For ant-protected plants, the best defense is a hungry offense

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Abstract. Animal foraging has been characterized as an attempt to maximize the intake of carbon and nitrogen at appropriate ratios. Plant species in over 90 families produce carbohydrate-rich extrafloral nectar (EFN), a resource attractive to ants and other omnivorous insects. This attraction can benefit the plant if those arthropods subsequently attack herbivores. This protective response has been attributed to the increased visitation and "ownership" of plants that provide a predictable source of fuel. Here, we propose and test an alternative (but non-mutually exclusive) hypothesis, that access to C-rich carbohydrates increases the ants' desire for N-rich protein and hence the likelihood that they will attack herbivorous insects on the host plant. This "deficit hypothesis" would be rejected if (1) EFN were itself a sufficiently balanced food source in terms of C and N, (2) ant dietary preferences were similar in the presence vs. absence of EFN, (3) protein-hungry ants were not more predaceous, or (4) ants provided access to protein were more aggressive toward potential prey items than were ants provided access to carbohydrates. We test these predictions in a protective mutualism between a guild of desert ants and the barrel cactus *Ferocactus wislizeni.* C:N ratios of EFN exceeded that of ants or potential prey items by an order of magnitude (i.e., EFN is an N-poor food for ants). Baiting studies demonstrated that plant-tending ant species recruited more workers to N-rich protein baits than to C-rich sugar baits; this difference was more pronounced when the ants had access to *F. wislizeni* EFN. From these data, we infer that protein is a valuable resource and that its relative value increases when carbohydrates are readily available. Moreover, ant colonies provided access to supplemental carbohydrates responded more aggressively to surrogate herbivores than did control colonies (to which no additional resources were provided) or colonies provided protein. These results support the predictions of the "deficit" hypothesis, wherein plant protection is elicited by plant-mediated dietary imbalances.

Key words: ants; barrel cactus; carbohydrates; C:N ratio; desert ant guild; Desert Laboratory, Tucson, Arizona, USA; extrafloral nectar; Ferocactus wislizeni; *foraging; mutualism; rewards.*

Introduction

Nutritional imbalances are prevalent in most food webs, and ecologists increasingly study interspecific and intraspecific interactions within the context of this variation (Elser et al. 2000, Cross et al. 2003, Grover et al. 2007). Autotrophs and consumers are characterized by dissimilar carbon to nitrogen ratios (hereafter C:N); C:N of carnivores and prey are more similar. Because consumer growth is progressively slowed as the C:N of its food and tissue become increasingly dissimilar (Elser et al. 2000), these differences offer challenges to herbivores and omnivores (Cook and Davidson 2006). These relationships between consumers and resources are often studied with respect to antagonistic interactions (e.g., Elser et al. 2000). Here, we focus on the consequences of these dissimilar ratios in mutually beneficial (mutualistic) consumer–resource interactions

(Holland et al. 2005) between plants and predaceous omnivores. Specifically, we test the hypothesis that ratios of carbon to nitrogen in plant-provided food rewards elicit behaviors that increase the benefits conferred to plants by their prospective mutualistic partners.

Omnivores collect carbohydrates from a variety of autotrophic organisms, with consequences that range from antagonistic to commensal to mutualistic. Plant-derived food resources contribute a significant fraction of the diet of many ant species (Tobin 1994, Davidson et al. 2003, Tillberg and Breed 2004), and >90 plant families attract ants to extrafloral nectaries (Koptur 1992), organs on the leaves, stems, and other nonreproductive plant tissues that exude carbohydrate-rich nectar (hereafter extrafloral nectar or EFN). These facultative ant–plant interactions are mutualistic when the omnivorous ants attack the plant's natural enemies, actions that can lead to an increase in plant survival or reproductive success. Such protective interactions are not limited to plants and ants; ants also protect certain other insects (e.g., hemipterans), and marine analogues

such as the interaction between *Pocillopora* corals and crab bodyguards (Stimson 1990, Pratchett 2001) also exist.

Three hypotheses that have been offered to explain this mutually beneficial interaction; we identify these as the ownership, fuel for foraging, and predictable rewards hypotheses. The ownership hypothesis (e.g., Janzen 1969, 1985, Davidson et al. 1988) proposes that valuable resources elicit ownership behavior by the omnivorous visitors, whose subsequent efforts to exclude other foragers so as to protect and dominate the resource benefits the host (here, the resources and other foragers are EFN and plant natural enemies, respectively). The fuel for foraging hypothesis (e.g., Carroll and Janzen 1973, Davidson 1998, Grover et al. 2007) proposes that the available carbohydrates provide fuel for metabolically expensive behaviors of omnivorous foragers, including heightened aggression and foraging for prey. The predictable rewards hypothesis (e.g., Janzen 1985, Smiley 1986), proposes that the presence of these carbohydrates, which are predictable in space and time, elicit repeated visits by foragers, thereby increasing the likelihood of serendipitous encounters with potential prey, including plant natural enemies, without requiring an increase in the activity or aggressiveness when foragers are visiting the reward producing plants.

Here, we test a fourth, not necessarily mutually exclusive, hypothesis that emphasizes the plant's role in manipulating the dietary preferences of visitors. We term this the deficit hypothesis. Although N-rich protein is often a more attractive resource than are carbohydrates (e.g., Savolainen and Vepsäläinen 1998), these preferences can be influenced by a forager's recent experiences. Ant colonies with access to carbohydrates exhibit stronger preferences for protein-rich resources than do colonies deprived of carbohydrates, and vice versa (Weseloh 1996, Engel et al. 2001, Offenberg 2001, Blüthgen and Fiedler 2004, Kay 2004). We predict that nutritional imbalances induced by access to plant-provided carbohydrates increases the ants' need for protein. This in turn should increase the likelihood that ants will attack herbivorous insects on the host plant, since they are likely to be the closest and most abundant forms of protein. Here, "deficit" refers to imbalances induced by an inadequate reward that is worth collecting nonetheless. We focus on the relative amounts of carbohydrates and protein, although any deficiency that can be remedied by predation (e.g., the presence of some but not all essential amino acids in nectar; Hagen 1986) could elicit the proposed responses. The deficit hypothesis would be rejected if one of the following were true: (1) EFN is itself a sufficiently balanced food source for omnivores, (2) access to EFN does not change dietary preferences for carbon-rich vs. protein-rich foods, (3) protein-hungry ants are not more predaceous (i.e., likely to engage in protein collecting behaviors that can benefit the plant host), or (4) ants provided access to protein are

more aggressive toward potential prey items than were ants provided access to carbohydrates. Our study tests these four predictions. We interpret the results in the context of the ownership, fuel for foraging, predictable rewards and deficit hypotheses to explain the incentives for plants and omnivores to engage in food-for-protection mutualisms.

EXPERIMENTAL SYSTEM AND METHODS

Ferocactus wislizeni (Cactaceae), an EFN-bearing cactus of the Sonoran Desert and desert grasslands of the southwestern United States, is typically tended by one of four ant species (*Crematogaster opuntiae*, *Solenopsis aurea*, *S. xyloni*, and *Forelius pruinosus*) at our study site at the Desert Laboratory in Tucson, Arizona, USA (32°13′11″ N; 111°00′14″ W). These four ant species differ in per-capita effectiveness as predators and hence as plant mutualists: nine *S. xyloni* workers kill *Manduca sexta* caterpillars at the same rate as 14 *S. aurea* workers, 19 *C. opuntiae* workers, or 31 *F. pruinosus* workers (Ness et al. 2006). Ant-tended *F. wislizeni* have fewer herbivores and successfully mature more flowers and fruits than do untended plants (Ness et al. 2006). Further details of this ant–cactus interaction are given in Morris et al. (2005), Ness et al. (2006) and Ness (2006). Although other plants at the study site have EFNs (e.g., staghorn chollas [*Opuntia versicolor*], chain-fruit chollas [*O. fulgida*], prickly pears [*O. phaecantha*], saguaros [*Cereus giganteus*], and white thorn acacia [*Acacia constricta*]), *F. wislizeni* is unique in producing nectar and conspicuously attracting ants year round, including the dry portion of the summer when most of this study was conducted. The most common *F. wislizeni* herbivore at the Desert Laboratory is the hemipteran *Narnia pallidicornis*, which attacks buds, fruit, and vegetative tissue with its sucking mouthparts. Ant tenders have been observed attacking *N. pallidicornis* and carrying fragments of individuals back to their nests (M. Lanan and J. L. Bronstein, *personal observations*).

Carbon and nitrogen sources and imbalances

To test the hypothesis that *F. wislizeni* nectar is not a sufficiently balanced food for supporting ants (prediction 1), we determined C:N for *F. wislizeni* somatic tissue and EFN extrafloral nectar ($n = 4$ plants), *F. wislizeni* herbivores ($n = 9$ *N. pallidicornis* individuals collected on *F. wislizeni*), and ants. The ants included eight species: three tending species collected from *F. wislizeni* (*C. opuntiae*, *S. aurea* and *S. xyloni*) and five locally abundant ant species rarely or never observed on *F. wislizeni* across three years of biweekly censuses of over 150 plants (*Tetramorium hispidum*, *Aphaenogaster cockerelli*, *Dorymyrmex bicolor*, *Camponotus laevigatus* and *C. ocreatus*). All samples were collected and analyzed in May–June 2005. Voucher specimens were collected in 2006 and deposited in the University of Arizona's insect collection.

We combined these analyses with stable isotope methods to test the hypothesis that a portion of the carbon in tending ants is derived from *F. wislizeni* and that this carbon signature differs from other common ants at our study site. Stable isotope ratios are expressed in delta (δ) notation, defined as parts per thousand deviation from a standard materials (Pee Dee belemnite limestone for C and atmospheric nitrogen for N). ^{13}C values are typically within 1‰ of food sources (DeNiro and Epstein 1978). If tending ants are supported by *F. wislizeni*-derived carbon to a greater degree than are other co-occuring ant species, their signatures should be more similar to the signature of *F. wislizeni* than are the signatures of other ant species. We used a one-sided *t* test to evaluate the hypothesis that the difference between the $\delta^{13}C$ values of *F. wislizeni* and the three tending ant species (*C. opuntiae*, *S. aurea*, and *S. xyloni*) would be less than the differences between *F. wislizeni* and the five locally abundant ant species rarely or never observed on *F. wislizeni*.

We also used stable isotope methods to test the hypothesis that the three ant species often observed tending *F. wislizeni* occupy a different trophic level from the herbivore *N. pallidicornis*. ^{15}N values are often enriched during a trophic transfer (e.g., as prey are converted to predator tissue) by 3‰ (Minagawa and Wada 1984), although the magnitude of that enrichment can vary (McCutchan et al. 2003). If the three ant species prey on *N. pallidicornis*, the $\delta^{15}N$ values should progressively increase from *F. wislizeni* to *N. pallidicornis* to the three ant species. We also provide $\delta^{15}N$ values for the other ants included in this study because that information may be valuable to subsequent researchers. Those values, however, have no relevance to our hypotheses, and ^{15}N enrichment of particular species, and hence their trophic position, cannot be inferred solely from their $\delta^{15}N$ values in the absence of information on more basal components of their particular food chain (i.e., *C. ocreatus* should not be classified an autotroph on the basis of similar $\delta^{15}N$ values for that ant and *F. wislizeni* in Fig. 1).

Elemental content, $\delta^{13}C$ and $\delta^{15}N$ were measured on a continuous-flow gas-ratio mass spectrometer (Finnigan Delta PlusXL, Valencia, California, USA) coupled to an elemental analyzer (Costech, Valencia, California, USA) at the University of Arizona Department of Geosciences. Precision was better than ±0.09‰ for $\delta^{13}C$ and ±0.2 for $\delta^{15}N$, based on repeated internal standards. Prior to the analysis, the sample materials were dried for >48 hours in a drying oven, and approximately 2 mg of sample materials were packed into tin capsules for isotopic analysis. This mass could be obtained from single individuals of the large-bodied ants *Pogonomyrmex, Aphaenogaster*, and *Camponotus* and the hemipteran *Narnia*, but required pooling multiple individuals of the other, smaller ant species. Our descriptions of the particular species were based on analysis of one sample of *A. cockerelli, T. hispidum, Camponotus laevigatus*, and
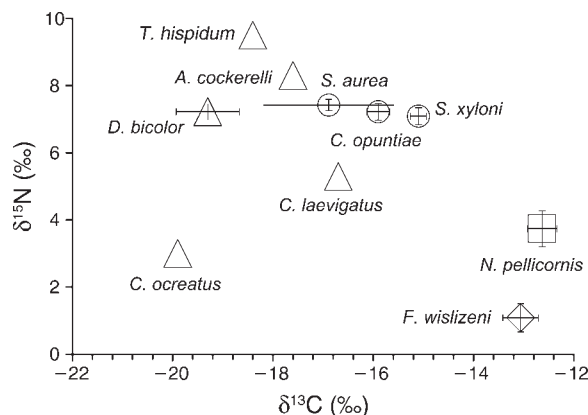

FIG. 1. Carbon and nitrogen stable isotope ratios of *Ferocactus wislizeni* (an extrafloral-nectary-bearing cactus, indicated by a diamond), *Narnia pallidicornis* (an herbivore of *F. wislizeni*, indicated by a square), three ant species that visit extrafloral nectaries (*Solenopsis xyloni, S. aurea*, and *Crematogaster opuntiae*, indicated by circles), and five other ant species (indicated by triangles) common at the study site in the Desert Laboratory, Tucson, Arizona, USA. Means are shown, and bars indicate standard errors.

C. ocreatus, four samples each of *D. bicolor* and *S. aurea*, seven samples of *Crematogaster opuntiae*, nine samples of *N. pallidicornis* and 12 samples of *S. xyloni*. Although we collected distinct samples from plants separated by >10–100 m, we refer to these units as "samples," rather than "colonies," because individual colonies occupy multiple cacti at one time, often linked by underground tunnels, making it difficult to identify the range or overlap of particular colonies (M. Lanan and J. L. Bronstein, *unpublished data*). Our analyses use average C:N, $\delta^{13}C$, and $\delta^{15}N$ values for a species when multiple samples were analyzed. To avoid measuring recently consumed food, gasters were removed from insects and discarded prior to analysis.

Dietary preference

To test the prediction that access to carbohydrates increases preference for protein (prediction 2), we contrasted ant dietary preference among microsites that differ in availability of EFN. We also sought to distinguish the effects of access to EFN from other conditions that might be associated with *F. wislizeni*. Baits of 4 g of processed meat (beef franks, sliced into 1-cm coins) were paired with a petri dish filled with 4 g of sugar dissolved in water. These were placed at the base of *F. wislizeni* and neighboring plants within 10 m that lacked EFN during the sampling period (*n* = 306 bait pairs in the 1 July–18 August 2003). Pared baits (meat and sugar water) were separated by approximately 20 cm at the base of the plants. We use these paired baits to address whether the decisions made by foragers differ based on their local environment, rather than to measure ant's overall preference for 4 g of meat vs. 4 g of sugar dissolved in water. As a result, any differences

in presentation between the bait types (e.g., surface area, ant's ability to manipulate or transport particular food, and so on) should not influence our results. Neighboring plants included *Fouquieria splendens*, *Larrea tridentata*, *Cercidium microphyllum*, *Acacia constricta*, *Opuntia fulgida*, *O. phaecantha*, *Cereus giganteus*, *Ambrosia deltoidea*, *O. versicolor*, and *Prosopis* sp. (n = 34, 21, 19, 18, 14, 13, 13, 10, 8, and 2, respectively). After 60 minutes, the identity and abundance of any ants present at either bait and on the plants were recorded (90 minutes if no ants observed at 60 minutes). We used two one-sided paired t tests (paired by ant species) to test the hypotheses that preferences for meat were greater beneath occupied *F. wislizeni* than beneath (1) unoccupied cacti and (2) other plants. To assuage concerns arising from our multiple comparisons involving the foragers at the base of occupied *F. wislizeni*, we also evaluate these results in the context of a sequential Bonferroni (as advocated by Rice 1989), although we note that Bonferroni corrections do not increase the rigor of a statistical test (merely decrease the likelihood of a Type I error at the expense of increased likelihood of a Type II error).

Prediction 2 would be rejected if ants were similarly likely to collect protein rich resource at the base of tended *F. wislizeni* and other plants. Means were arcsine-transformed prior to analysis. Our estimates of dietary preference were based on 26 ± 16 (mean ± SD) baiting pair records per species at the base of occupied *F. wislizeni*, 7 ± 3 records at the base of non-occupied *F. wislizeni*, and 29 ± 16 records at the base of other plants.

We also used a simple linear regression to test the hypothesis that species with stronger preferences for protein baits would be more effective bodyguards on a per capita basis (prediction 3). This effectiveness was measured inversely as the number of ants required to remove caterpillars placed on the plants within in a constrained time interval. It was estimated as the half-saturation constant of the Michaelis-Menten equation fit to data from another experiment performed during the same summer (see results described in *Experimental system* and reported in Ness et al. 2006). Because these estimates were derived from experiments with tending ants on *F. wislizeni*, we used the percentage of ants allocated to meat baits beneath occupied plants as the independent variable.

Dietary history and ant aggressiveness

We performed two experiments to test whether ants provided with access to protein were more aggressive toward potential prey items than were ants provided access to carbohydrates (prediction 4). The first experiment explored the effect of carbohydrate additions, relative to control plants. The second contrasted the effects of adding different types of resources (carbohydrate-rich vs. protein-rich). Because our intent was to measure the effect of altering the colonies' perception of available resources, rather than measuring the immedi-

ate effect of access to resources on the behavior of an individual at that time, both experiments separated the provision of resources and the measurement of aggression by several days. We could not identify whether the same workers foraged on the plant on both days, although plants were tended by the same ant species, and likely the same colony, throughout the duration of the experiment.

In experiment 1, we measured the effect of dietary history by contrasting ant aggressiveness on plants with and without carbohydrates added. Carbohydrate-addition cacti received five drops (~1.5 mL) of syrup added to the top of the cacti (the location of the plant's extrafloral nectaries and most intense ant foraging activity), while control plants received no additional resources (n = 12 and 15 plants, respectively). Five days later, eight laboratory-reared second- and third-instar *Manduca sexta* (Lepidoptera: Sphingidae) caterpillars were added to the plants. While *M. sexta* do not feed on cacti in nature, they are palatable and relatively sedentary herbivores, and thus effective for use in a bioassay such as this one. The use of surrogate herbivores is well established in research on ant–plant mutualisms (discussed in Ness et al. 2006). We separated the syrup provision and caterpillar addition by five days to highlight the effects of resource provisioning, rather than recruitment to food resources, on caterpillar mortality rates. Twenty minutes after adding the caterpillars, the number of ants on the plant and the number of incapacitated caterpillars were recorded. The method of maximum likelihood, assuming binomially distributed errors, was used to fit the Michaelis-Menten equation ($F = A/[b + A]$) describing F, the fraction of the eight caterpillars that were incapacitated by ants during a trial, as a function of A, the number of ants observed on the cactus, where b is the number of ants at which half of the caterpillars were incapacitated in 20 minutes (Ness et al. 2006). The reciprocal of b measures the per-capita ant effectiveness when ant numbers are low. That is, smaller values of b indicate higher aggression (fewer ants are needed to provide similar levels of defense to the plant). We used the Michaelis-Menten equation because it allowed us to distinguish the effects of ant per-capita effectiveness and ant abundance, accounting for the fact that F must asymptote at 1 as the number of ants increases.

In experiment 2, we contrasted the effects of adding protein-rich vs. carbohydrate-rich supplements to plants (3 g of processed meat (beef franks) and four drops (~1 mL) of commercial honey, respectively, n = 8 plants per treatment). As in the previous experiment, both resources were added to the top of the cacti. The meat was impaled on cactus spines to decrease the likelihood of it being dislodged from the plant. Seven days later, 10 *M. sexta* caterpillars were placed on the plants and monitored for 30 minutes. Maximum likelihood estimates of b were calculated as above. Although our formal comparisons were limited to contrasting the two

resource-addition treatments, we also added caterpillars to control plants ($n = 12$) to verify that b values on these plants were intermediate to the carbohydrate- and meat-addition plants.

No significance should be ascribed to among-experiment differences in b values. These two experiments used different numbers of caterpillars (due to differences in their availability), monitored ant responses for different durations (due to varying availability of caterpillars), and included different subsets of ant attendants. The first trial was performed on plants tended by *C. opuntiae* ants. The second trial was performed on plants tended by either *C. opuntiae* or *S. xyloni*, with all three treatments evenly allocated between plants tended by those two ant species. The identity of the tending ant did not change on any of the plants during the duration of this study.

RESULTS

Carbon and nitrogen

The extrafloral nectar of *F. wislizeni* is nitrogen poor (C:N = 204.5 ± 20.5 [mean ± SE], $n = 4$ plants) relative to *F. wislizeni* plant tissue (49.6 ± 10.9, $n = 4$ plants), *Narnia pallidicornis* (5.1 ± 0.1, $n = 9$ individuals) collected on the plant, and the ants (4.7 ± 0.1, $n = 8$ species). Herbivores are compositionally more similar to omnivorous ants than are plants or EFN (supporting prediction 1).

$\delta^{13}C$ values for the cactus-tending ant species *C. opuntiae*, *S. aurea*, and *S. xyloni* (as a group, 15.9‰ ± 0.5‰ [mean ± SE]) were significantly closer to those of *F. wislizeni* (EFN = 12.3‰ ± 0.3‰, tissue = 13.1‰ ± 0.4‰) and its herbivores than were those of the other five other ant species (18.9‰ ± 0.7‰) rarely observed on *F. wislizeni* plants (one-sided t test, $t = 2.83$ df = 6, $P = 0.015$; Fig. 1).

$\delta^{15}N$ values of *C. opuntiae*, *S. aurea* and *S. xyloni* were higher than those of *F. wislizeni* herbivores. The difference (3.49‰ ± 0.1‰) between those three ant species and the herbivore was typical of that reported for a predator and their prey (Fig. 1).

Dietary preference

Ants foraging at the base of tended *F. wislizeni* plants allocated a significantly greater proportion of workers toward collecting meat than did ants foraging at baits at the base of *F. wislizeni* plants that were untended or tended by other ant species (0.74 and 0.57, respectively; one-sided paired $t = 5.51$, df = 3, $P = 0.006$) or ants foraging at baits at the base of other neighboring plants (0.74 and 0.64; one-sided paired $t = 2.92$, df = 3, $P = 0.031$). Both P values were sufficiently small to be judged significant at <0.05 (threshold values for sequential Bonferroni with two comparisons = 0.025 and 0.05, respectively). The proportions foraging at the meat baits at untended *F. wislizeni* and neighboring plants were similar (0.57 and 0.64, respectively). More workers were attracted to the meat bait than to the sugar bait at 79%
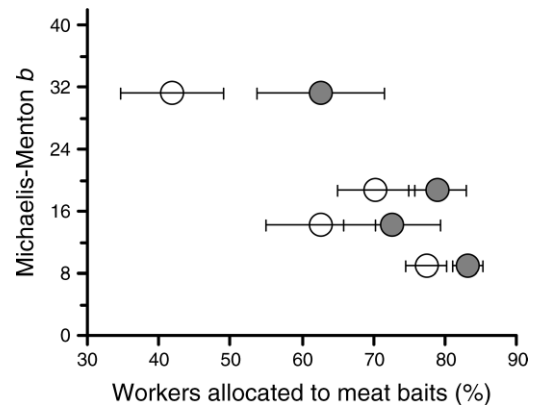

FIG. 2. The percentage of ant workers allocated to collecting meat (rather than carbohydrates) varies among ant species and among microhabitats and is positively correlated with per capita effectiveness of ants as predators. Solid circles indicate allocations of workers at the base of *F. wislizeni* cactus with extrafloral nectaries tended by the focal ant species (left to right: *Forelius pruinosus*, *C. opuntiae*, *S. aurea*, and *S. xyloni*); open circles indicate allocations of workers beneath other plants (non-tended *F. wislizeni* or other plants). Aggressiveness toward potential prey is described here as the Michaelis-Menton b, the number of workers required to kill 10 *Manduca sexta* caterpillars in 30 minutes (estimated in Ness et al. [2006]). The reciprocal of b measures the per capita ant effectiveness when ant numbers are low. That is, smaller values of b indicate higher aggression (fewer ants are needed to provide similar levels of defense to the plant). Error bars indicate ±SE.

of the occupied bait pairs. Furthermore, all four ant species allocated a greater proportion of foragers to meat baits when foraging at the base of *F. wislizeni* plants tended by that species (Fig. 2).

The proportion of workers allocated to meat baits at the base of occupied plants predicted much of the variation ($r^2 = 0.78$) in the differences in per-capita effectiveness of the tending ant species, although the pattern was not statistically significant (simple linear regression, $F = 7.1$, df = 1, 3, $P = 0.11$). The ant species that allocated the greatest proportion of workers to collecting meat (*S. xyloni*) required fewer workers to kill *M. sexta* caterpillars than did less meat-hungry species (particularly *F. pruinosus*; Fig. 2).

Dietary history and ant aggressiveness

Experiment 1 contrasted aggressiveness of ants on carbohydrate-added plants compared to plants with no added food. Fewer ants were required to incapacitate four caterpillars within 20 minutes on carbohydrate-added plants than that on control plants (maximum likelihood estimates of $b = 6.1$ and 14.6, respectively; Fig. 3A). The maximum likelihood estimate of b for each treatment lay outside of the 95% confidence interval of b for the other treatment. In Experiment 2, aggressiveness was compared between ants on carbohydrate-added and protein-added plants. In this case too, aggressiveness was higher on carbohydrate-added plants. Fewer ants were required to incapacitate five caterpillars within 30
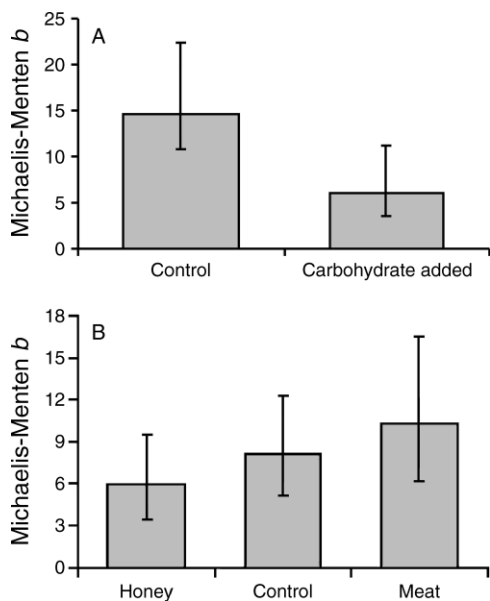
Fig. 3. Exposure to food resources alters the per capita effectiveness, described as *b* in the Michaelis-Menten equation, of omnivorous ants as predators of prospective natural enemies of plants. (A) The addition of carbohydrates (sugar water) decreased the number of workers required to kill four caterpillars in 20 minutes five days later, relative to control plants. (B) The addition of carbohydrates (honey) decreased the number of workers required to kill five caterpillars in 30 minutes one week later, relative to control plants or those where meat was added. Bars indicate 95% confidence intervals.

minutes on carbohydrate-added plants than that on protein-added plants ($b = 5.85$ and 10.2, respectively), and the maximum likelihood estimate of either *b* lay outside of the 95% confidence interval of *b* for the other treatment. Michaelis-Menten *b* values were intermediate on control plants (with no resource added; Fig. 3B). In both experiments, ant foragers dismembered the caterpillars and carried them off the plants, presumably to their nests.

DISCUSSION

Our results reject all four conditions that would invalidate the deficit hypothesis of plant protection by omnivores. First, the C:N ratio of *F. wislizeni* nectar was ill suited to support ant colonies in the absence of complementary resources with C:N more similar to the ants themselves. Second, access to carbohydrates increased the proportion of foragers allocated toward the collection of N-rich resources (*F. wislizeni* nectar and meat baits, respectively). Third, the most protein-hungry ant species were the most effective bodyguards on a per capita basis. Fourth, access to relatively carbohydrate-rich resources (syrup vs. water, or honey vs. meat) increased the per capita effectiveness of ants as attackers of herbivore surrogates (*M. sexta* caterpillars). In addition, the stable isotope data presented here support the hypothesis that the ants are nourished (directly or

indirectly) by cactus-derived carbon (but see Mooney et al. [1989] and Markow et al. [2000] regarding other plant species in our study site with similar ^{13}C signatures), and that they forage at a higher trophic level than do herbivores of *F. wislizeni* (i.e., the ants are omnivorous). Below, we discuss the implications of these results, and alternative explanations.

This study highlights the important role that dietary requirements may play in mediating aggressiveness, and the role that plants may play in mediating the foraging preferences of omnivores. Omnivores provided with a surplus of carbohydrates have previously been shown to become more predaceous, increasing their protein intake and thereby restoring metabolic equilibrium (Pontin 1958, Engel et al. 2001, Offenberg 2001). For example, ant colonies provided access to carbohydrates interact differently with prospective hemipteran mutualists, often treating them as prey instead of solely as a long-term source of carbohydrates (Way 1954, Engel et al. 2001, Offenberg 2001). To our knowledge, the results presented here are the first to demonstrate that ant dietary preference differs when in the immediate vicinity of EFN-bearing vs. EFN-lacking plants (here, *F. wislizeni* vs. neighboring plants). Ants may be differently inclined toward acting as predators on those two types of plants due to plant-induced changes in their dietary preferences. As variation in protector aggressiveness and the extent of host protection appear to be causally linked in some protection mutualisms (see Ness et al. 2006 regarding the *F. wislizeni* system; Rico-Gray and Thien 1989, Itioka et al. 2000, Pratchett 2001), these changes may benefit plants. Intriguingly, the results also hint that the protective services received by plants could be influenced by fluctuations in the dietary preferences of the ant colonies (due to reproduction, availability of prey in other microhabitats, satiation, and other factors). In such a case, both the perceived value of plant-produced rewards and the malleability of dietary preference would be influenced by those drivers.

The dissimilar C:N of insects and EFN, as well as evidence that the omnivorous foragers collect plant-derived carbohydrates and attack natural enemies (here, suggested by stable isotope analyses) are necessary preconditions for the deficit hypothesis to be supported. However, they do not distinguish that hypothesis from alternative explanations for why ants aggressively defend these plants. Below, we interpret other portions of our study, particularly the baiting studies and resource supplementation experiments, with regard to the relative support they provide the deficit hypothesis vs. the ownership, fuel for foraging, and predictable rewards hypotheses.

The predictable rewards hypothesis recognizes that rewards offered to putative protectors can elicit greater visitation by foragers, perhaps thereby increasing the likelihood that omnivores encounter prey (some of which are natural enemies of the reward producer). However, it does not predict that omnivores will be

more active or aggressive when they visit a rewarding plant. We used the Michaelis-Menten model to account for any numerical differences in the number of ants on plants. Our results show that access to carbohydrates influences per capita effectiveness (b) of foraging ants. However, nectar does both attract ants and modify their behavior. In some contexts, nectar's influence on ant abundance will have greater influence on the degree of plant protection conferred by the ants than it will on the behavior of individual ants. From the plant's perspective, the ideal reward might engender predictable foraging by protein-deficient ants. Although our study focused on the effects of rewards on per capita effectiveness, that relationship between abundance and effectiveness is explicit in our Michaelis-Menten model. A change in one variable need not obviate the other, and evidence supporting of the deficit hypothesis does not lessen support for the predictable rewards hypothesis.

The ownership hypothesis predicts that valuable rewards elicit ownership behavior by omnivorous visitors, and that those foragers may subsequently work to exclude other visitors, including plant natural enemies. In isolation, the greater per capita protection provided to honey-supplemented plants, relative to controls, that we documented could provide support for both the ownership and deficit hypotheses. However, the deficit hypothesis received stronger support because our carbohydrate additions elicited greater protec-fj;tive behavior than did the meat additions (Fig. 3B). Critically, this change occurs in an ecological setting in which meat is a more attractive resource than are carbohydrates (as demonstrated by the baiting study). This greater protection in the presence of a less valuable resource is contrary to the predictions of the ownership hypothesis that protection increases in proportion to the perceived value of the resource.

The fuel for foraging hypothesis (Davidson 1998) predicts that carbohydrates provide ants with fuel for metabolically expensive behaviors, possibly including heightened foraging and aggression (Grover et al. 2007). We interpreted the carbohydrate-mediated change in ant per-capita effectiveness (b) as an increase in aggressiveness. An alternative interpretation is that access to carbohydrates altered foraging rates in a manner that increased the likelihood of ant–herbivore encounters (e.g., fueling more active foraging by the same number of ants). Grover et al. (2007) demonstrated that access to sucrose could increase ant activity levels. However, in their study the activity of ant colonies provisioned with the greatest amounts of sucrose was indistinguishable from that of control colonies. In the present study, intriguingly, of the three resources we added to the plants, the meat additions likely provided the most fuel to ant foragers (3 g of beef frank, 2 mL of syrup, and 1 mL of commercial honey provide approximately 10, 6, and 4 kcal, respectively), even as it was the only item of the three to include any protein (U.S. Department of Agriculture 2005). Our demonstration that ants provided

with intermediate amounts of fuel have greater per capita effectiveness (as predators) that do ants provided greater amounts of fuel in a more balanced form (i.e., provided with protein) is counter to some aspects of the fuel for foraging hypothesis, and supports the deficit hypothesis.

Recognizing that these four hypotheses are nonexclusive, predictions of the deficit hypothesis are well supported in the *F. wislizeni*–ant interaction and by natural experiments in other ecological settings. Within the continuum of rewards produced by plants for animals, extrafloral nectar is particularly carbohydrate rich. It often has higher sugar concentrations, fewer amino acids, and lower amino acid concentrations than does floral nectar intended for pollinators produced by the same plant species (Koptur 1994, Blüthgen et al. 2004; but see Baker et al. 1978 regarding cysteine and nonprotein amino acids). The extrafloral nectar collected by rain forest ants is nutritionally insufficient to support them (C:N = ~200; Tillberg and Breed 2004) and ants of the EFN-rich rain forest canopy (Blüthgen et al. 2000) are typically more protein hungry than their terrestrial counterparts (Kaspari and Yanoviak 2001, Hahn and Wheeler 2002). Rainforest ants that tend Hemiptera or forage on leaves are also reported to be particularly predaceous, relative to congeners (Davidson 2005). The availability of rewards can also differ among plant congeners within a habitat, and these differences can influence the ant–plant interaction. Different species of ant-hosting *Macaranga* offer food rewards that differ in C:N (Hatada et al. 2002), and the ant attendants on the plant species offering the most carbohydrate-rich food bodies are more aggressive than those on the plant species offering the most nitrogen-rich rewards (*M. trachyphylla* and *M. beccarina*, respectively; Itioka et al. 2000). Last, damage to plants can induce increases in nectary number (Mondor and Addicott 2003) or in sugar production by existing nectaries (Ness 2003), responses that augment (and modify) the rewards offered to prospective bodyguards.

Some ant-tended plants, particularly myrmecophytes (i.e., those with relatively obligate ant associations that house as well as feed their ants), may have greater incentives to produce N-rich food rewards for their attendants. Rewards produced by myrmecophytic plants typically contain a complement of amino acids and fatty acids more suitable to support insects (Heil et al. 1998, 2004). This feeding and housing of the bodyguards may have two effects: (1) plant rewards may need to be sufficiently N-rich to maintain healthy colonies even in periods when herbivores are rare or absent (i.e., to prevent abandonment by colonies in search of N), and (2) ants may defend the plant as a home (rather than solely as a source of energy or nutrients). Intriguingly, these plant-dwelling ants often evict herbivores from plants rather than kill and eat them (e.g., Carroll and Janzen 1973, Fonseca 1993, Heil and McKey 2003), suggesting that ownership rather than dietary needs may be the primary driver of these behaviors.

From the perspective of each partner, a successful mutualism will maximize the ratio of benefits to costs and be minimally susceptible to cheating. From a plant perspective, the high C:N of EFN rewards may include all of these characteristics. Carbon-rich defenses (direct or indirect) should be less costly for plants to produce in relatively N-limited habitats, where C is in excess (Folgarait and Davidson 1994). EFN-bearing plants are in fact common in sunlight-rich habitats such as rain forest canopies (Blüthgen et al. 2000), forest edges (Bentley 1976) and deserts (Pemberton 1988). Further, allocating more nitrogen to those rewards (i.e., increasing costs where nitrogen is more limiting than carbon) may not improve the quality of service, and will perhaps decrease it. In this case, natural selection may favor sugary EFN, the least costly reward that can also be highly effective. The likelihood and power of such selection, however, will depend on the poorly understood relative influences of genotype vs. environment on nectar characteristics (e.g., production phenology, concentration, and composition) (Mitchell 2004). Last, our results suggest that the consumption of carbohydrates increases the incentives for omnivores to act as carnivores. Collection of the reward inevitably engenders responses favorable to the reward-producing plants, and increases the mutualistic effect of perhaps otherwise mediocre (i.e., less aggressive) partners.

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