

Carbohydrate as Fuel for Foraging, Resource Defense and Colony Growth – a Long-term Experiment with the Plant-ant *Crematogaster nigriceps*

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ABSTRACT

Mismatches in nutrient composition (*e.g.*, protein, carbohydrates, lipids, etc.) between consumers and the resources they depend on can have ecological consequences, affecting traits from individual behavior to community structure. In many terrestrial ecosystems, ants depend on plant and insect mutualist partners for carbohydrate-rich rewards that are nutritionally unbalanced (especially in protein) relative to colony needs. Despite imbalances, many carbohydrate-feeding ant mutualists dominate communities—both competitively and numerically—raising the question of whether excess carbohydrates ‘fuel’ colony acquisition of limiting resources and growth. In a 10-month field study, we manipulated carbohydrate access for the obligate plant-ant *Crematogaster nigriceps* to test whether carbohydrate availability could be mechanistically linked to ecological dominance via heightened territory defense, increased protein foraging, and colony growth. Supplementation increased aggressive defense of hosts after only two weeks, but was also strongly linked to variation in rainfall. Contrary to predictions, we did not find that supplemented colonies increased protein foraging. Instead, colonies with reduced carbohydrate access discovered a greater proportion of protein baits, suggesting that carbohydrate deprivation increases foraging intensity. We found no significant effect of carbohydrate manipulation on brood or alate production. These results contrast with findings from several recent short-term and lab-based nutrient supplementation studies and highlight the role of seasonality and biotic context in colony-foraging and reproductive decisions. These factors may be essential to understanding the consequences of carbohydrate access in natural plant-ant systems.

Key words: *Acacia drepanolobium*; ant-plant; East Africa; ecological stoichiometry; extra-floral nectar; mutualism; savanna.

CARBOHYDRATES ARE PERHAPS THE MOST UBIQUITOUS CURRENCY IN MUTUALISMS. Plants, algae, and some insects reward their mutualist pollinators, defenders, root symbionts, corals, and seed dispersers with nectar, photosynthate, or fruit pulp dominated by carbohydrates, especially simple sugars. As basic products of photosynthesis in plants or the byproducts of phloem-feeding (*e.g.*, scale insects, aphids), carbohydrates are metabolically inexpensive offerings relative to lipids or proteins (Lambers *et al.* 2008). High ratios of carbohydrates relative to other nutrients in rewards allow producers to minimize the cost of paying for the beneficial goods or services they receive. For consumer partners, however, these carbohydrate-skewed rewards are often out of balance with their own nutritional requirements for growth, most notably these rewards are deficient in protein (Davidson 2005).

Nutrient imbalances in rewards for ant defenders of plants and hemipterans can be particularly striking, yet many such mutualistic ant species (*e.g.*, numerous tropical canopy ants, invasive Argentine and fire ants) achieve high levels of competitive and numerical dominance (Davidson *et al.* 2003, Bluthgen & Fiedler 2004, Tillberg *et al.* 2007). How do these species manage to dominate communities, despite potential nutrient imbalances in plant or insect exudates? Do these abundant carbohydrate rewards

promote acquisition by ants of the complementary protein-rich resources that are required for colony reproduction and growth?

Davidson (1997) proposed the ‘resource imbalance hypothesis’, a mechanistic explanation for how the availability of carbohydrate-biased rewards could lead to ecological dominance of mutualistic ant species. This hypothesis suggests that abundant carbohydrate resources ‘fuel’ increased protein acquisition by ants. Although formulated as an evolutionary hypothesis, several fundamental predictions can be tested on ecological time scales, in particular, the expectations that excess carbohydrates: (1) fuel ‘high tempo’ foraging and aggressive defense of resource territories; and (2) increase acquisition of non-reward based nutrients; notably protein and that (3) greater protein acquisition accelerates worker and alate production, resulting in positive feedbacks to colony growth.

Recent lab-based and short-term field studies have found support for these predictions. Carbohydrate augmentation can fuel individual worker activity (Grover *et al.* 2007, Schilman & Roces 2006), foraging for protein sources (Ness *et al.* 2009) and aggressive defense of resource territories (Ness *et al.* 2009, Pringle *et al.* 2011). Abundant carbohydrates can also increase worker preference for protein sources and change foraging patterns to insure that target intake ratios of carbohydrates and protein are maintained (Kay 2004, Cook & Behmer 2010, Cook *et al.* 2010, Wilder and Eubanks 2010). Furthermore, the size and number of new workers and alates have been increased by experimentally

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supplementing carbohydrates (Kay *et al.* 2006, Grover *et al.* 2007, Wilder *et al.* 2010, Kay *et al.* 2011). While these studies show that carbohydrate resources can affect worker behavior and colony demography over shorter timescales (3 days to 3 mo) and under relatively constant conditions, we know less about how carbohydrate rewards influence ant mutualist colonies under more typical field conditions where interactions take place over long timescales within temporally variable environments.

Our 10-month field manipulation of carbohydrate availability examined colony responses within the context of naturally fluctuating temperature and precipitation, seasonal availability of resources, and competition, which have all been shown to modify ant behavior and colony decisions (Rico-Gray & Garcia-Franco 1998, Kaspari & Weiser 2000, Hahn & Wheeler 2002, Diaz-Castelazo *et al.* 2004). We used the obligate plant-ant *Crematogaster nigriceps*, an abundant mutualist of the tropical tree *Acacia drepanolobium* throughout East Africa, to test whether carbohydrate augmentation or deprivation resulted in changes in: (1) ant defense of resource territories (*i.e.*, host plants); (2) rates of resource discovery and recruitment; and (3) colony growth and demography.

METHODS

STUDY SYSTEM.—This study was conducted from August 2008 to June 2009 at the Mpala Research Centre in Laikipia, Kenya (37°53' E, 0°17' N; MAP ~500 mm). In the region, the ant-plant *A. drepanolobium* dominates 'black cotton' savannas, and accounts for 97 percent of the woody cover (Young *et al.* 1997). Colonies of four ant species compete for sole control of nest space (swollen thorn domatia) and/or food provided by individual *A. drepanolobium* trees. Ant colonies frequently span multiple tree canopies, but very rarely share territory within a canopy. Each ant species differs strongly in behavior, their use of extrafloral nectar (EFN), the number and size of trees inhabited by a single colony and the degree to which they manipulate trees to gain additional food or housing (for a complete description of the system, see Palmer *et al.* 2000, Palmer 2003). *Crematogaster nigriceps*, an ecologically dominant ant (inhabiting over 50% of trees in many areas (Hocking 1970, Stapley 1998, Palmer 2004, K. Rudolph unpubl. data)), is uniquely suited for this study because workers of this species appear strongly reliant on carbohydrate-rich, nitrogen-poor EFN (Brody & Palmer 2007). *Crematogaster nigriceps* colonies do not access additional carbohydrates by tending phloem-feeding insects and routinely scavenge for prey

off-tree because *A. drepanolobium* does not produce N-rich food bodies (Palmer *et al.* 2008).

EXPERIMENTAL COLONIES.—We chose focal colonies based on three criteria: (1) located > 10 m from termite mounds (to limit variation related to proximity to nutrient-rich termite mounds [Palmer 2003]); (2) located > 20 m from one another; and (3) inhabiting < 3 individual trees (each < 3 m in height). We delineated colony boundaries by reciprocally transplanting workers and observing the presence/absence of subsequent conflict (*cf.* Palmer 2004, Palmer *et al.* 2008). Twelve colonies were assigned to each of three treatments (carbohydrate supplemented, carbohydrate removal, and controls; 36 colonies total) in a stratified random design. Treatments were imposed on all trees inhabited by the focal colony, and responses were measured at only the largest tree within the colony (~1.5–3 m in height). Colonies varied in estimated size from 4000 to 9000 workers (Palmer 2004) and were all mature (*i.e.*, alates present in domatia of focal trees).

TREATMENTS.—All treatments were maintained for 10 mo. For the Carbohydrate Supplementation Treatment (Carb+) (see Table 1 for comparison of treatment manipulations), we augmented natural EFN with a 20 percent solution of cane sugar (sucrose) and filtered rainwater. Artificial nectaries were made from 35 mm film canisters (~30 ml) fitted with 7 cm absorbent Water Wicks (Pepperell Braiding Co.) (for an alternative design see Rowles & Silverman 2009)(Fig. S1). These were attached to tree stems or large lateral branches and refilled with sucrose solution every 4 days, with little disturbance to resident ants. We supplemented carbohydrate in proportion to colony size, using total tree height per colony as a proxy for worker number (*cf.* Palmer 2004). We added 1.5 artificial nectaries for each 1.0 m tree height, or ~45 ml additional nectar for each estimated 3800 workers.

We curtailed colony access to tree-provisioned carbohydrates by removing extrafloral nectaries in the Carbohydrate Removal Treatments (Carb-). After each flush of new growth (following seasonal rains), we scanned the petioles of recently expanded leaves for active nectaries (raised, saucer-like structures ~3–4 mm in diameter) and pinched them off with forceps. This technique permanently eliminated EFN access at those leaf sites (*cf.* Palmer *et al.* 2002). We manipulated trees during cool, early mornings when ant activity is low. While we cannot discount the possibility that some nectaries remained after our thorough checks (focal trees are covered with hundreds of small leaves) or

TABLE 1. Experimental design and colony demography means (\pm SE) after 10 mo of manipulation.

	Manipulations		Colony Demography			
	EFN access	Artificial Nectary Contents	Eggs	Brood	Alates	Workers
Supplemented (Carb+)	Intact	20% sucrose solution	98 (47)	159 (22)	32 (8)	651 (82)
Removal (Carb-)	Removed	Water	92 (30)	183 (23)	37 (11)	586 (40)
Control	Intact	Water	94 (50)	142 (23)	43 (8)	590 (57)

that ants accessed carbohydrates from new nectaries before we removed them, it is clear that colony access to EFN was severely reduced as a result of our manipulation.

The liquid carbohydrates manipulated in this study (artificial and natural) are a coupled resource, containing carbohydrate and water. To isolate the effects of carbohydrate versus water, the latter predicted to be an independent and important resource in this seasonally dry system, we installed 'water only' artificial nectaries on (Carb-) and Control trees and refilled these every 4 days. This insured that we were only manipulating access to carbohydrates. We could not feasibly estimate the volume used or added, but given the frequency of refilling, considered the nectar and water an *ad libitum* resource. We also controlled for the tissue loss associated with destruction of nectaries on (Carb-) trees by damaging an equivalent amount of leaf tissue on (Carb+) and Control trees.

TREATMENT RESPONSES.—In contrast to aggression and territorial defense assays in other studies that focus on individual worker behavior (Grover *et al.* 2007, Kay *et al.* 2010), our measures focus on whole colony responses as whole colonies are the predominant level of selection in social insects (Kay *et al.* 2011) and individual behaviors frequently change outside the context of the colony (K. Rudolph pers. comm.) Every 1–3 months, we assayed the defensive behavior of *C. nigriceps* workers, who rush up thorn tips and attack animals that disturb their host plants. We quantified levels of aggressive defense using established methods (Palmer *et al.* 2008). Observers haphazardly selected three domatia on each tree and after synchronously tapping each five times with a pencil, recorded the number of ants reaching the tip of a focal thorn within 30 sec (including returning individuals). Assays took place between 1100 h and 1300 h on four consecutive days. Counts were averaged over the three domatia and four sampling days for each tree. This design was repeated in June, August and October 2008 and January–April 2009.

FORAGING.—'High tempo' foraging in many exudate-feeding ants is associated with long-distance resource discovery and dominance (Davidson 1997). To test the effects of carbohydrate access on foraging distance, protein discovery, and recruitment, we monitored worker presence at one protein bait located at either 0.5 m or 1.0 m from each focal tree on two consecutive days at three separate times, August and October 2008, and January 2009. *Crematogaster nigriceps* is known to regularly visit protein baits 40 cm from host tree stems (Palmer 2003). Monitoring baits at 0.5 m and 1 m from trees allowed us to test if carbohydrate access affects foraging distance. We randomly assigned both a location (in one of two cardinal directions, either due north or south of tree stem) and day of baiting (first or second) for each bait distance. Baits consisted of ~ 4 g canned tuna fish (~ 60% water, 26% protein and 12% lipids [Rasmussen & Morrissey 2007]) placed on laminated 7.6 × 7.6 cm cards and shaded from the sun. Baiting commenced at 0800 h, with observers recording the number of *C. nigriceps* present at 30 min intervals. Each bait was checked five times (such that trials lasted 150 min) after

which any remaining tuna was removed. Baits were scored as 'discovered; if one or more ants were observed feeding on tuna during the 150 min trial. The total number of ants recorded at baits within each 150 min trial was used as a measure of colony 'recruitment'.

To estimate colony growth, we collected two domatia from four zones within each focal tree's canopy (top and middle of canopy, both proximate to and distal from main stem, $N = 8$ per tree). Domatia contents were counted as eggs, brood (larvae/pupae), reproductives (queens/males), or workers.

To explore potential diet changes in focal colonies (Menke *et al.* 2010) and determine whether supplementation affected worker tissue production and maintenance, we analyzed the stable isotope content of workers from each colony after treatment manipulation ended (9 mo after initiation) using stable isotope. C4 plants (*i.e.*, sugar cane, $\delta^{13}\text{C} = -12.61$) display distinctly less negative carbon isotope signatures compared with C3 plants (*i.e.*, *Acacia drepanolobium*, $\delta^{13}\text{C} = -26.402$) and those differences are passed on to consumers without further isotope discrimination (Lambers *et al.* 2008). As all the sugar used in the nectar manipulations was from sugar cane, less negative $\delta^{13}\text{C}$ values in supplemented ants would reveal artificial nectar use and show that nectar carbon can be incorporated into worker tissue. Nitrogen isotopes are common indicators of the trophic position of diet items. Although not expected, if natural colony protein sources (*e.g.*, herbivorous vs predatory insects) varied by treatment, we would see differences in worker $\delta^{15}\text{N}$ (Ness *et al.* 2009, Palmer 2003). To remove potential contamination from gut contents, we removed dried worker heads from thorax and gasters. We pooled 4–5 ant heads from each colony ($N = 35$) and ran analysis at the University of Florida's Department of Geology Stable Isotope Facility.

Rain gauges near the study site are checked manually after each rainfall event (T.P. Young unpubl. data). We summed daily rainfall measures for the 30 days before each resource defense survey and included these values in our statistical models.

STATISTICAL ANALYSES.—All analyses were performed in JMP (v. 8.0.2; SAS Institute 2009 [SAS Institute, Cary, North Carolina, U.S.A.] – default type II Sums of Squares [SS]) except analysis of bait discovery for which we used R v. 2.15.1 (R Development Core Team 2010 – type I SS). Resource defense responses and colony growth measures were square root transformed and counts of recruits to baits were cube root transformed to meet assumptions of normality.

We modeled the number of ants responding to simulated herbivory (resource defense) using a general linear mixed model (GLMM) with restricted maximum likelihood (REML) procedure in JMP. We treated individual colonies as a random effect in the analysis to control for pseudo-replication stemming from our repeated measures design. Treatment (Carb+, Carb- and control) and monthly rainfall were included as fixed effects in the model as was the two-way interaction between these factors (Table 2). No violation of sphericity (unequal variance of differences between groups) was detected (Mauchly's sphericity tests,

TABLE 2. Linear mixed model results for measures of resource defense and worker recruitment to protein baits. (dfDen is the abbreviation for degrees of freedom denominator).

Source	df	dfDen	F-ratio	P
Resource defense				
treatment (tr)	2	33	5.16	0.0112*
rainfall	1	177	63.04	<0.0001*
tr × rainfall	2	177	1.12	0.3296
Recruitment to baits				
treatment (tr)	2	21	0.14	0.8540
distance (0.5 or 1 m)	1	69	3.36	0.0722
tr × distance	2	65	0.54	0.5759
Bait discovery				
			Z- value	P
Intercept			-0.437	0.662
(Carb-)			1.896	0.058
(Carb +)			-1.096	0.273
distance			-3.486	0.0005*

$P > 0.5$). We compared least square mean differences between groups with Student's *t*. The univariate method used here is robust to missing data (*e.g.*, when one or more repeated measures within a replicate are missing). This was important for our analysis because one (Carb-) colony was displaced by a *C. mimosae* colony after our January 2009 survey. As a consequence, there were no responses from that replicate in subsequent surveys.

Binary bait discovery data (bait found/bait not found) were analyzed with a generalized linear mixed model GLMM in R (*lmer* function in *lme4* package). Treatment and bait distance (1 m and 0.5 m) were included as fixed effects (in that order) and colony was included as a random effect to account for repeated measures design. Because colony responses to baiting were measured in only 3 mo, we did not have sufficient data to estimate a rainfall effect.

If baits were discovered, the response in terms of number of ants recruiting over the 150 min survey was modeled using the mixed model procedure described for resource defense above. Here, we assigned treatment and bait distance as fixed effects and individual colonies as the random effect.

Colony growth data were square root transformed. Whether carbohydrate manipulation affected the number of eggs, brood, reproductive, or workers and separately $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope signatures was tested using MANOVA. We used Dunnett's *post-hoc* test to compare treatment means.

RESULTS

Consistent with previous lab and short-term experimental findings demonstrating increased aggressive resource defense with carbohydrate supplementation, the (Carb+) treatment in this study was associated with an increase in the number of workers swarming thorn tips (a measure of host plant defense) ($P = 0.01$) (Fig. 1; Table 2). This positive effect was detected within 2 weeks of treatment initiation and remained pronounced through the wet

season (Fig. 1). Removing nectar from host plants (Carb-), however, was not associated with a significant reduction in host plant defense. Defensive responses were strongly affected by rainfall in the 30 days preceding surveys ($P < 0.0001$) (Table 2). Coincident with severe and prolonged dry conditions (see Fig. 1), aggressive defense by ants in all groups decreased, and carbohydrate supplementation did not buffer colonies against this decline.

The ranking of effects of treatment on the proportion of baits discovered was from low to high; (Carb+), control, (Carb-) (Fig. 2). Contrary to predictions, the proportion of baits discovered by (Carb+) was not significantly different from controls ($Z = -1.09$; $P = 0.27$) (Table 2), while (Carb-) was marginally different from controls ($Z = 1.90$; $P = 0.06$). We re-ran this analysis after combining groups that did not differ significantly in the first analysis (control and Carb+) colonies. We found a significant difference between the combined group and (Carb-) ($Z = 2.76$; $P = 0.006$). Baits placed 1 m from host trees were significantly less likely to be found than those only half a meter away ($Z = -3.486$, $P = 0.0005$). Despite increased bait discovery, (Carb-) colonies did not recruit more workers to baits after locating them. We found a marginal effect of bait distance ($P = 0.07$) and no significant effect of treatment ($P = 0.85$) on the number of workers at baits (Table 2).

Domatia contents were not affected by supplementation or removal of carbohydrates (Table 1). We found no significant

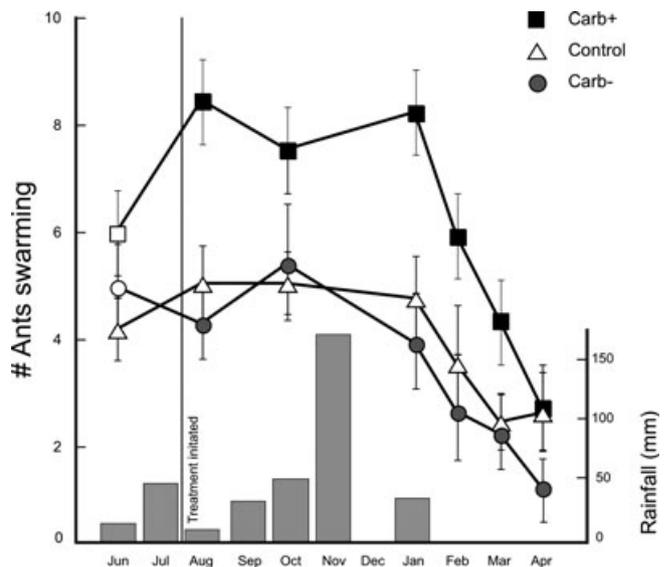


FIGURE 1. Effect of carbohydrate manipulation on ant aggressive defense of host plant resources, and total monthly rainfall. Aggressive defense is reported as the mean (\pm SE) number of *C. nigripes* recruiting to thorn tips following domatia taps. Carbohydrate availability significantly influenced ant defense of host resources, with supplemented colonies (square) showing higher levels of defense than removals (circle), and controls (open triangle) intermediate between the two. Pre-treatment responses (open symbols in June 2008) were not significantly different between groups ($F_{2,33} = 1.35$; $P = 0.27$). Gray histogram bars report total monthly rainfall in mm.

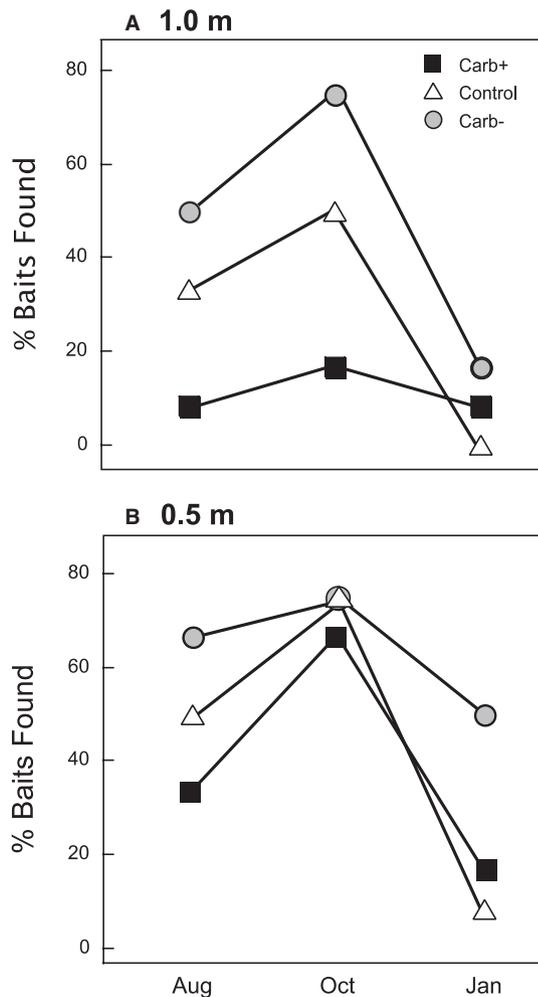


FIGURE 2. Ant discovery of baits located 0.5 and 1.0 m from colony focal trees. Tuna baits were ‘found’ if one or more *C. nigricipes* worker(s) were present at a bait during the 150-min observation period. a) Baits found at 1.0 m distance from focal tree. b) Baits found at 0.5 m distance from tree. Carbohydrate supplemented (square), control (triangle), removal (circle).

differences in the mean number of eggs ($F_{2,32} = 0.01$; $P = 0.99$), brood ($F_{2,32} = 0.86$; $P = 0.43$), reproductives ($F_{2,32} = 0.42$; $P = 0.67$), workers ($F_{2,32} = 0.14$; $P = 0.86$), or ratios of brood:workers ($F_{2,32} = 1.26$; $P = 0.29$), and brood+eggs:workers ($F_{2,32} = 0.58$; $P = 0.56$).

Stable isotope analysis of $\delta^{13}\text{C}$ confirmed that additional carbohydrates (from C4 sugar solution) were incorporated into the diet and ultimately the body tissue of (Carb+) ants ($F_{2,32} = 13.92$; $P < 0.0001$) (Fig. 3). $\delta^{15}\text{N}$ signatures did not indicate that (Carb+) colonies differed in the trophic position of nitrogen sources relative to (Carb-) or controls ($F_{2,32} = 0.31$; $P = 0.73$).

DISCUSSION

Results from our long-term field study support the prediction and earlier findings that abundant carbohydrates fuel increased

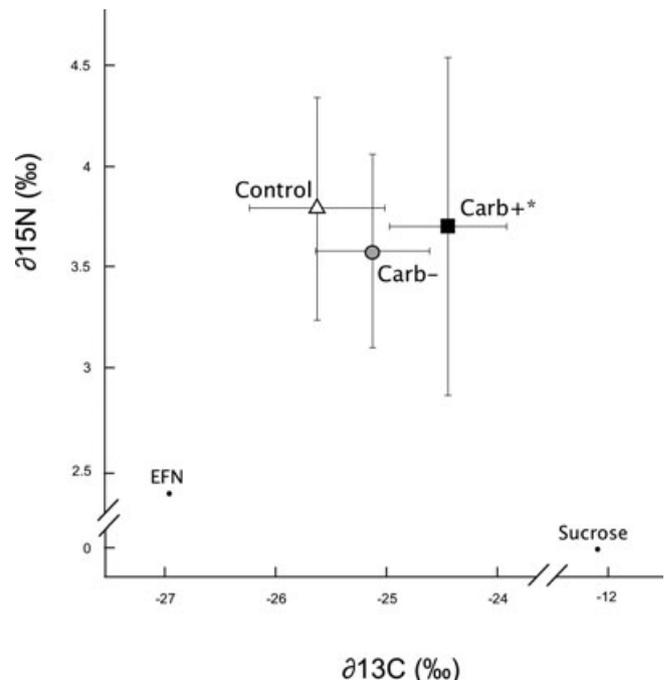


FIGURE 3. Carbon and nitrogen stable isotope ratios for *C. nigricipes* workers compared with natural (extrafloral nectar) and artificial (sucrose) carbohydrate sources. Mean (\pm SD) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by treatment: Carbohydrates supplemented (Carb+), removal (Carb-), and control (C). Asterisk indicates that (Carb+) was significantly different from removals and controls in $\delta^{13}\text{C}$ values. Mean for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ of Sucrose [-12.61 , 0]. Mean (\pm SD) for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ of EFN [-26.96 (± 1.62), 2.38 (± 1.55)].

aggressive resource defense in ants (Ness *et al.* 2009, Kay *et al.* 2010, Pringle *et al.* but see Grover *et al.* 2007). Removing carbohydrates, however, was not associated with an equivalent decrease in defense response to disturbance (Fig. 1). While a significant increase in colony defense of thorn tips was observed just 2 weeks into (Carb+) treatments, it is striking that these high absolute levels of aggressive defense did not persist over the 10-month span of the experiment (Fig. 1). This may be due to factors associated with rainfall (a significant factor in our analysis of resource defense, $P < 0.0001$). For all treatments, 3 mo without rain corresponded to a more than two-fold decline in levels of aggressive defense of host plants, suggesting that for a system characterized by wet and dry seasons and periodic drought, aggressive defense of resources is limited by environmental conditions. In the field, factors such as low relative humidity and desiccation risk (Rico-Gray & Garcia-Franco 1998, Kaspari & Weiser 2000) may alter worker ability or motivation to react defensively irrespective of carbohydrate supplementation.

We did not find evidence consistent with the prediction that carbohydrate augmentation fuels colony acquisition of protein (Davidson 1997). In fact, (Carb-) colonies discovered more tuna baits relative to (Carb+) or control colonies (Fig. 2). (Carb-) colonies' increased foraging could be explained by more workers forced to forage off-tree to compensate for on-tree food losses,

yielding the increased level of bait discovery we observed. Increased discovery was not associated with higher recruitment and this may reflect workers specifically scouting for carbohydrate-rich resources or exploring the environment for new, higher quality hosts. Worker specialization to particular tasks and food types is common in ants (Holldobler & Wilson 1990, citations in Portha *et al.* 2002) and (Carb-) colonies lacking EFN resources may prioritize carbohydrate collection over that of protein.

(Carb+) colonies' low rates of bait discovery especially at longer distances may be due to colonies prioritizing on-tree or near-tree defense aboveground foraging for protein. *Crematogaster nigriceps* colonies are prone to takeovers by both *C. sjostedti* and *C. mimosae* (Palmer *et al.* 2000) that occur disproportionately during the wet season (K. Rudolph, unpubl. data). Palmer *et al.* (2002) showed that subordinate colonies of *Tetraponera penzigi* were more often supplanted by larger neighboring colonies when their host trees are supplemented with artificial nectar. (Carb+) colonies may be similarly vulnerable and respond by allocating more workers to protect high-quality hosts and fewer workers to longer distance off-tree foraging. This could decrease the frequency of finding tuna baits and may help explain elevated aggressive defense of host trees (Fig. 1). As with aggressive territory defense, we found that foraging is linked to rainfall, with the greatest bait discovery occurring during the wettest baiting period (October, Fig. 2). Cook *et al.* (2011) demonstrated that in addition to food quality effects on ant foraging, seasonality *per se* influences foraging choices. Our findings join this work in suggesting that colony decisions can be shaped by both food availability and environmental conditions to produce not one predictable response but instead temporally shifting behaviors.

In contrast to colony container studies showing effects of carbohydrate imbalance on ant brood mass, worker number, and worker survival (Grover *et al.* 2007, Dussutour & Simpson 2008, Lach *et al.* 2009, Cook *et al.* 2010, Kay *et al.* 2011), we found no significant effect of carbohydrate manipulation on *C. nigriceps* colony growth (number of workers, eggs or brood) or colony reproduction (number of reproductives). Laboratory studies monitoring development time in *C. mimosae*, another Acacia-ant found on *A. drepanolobium*, show that larvae emerge as workers after 40–50 days (K. Rudolph unpubl. data). In the temperate ant *Crematogaster ashmeadi*, eggs become workers in about 50 days (Tschinkel 2002). Like many tropical ant species, *C. nigriceps* colonies appear to have homodynamous development (all developmental stages are present in nests at all times of year) (Kipyatkov 1993, Isbell & Young 2007), suggesting that brood production is continuous. Therefore, setting generation time of *C. nigriceps* in the field at 60 days, we estimate 4–5 generations of workers over the course of this study. If carbohydrate augmentation strongly influenced worker or brood production, we expected 10 mo to be a sufficient period over which to detect an effect.

A field study of non-plant-ants (Bono & Herbers 2003) also did not observe effects of carbohydrate or protein augmentation on the production of *Myrmica brevispinosa* workers or reproductives, but did report sex ratio changes in reproductives. It remains unclear why resource-supplemented colonies in natural settings

would not produce more individuals, but tentatively points to the importance of factors absent from laboratory studies (*e.g.*, variable precipitation or competition) in mediating colony growth. Alternatively, our failure to find treatment differences may have been affected by our sampling. Synchronizing domatia collection with reproductive bouts was not possible because *C. nigriceps* reproduction is temporally unpredictable (Isbell & Young 2007). Therefore, seasonal pulses in brood or alates may have been missed, although we did not observe differential release of alates during frequent artificial nectary refilling events. Colony growth was likely not constrained by nesting space (Fonseca 1999) because for 50% of colonies (regardless of treatment), one of eight sampled domatia was nearly empty (*i.e.*, contained < 5 immature ants [overall mean # immature ants per domatia = 42]). In contrast to the study of Grover *et al.* 2007, our (Carb-) colonies deprived of natural carbohydrate resources continually produced brood, demonstrating an ability to compensate for the loss of tree-provisioned resources, perhaps by foraging more off of host trees.

Combining our results with others from this system (Brody & Palmer 2007), we find that carbohydrate augmentation increases ant aggression on host *Acacia* trees, which in turn is associated with decreased herbivory on *A. drepanolobium*. This suggests that plants could directly modify ant defensive behavior and influence protection benefits (an indirect defense) via changes in EFN production (Ness *et al.* 2009). Increasing carbohydrate production or concentration is not possible in dry seasons when *A. drepanolobium* trees abscise leaves with nectaries attached. However, in the wet season, when new branch tissue is expanding and vulnerable to herbivory, trees routing photosynthate to nectar should experience a timely up-regulation of resident ants' defense behavior. The degree to which trees versus ant controls the volume and quality of rewards within this partnership remains unknown.

Our 10-month study maintained fixed supplementation levels and standardized baiting protocols throughout; yet we found dramatic differences in protein bait discovery and recruitment across months as well as changes through time in resource defense (Figs. 1 and 2). Together, these highlight the inconsistency of colony responses to carbohydrate manipulation. Reduction in resource defense was significantly associated with declines in rainfall at the end of the study. We suspect that other unmeasured environmental features (*e.g.*, competition with neighboring colonies or daily temperature fluxes) constrain colonies' ability to forage, defend territory, and reproduce, and may have contributed to differences between observed and predicted responses. The biological realism included in this field manipulation has been largely missing from previous investigations of diet balancing where food supply is unlimited and conditions are held constant. More explicit consideration of environmental variation and how it interacts with diet balancing (Cook *et al.* 2011) will improve the accuracy of our predictions about the relationship between plant-provided carbohydrates and colony behavior and demography, and how feedbacks between these may contribute to the ecological dominance of nectar-feeding ant species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

FIGURE S1. *Crematogaster nigriceps* feeding at artificial nectary.

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