to predict protection*

Plants were predicted to receive the greatest average protection when tended by *S. xyloni*, a result attribut-

able to that ant's relatively high abundance when visiting plants (Appendix A) and high per capita aggressiveness (i.e.,  $1/b$ ; Fig. 2). The average protection conferred by *S. aurea* and *C. opuntiae* were similar, and all three species conferred greater protection, on average, than did *Forelius* sp. (the ant both least aggressive and least abundant during visits; Appendix B). This hierarchy was consistent whether the fraction of caterpillars killed is estimated using the maximum likelihood estimate of  $b$ , or using the upper or lower 95% confidence limits. It was also consistent across April–June and July–September, although each species provided greater protection in the later season due to more workers per visit during that time.

*Ant effectiveness and plant reproduction*

Plants that were tended by ants for at least half of the April–June surveys produced more buds than did plants not tended by ants for greater than one-half of the surveys (Appendix B; one-sided  $t$  test assuming unequal

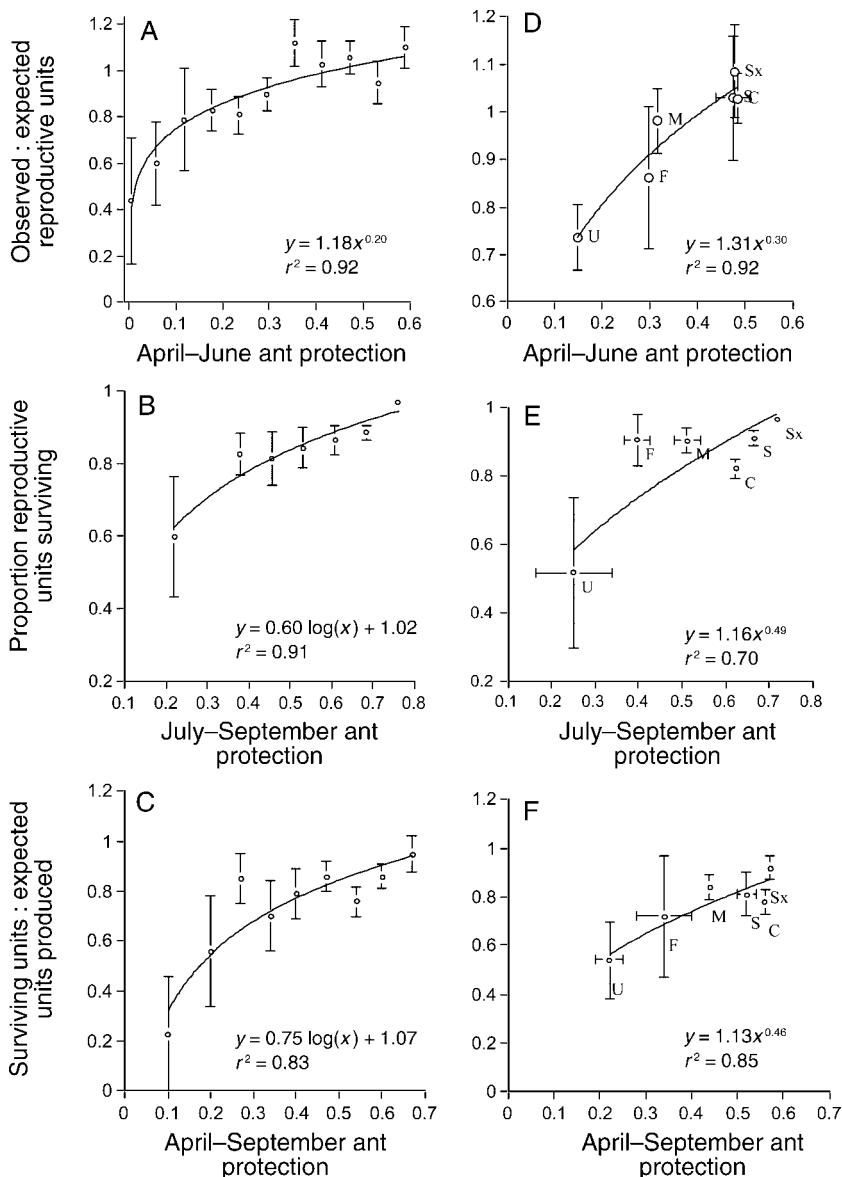


FIG. 3. Components of reproduction for plants that differ in protection received from ants. Protection is estimated as the proportion of 10 caterpillars expected to be removed from the plant in 30 minutes, based on the tending history of plants. Plants are grouped by the level of protection conferred by all ant bodyguards in panels A, B, and C, and by the identity of the most common tending ant in panels D, E, and F (codes: C, *C. opuntiae*; F, *Forelius* sp.; Sa, *S. aurea*; Sx, *S. xyloni*; U, untended plants; M, mixed plants, which none of the four ant species tended on more than half of the surveys). Best-fit lines were drawn through group means ( $\pm$ SE). (A) Variation in the ratio of observed to expected buds on plants, as a function of ant protection in April–June (a period of bud production and maturation). Where the ratio exceeds 1, plants are producing more reproductive units than expected, based on their size. (B) Variation in the proportion of reproductive units lost (to abortion, herbivory, and other factors) as a function of ant protection in July–September (a period of bud maturation, flowering, and fruit maturation). Ant protection is presented as in panel A. (C) Variation in fruit production linked to protection by ants from April to September. Fruit production is presented as the ratio of number of fruits produced to the number of buds expected to be produced based on plant size. Dependent and independent variables in panels D, E, and F follow panels A, B, and C, respectively.

variance,  $t = 3.51$ ,  $df = 83$ ,  $P = 0.0004$ ). During those surveys, phytophagous hemipterans, including *Cheliniidea vittiger*, *Leptoglossus phyllopus*, and *Narnia* sp., were five times more abundant on untended plants, relative to plants with ants at the time of inspection ( $0.02 \pm 0.001$  and  $0.12 \pm 0.06$  individuals/plant/survey, mean  $\pm$  SE;

one-sided  $t = 1.65$ ,  $df = 275$ ,  $P = 0.049$ ). Hemipterans were less abundant on plants tended by only *S. xyloni* at the time of inspection, relative to plants tended by any of the other three ant species (one-sided  $t = 2.57$ ,  $df = 469$ ,  $P = 0.005$ ). When protection was integrated for each plant from April to June, the ratio of observed to expected

buds increased with plant protection by ants, whether plants were grouped by integrated measures of protection (simple linear regression,  $F = 31.2$ ,  $df = 1, 9$ ,  $P = 0.0004$ ) or by their most common attendant ( $F = 33.4$ ,  $df = 1, 4$ ,  $P = 0.004$ ). Those relationships were best described by decelerating power functions (Fig. 3a, d, respectively).

Plants tended by ants in July–September matured a greater proportion of buds into mature fruits, relative to untended plants ( $0.89 \pm 0.03$  fruits/bud,  $n = 227$  plants and  $0.52 \pm 0.22$  fruits/bud,  $n = 4$  plants, respectively); one-sided  $t$  test assuming unequal variance,  $t = 1.79$ ,  $df = 4$ ,  $P = 0.07$ ). Differences in the mean proportion of buds surviving to fruit were accurately predicted ( $r^2 > 0.9$ ) whether plants were grouped by integrated measures of protection (simple linear regression,  $F = 28.4$ ,  $df = 1, 5$ ,  $P = 0.003$ ) or by their most common attendant ( $F = 5.73$ ,  $df = 1, 4$ ,  $P = 0.07$ ). Those relationships were best described by logarithmic (Fig. 3b) and decelerating power functions (Fig. 3e), respectively.

Ultimately, the production of mature fruit differed among plants with different ant tending histories from April to September. The magnitude of these differences was accurately predicted ( $r^2 > 0.8$ ) whether plants were grouped by integrated measures of protection (simple linear regression,  $F = 13.5$ ,  $df = 1, 7$ ,  $P = 0.008$ ; Fig. 3c) or by their most common attendant ( $F = 14.5$ ,  $df = 1, 4$ ,  $P = 0.02$ ; Fig. 3f). The relationships were best described by logarithmic and decelerating power functions, respectively.

#### DISCUSSION

The overall effect of the interactions between mutualists is determined by both interaction frequency and net effects per interaction event. In cases such as the interaction between ants and EFN-bearing plants, where each event may involve multiple individual ants from the same colony interacting with an individual plant, the net effect for the plant may depend on both the number of ants and their per capita effect. In our study, we found evidence of variation among potential mutualist species in their numbers of individuals per interaction event, per capita effects, and in the frequency of those interactions. Recognizing these several ways that ant partner species can differ helped us to predict variation in the reproductive output among plants based on their different histories of interactions.

Although it is well appreciated that prospective partner species can differ in their effectiveness as mutualists, the components of this variation are rarely dissected. For example, the ant bodyguards of plants are often ranked by a gestalt approach, one that combines observations of clearly aggressive behaviors (bites and sprays) with more ambiguous behaviors, such as recruitment to disturbance (e.g., Buckley and Gullan 1991, Itioka et al. 2000, Michelangeli 2003). A more quantitative approach involves adding surrogate herbivores that are available in abundance but do not damage plants to explore the degree of protection conferred by ant attendants (e.g., Inouye and Taylor 1979, Koptur 1984, Cronin 1998). This technique standardizes trials

with respect to herbivore size, condition, and abundance, although it is uncertain whether the interactions between an ant and these surrogates mimic interactions with the natural herbivore communities. We used the Michaelis-Menten function to quantify the per capita effectiveness of ants in finding and attacking surrogate herbivores, and to describe those species on a continuous (rather than ordinal) scale. This technique allowed us to assess the functional significance of differences among ant species, and facilitated accurate predictions regarding the protection these ants provide in the wild. Further, it illustrates that relatively few aggressive individuals and a larger number of milder ants can provide similar protection (Janzen 1972), particularly if the latter are more likely to discover herbivores (Koptur 1984). Last, this technique recognizes that the protective services conferred by ants saturates with increasing ant abundance (e.g., Inouye and Taylor 1979, Rocha and Bergallo 1992). Although our methodology places a ceiling on these benefits, because we only added 10 caterpillars per plant for the ants to subdue, this caterpillar density exceeds the density of native herbivores observed in the wild (McIntosh 2002). We chose to add surrogate herbivores at higher densities for ecological and methodological reasons. First, a surrogate herbivore may be easier for bodyguards to deter or kill than is a natural, and perhaps ant-adapted, herbivore (e.g., Freitas and Oliveira 1996). Second, because the Michaelis-Menten function relates the species-specific  $b$  to the proportion of a service completed,  $b$  can be estimated with greater accuracy when (1) the criteria for “complete service” (here, incapacitating all caterpillars) is more stringent, and (2) the proportions can be distinguished in progressively smaller increments.

Because the four ant species that interact most frequently with *F. wislizeni* at our site are found in different abundances, and differ in their per capita effectiveness when challenged to remove herbivores, we can describe a hierarchy of protection offered by these ant species when visiting the plants: *S. xyloni* > *S. aurea* = *C. opuntiae* > *Forelius* sp. (Appendix B). However, that hierarchy fails to incorporate the frequency at which those ants may defend the plants, a second component of service quantity (Herrera 1989). Contrast an ant species that visits plants regularly with a more sporadic attendant. For example, *C. opuntiae* and *S. xyloni* were present in 76% and 3% of the April–June 2003 surveys at which ants were present, respectively. If the service provided to the *F. wislizeni* population is the product of service per visit ( $F$  from Eq. 1) and visit frequency (proportion of all visits), the *C. opuntiae* population provided ~25 times as much protection as did the *S. xyloni* population in April–June, 2003. Incorporating visit frequency, then, changes the interpretation of the importance of *C. opuntiae*, an ant species that is an “average bodyguard” on a per visit basis but that interacts more frequently with *F. wislizeni*. Interaction frequency may be very important to herbivores that are deterred by ants yet remain capable

of returning to the plant (as in Inouye and Taylor 1979, Koptur 1984, Rocha and Bergallo 1992), in situations where new herbivores continually attack the plant, or where the presence of ants discourages oviposition by adult herbivores (Freitas and Oliveira 1996). Against these herbivores, the protection that ants provide the plant may saturate with the quantity of workers on the plant at any one time (a "visit") yet still increase continually with the frequency of visits.

Whether a visit by a given ant species truly confers protection to the plant may also be influenced by the correspondence between the phenologies of a plant's bodyguards and its natural enemies, because bodyguards may provide little benefit when plants do not need protection. Analogously, two pollinators can differ in quality simply because they visit flower stages of differing receptivity to pollen (e.g., Herrera 1987). We distinguished two three-month periods to account for the variation *F. wislizeni* may experience in vulnerability, worker abundance per interaction, and interaction frequency over time. Our results (Fig. 3) suggest that plants benefited from ant tending in both April–June and July–September, although estimated protection was greater in the later season, due to an increase in visit frequency, workers per visit, and more frequent visitation by *S. xyloni*. Many EFN-bearing plants, including a congener of *F. wislizeni* (Rufner and Clark 1986), can change the nectar rewards they offer among seasons, perhaps explaining some of the temporal variation in interaction frequency, worker abundance, and partner identity observed in this system (Morris et al. 2006). We note, however, that even if a greater proportion of plants are tended by *C. opuntiae* in one season or *S. xyloni* in another, that distinction may be of negligible importance to an individual plant if those species provide similar levels of protection. Further, the saturating relationship we find between ant protection and plant benefit suggest that the importance of variation in plant protection decreases as plants become progressively better defended.

We view the strong correlations between the protection provided by ants and the successful production of flowers and fruits by plants as evidence that the ant–cactus interaction is in fact a mutualism, in which ants benefit from a carbohydrate food source and cacti benefit by protection from natural enemies. An alternative hypothesis is that the relationship between plant reproduction and ant tending is coincidental rather than causal. Interestingly, reproduction by the subset of plants tended by rarer ant species (i.e., *Camponotus*, *Dorymyrmex*, *Myrmecosytus*, and *Monomorium* spp. as a group) in greater than half of the inspections was similar to that by untended plants ( $60 \pm 20\%$  of buds predicted from plant size, and  $61 \pm 12.4\%$  of buds became mature fruits;  $n = 4$  plants in April–June, 7 plants in July–September, respectively). The fact that a particular subset of ant-tended plants reproduce no better than do untended plants suggests that variation among ant species influences plant reproduction. This

variation may be due to among-ant differences in both the costs and benefits plants can incur by participating in the mutualism. Our current study explores how bodyguards deter herbivores and increase flower and fruit production, two positive effects on plant fitness components. A companion study (Ness 2006) explores whether the more aggressive ant species may also deter pollinators, perhaps thereby altering the quantity of pollen delivered to, or donated by, host plants, as well as seed production arising from those individual flowers. Although spatiotemporal variation in the relative magnitudes of these costs and benefits may be considerable (e.g., due to fluctuating herbivore and pollinator populations), we believe the net effects of this ant–plant interaction are typically positive.

If we view quantity and quality as the two axes along which multiple partner species in generalized mutualisms may differ, we must recognize that these two axes often will not be independent. Although we expect in general that resource limitation will cause quality to decline as quantity increases, details are likely to differ for different types of mutualism (Holland et al. 2002). Mutualisms may also differ in the components that constitute the quantity of interaction. For example, when animal partners forage singly, as in most pollination and seed dispersal mutualisms, the quantity of service is simply the number of individual partners arriving at a plant per unit time. In most ant protection mutualisms, both the proportion of time ants are present and the number of workers when present contribute to the quantity of interaction. We found evidence that the quality of protection service may saturate with an increasing quantity of service measured at both of these levels: herbivore removal saturates as the number of workers increase, and plant reproduction saturates as overall protection (which incorporates the proportion of time plants are tended) increases. The ways that quantity and quality of service interact offer an avenue for exploring similarities and differences between generalized mutualisms of different kinds. The quantitative approaches we have used to describe and integrate quantity and quality of service may therefore be useful in studying other types of mutualism.

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#### APPENDIX A

The number of ant workers foraging on *Ferocactus wislizeni* plants during April–June 2003 and July–September 2003 (*Ecological Archives* E087-053-A1).

#### APPENDIX B

Summary data distinguishing four ant species that visit the extrafloral nectaries of *Ferocactus wislizeni*, and reproduction on plants typically tended by those ants (*Ecological Archives* E087-053-A2).