CONSEQUENCES OF OBLIGATE ASSOCIATION FOR HOSTS AND DEFENDERS IN AN AFRICAN ACACIA-ANT MUTUALISM

By

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To my parents who gave me the freedom and encouragement to explore
To Jay who adventures with me
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Understanding how interactions between individuals shape the distribution and abundance of species is an important challenge for biologists. Some interactions can be mutually beneficial and lead to the close association between organisms over evolutionary time. To explore the consequences of such obligate mutualisms for partners and ecological communities, I conducted experiments with *Crematogaster* ants that reside in domatia produced by *Acacia drepanolobium* hosts.

In addition to housing, trees exchange carbohydrate-rich nectar for ant defense against herbivores. In Chapter 2 I tested whether this nutritionally imbalanced reward influences worker behavior, foraging, and ultimately colony growth. Increased access to carbohydrates enhanced ant defense of resources, however I did not find evidence supporting the hypothesis that carbohydrate augmentation fuels increased levels of protein foraging or colony growth.

In Chapter 3, I explored how *Acacia* ants’ extreme reliance on host rewards influences intraspecific colony dynamics. Conflicts over tree territories resulted in substantial worker death for both winning and losing colonies. Following costly fights,
victorious colonies appear less able to defend hosts against browsing herbivores and takeover by neighboring colonies. I discovered that during recovery from conflict, some winner colonies build back their workforce by incorporating members of the losing colony, most likely brood left behind in domatia. Other conflicts appear to end in truces, with unrelated queens and workers persisting together 8 months after fights end.

Sustaining resident ant defenders can be expensive to hosts. I investigated how payment for protection affects tree performance in Chapter 4. Removal of ants increased herbivory and decreased tree growth consistently across a productivity gradient. I compared this effect of ants to the effect of another important savanna tree interaction: competition with grass. My manipulations indicated that mutualists rather than competitors had a greater impact on *Acacia drepanolobium* performance.

My experiments (and methodological tools described in Chapter 5) provide new insights into dynamics within an economically and ecologically important savanna system. In a region where ant defenders influence community level patterns and processes, understanding the forces shaping interactions and decision-making within colonies will be a promising area of further research.
CHAPTER 1
WHY STUDY PLANT-ANT PARTNERSHIPS?

Mutually beneficial interactions between two or more species occur across the
diversity of life (Boucher et al. 1982) in environments ranging from deep sea vents to
alpine landscapes (Dubilier et al. 2008, Bingham & Orthner 1998). These mutualisms
can be lose associations (facultative) or essential for the survival one or both
participants (obligate). One particularly well-studied example of the latter case is the
coevolved relationship between plants and ants. Here the plants provide food and
housing in return for herbivory defense by ant species that cannot live apart from hosts.
Recorded interest in this interaction dates to the arrival of Spanish conquistadors in
Mexico where they discovered ants bodyguards living in the hollow swollen thorns of
Acacia trees (Uphof 1942). Centuries later, manipulations of these vary same ants and
plants contributed to the advent of modern experimental ecology (Janzen 1966).

After years of examining the origins and nature of this partnership D.H. Janzen
stated “Mutualisms have been thought to death; what we need are solid descriptions of
how organisms actually interact, experiments with what happens when a potential
mutualist is removed” (Bronstein 1994). A more recent report identifying important future
directions in community ecology research reiterates the need for direct investigations of
mutualistic interactions and stresses a focus on feedbacks, the context dependence of
outcomes and the broader impacts of these interactions in communities (Agrawal et al.
2007).

Motivated by these authors, my dissertation seeks to investigate unexplored
consequences of plant-ant partnership using the East African savanna tree Acacia
drepanolobium and its symbiotic Crematogaster ants. I begin in Chapter 2 by testing the
underlying assumptions of an evolutionary hypothesis proposed to explain the
dominance of nectar feeding ants in the tropics. Davidson (1997) theorized that extreme
bias in nutrient ratios (high carbon: nitrogen) in liquid rewards offered by plants to ant
defenders represents an incomplete food source. As a consequence, these ants are
predicted to use excess carbohydrates as fuel to increase aggressive defense of
resource territories. Carbohydrates should also fuel worker discovery and dominance of
protein sources that can help balance their diet. Greater protein acquisition by colonies
may then feedback to stimulate worker production and colony growth, and ultimately
contribute to the numerical dominance of these ant species. I conducted long term field
manipulations both augmenting and removing carbohydrate access to *C. nigriceps*
colonies within *A. drepanolobium* hosts to experimentally test these predictions.
Determining if and how feedbacks between rewards of mutualism affect partner
behavior and ecology has important implications for understanding how stoichiometry
affects the ecology and evolution of ants.

In Chapter 3, I explore how ants’ extreme dependence on tree housing (obligate
association) influences intraspecific colony dynamics. Conflicts between unrelated
colonies to gain or maintain tree housing leads to massive worker death (Palmer 2004).
I predicted that as a result of these conflicts queens of the loser colonies would be killed
or expelled and that winners of these ‘ant wars’ would incur significant losses. Reduced
colony size should also mean that winner tree territories (both old and new) become
vulnerable to browsing by large mammalian herbivores and/or subsequent takeover by
neighboring colonies. I experimentally induced fights between neighboring colonies to
test this. I predicted that orphaned loser brood remain on trees after fights and
represent a resource that can be used by winners to offset losses and build back worker number. I used molecular markers to test whether individuals from the loser colony are adopted or enslaved by the winners during colony recovery from conflict.

Shifting focus to the consequences of mutualism for tree partners, I heeded the call to move beyond studying simple pairwise species interactions in a single location. We know from other studies that maintaining ant protection can be expensive (Stanton & Palmer 2011) but that such interactions do not occur in a vacuum. While provisioning ants with rewards, host plants are simultaneously allocating carbohydrates to other processes and partnerships (e.g. pollinators, nitrogen fixing bacteria) and losing the water and nutrients needed for carbon fixation via still other species interactions (e.g. grass competitors). Determining how the strength of different species interactions compare and change with environmental context is essential for understanding and predicting broader community dynamics. In Chapter 4, I report tree growth and reproduction responses to a factorial manipulation of grass and ant removal. This design allowed me to ask how the relative costs of mutualism and competition compare for tree partners and whether these effects change across a heterogeneous savanna landscape.

In Chapter 5 I describe techniques developed to aid in my investigation of research questions. Detailed methods will enable others to produce the various tools I have used to 1) facilitate manipulation of ants within host trees in the field and 2) enable successful relocation of colonies into the lab.
CHAPTER 2
CARBOHYDRATE AS FUEL FOR FORAGING, RESOURCE DEFENSE AND COLONY GROWTH – A LONG-TERM EXPERIMENT WITH THE PLANT-ANT CREMATOGASTER NIGRICEPS

Introduction

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, Chapin & Pons 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. While formulated as an evolutionary hypothesis, several fundamental predictions can be tested on ecological time scales, in particular the expectations that excess carbohydrates 1) fuels ‘high tempo’ foraging and aggressive defense of resource territories and 2) increases 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, Morris, & Bronstein 2009) and aggressive defense of resource territories (Ness, Morris, & Bronstein 2009, Pringle, Dirzo & Gordon 2011). Abundant carbohydrates can also increase worker preference for protein sources and change foraging patterns to ensure target intake ratios of carbohydrates and protein are maintained (Kay 2004, Cook & Behmer 2010, Cook et al. 2010). Further, the size and number of new workers and alates have been increased by experimentally supplementing carbohydrates (Kay, Rostampour & Sterner 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 months) 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 ~500mm). In the region, the ant-plant *A. drepanolobium* dominates ‘black cotton’ savannas, and accounts for 97% 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 extra floral 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 unpub. data) is uniquely suited for this study because workers of this species appear strongly reliant on carbohydrate-rich, nitrogen-poor EFN (Brody & Palmer 2007). *C. 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 > 20m from one another, and 3) inhabiting < 3 individual trees (each < 3m 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 (~1.5-3m in height). Colonies varied in estimated size from 4,000-9,000 workers (Palmer 2004) and were all mature (i.e. alates present in domatia of focal trees).

**Treatments**

All treatments were maintained for 10 months. For the Carbohydrate Supplementation Treatment (Carb+) (see Table 1 for comparison of treatment manipulations), we augmented natural EFN with a 20% solution of cane sugar (sucrose) and filtered rainwater. Artificial nectaries were made from 35mm film canisters (~ 30ml) fitted with 7cm absorbent Water Wicks (Pepperell Braiding Co.) (for an alternative
design see Rowles & Silverman 2009)(Figure 2-1). 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 ~ 45ml additional nectar for each estimated 3,800 workers.

We curtailed colony access to tree-provisioned carbohydrates by removing extra-floral 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 ~ 3-4mm in diameter) and pinched them off with forceps. This technique permanently eliminated EFN access at those leaf sites (c.f. Palmer, Young & Stanton 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 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 vs. water, the latter predicted to itself be an important resource in this seasonally dry system, we installed ‘water only’ artificial nectaries on (Carb-) and Control trees and refilled these every four 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
libitium 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

Resource defense – While aggression/ territorial defense of resources in other
studies have focused 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 one to three 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 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 0.5m or 1.0m from each
focal tree on two consecutive days at 3 separate times, August and October 2008 and
January 2009. *C. nigriceps* is known to regularly visit protein baits 40cm from host tree stems (Palmer 2003). Monitoring baits at 0.5m and 1m 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 ~ 4g canned tuna fish (~ 60% water, 26% protein and 12% lipids (Rasmussen & Morrissey 2007)) placed on laminated 7.6 x 7.6 cm cards and shaded from the sun. Baiting commenced at 800 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”.

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.

Stable isotope analysis – To reveal potential diet changes in focal colonies (Menke et al. 2010) and determine whether supplementation affected worker tissue production and maintenance, we analyzed ten workers from each colony after treatment manipulation ended (nine months after initiation). C4 plants (i.e. sugar cane, δ^{13}C = -12.61) display distinctly less negative carbon isotope signatures compared with C3 plants (i.e. *Acacia drepanolobium*, δ^{13}C = -26.402) and those differences are passed on to consumers without further isotope discrimination (Lambers et al. 2008). Since all the
sugar used in my nectar manipulations was from sugar cane, less negative $\delta^{13}$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. Though not expected, if natural colony protein sources (e.g., herbivorous vs. predatory insects) varied by treatment, we would see differences in worker $\delta^{15}$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.

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

**Data Analysis**

All analyses were performed in JMP (version 8.0.2; SAS Institute 2009 – default type II Sums of Squares (SS)) except analysis of bait discovery for which we used R version 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 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 (Mauchley's sphericity tests, \( P > 0.5 \)). 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.

We fit binary bait discovery data (baits found / not found) using a linear mixed model in R (lmer function in lme4 package). Treatment and bait distance were included as fixed effects and colony was included as a random effect to account for repeated measures design. Because colony responses to baiting were measured in only 3 months, 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 \( \delta^{15}N \) and \( \delta^{13}C \) 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) \( (P = 0.01) \) (Figure 2-2, Table 2-2). This positive effect was detected within two weeks of treatment initiation and remained pronounced through the wet season (Figure 2-2). 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 2-2). Coincident with severe and prolonged dry conditions (see Figure 2-1), aggressive defense by ants in all groups decreased, and carbohydrate supplementation did not buffer colonies against this decline.

Contrary to predictions, the proportion of baits discovered appeared to be higher for (Carb-) colonies than for colonies supplemented with artificial nectar and controls \( (Z = 1.90; P = 0.06) \) (Figure 2-3, Table 2). Baits placed 1m 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 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 δ13C 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) (Figure 2-4). δ15N 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

Aggressive Resource Defense

Results from our long-term field study support the prediction and earlier findings that abundant carbohydrates fuel increased 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 (Figure 2-2). While a significant increase in colony defense of thorn tips was observed just two 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 (Figure 2-2). This may be due to factors associated with rainfall (a significant factor in our analysis of resource defense, P < 0.0001). For all treatments, three months 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.

**Treatments and Protein Foraging Responses**

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 (Figure 2-3). (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 above ground foraging for prey. *Crematogaster nigriceps* is prone to takeovers by both *C. sjostedti* and *C. mimosae* (Palmer et al. 2000) that occur disproportionately during the wet season (Kathleen Rudolph, unpub. 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. 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, Figure 2-2). Cook et al. (2011) demonstrated that in addition to food quality effects on ant foraging, seasonality per se influences foraging choices (Cook et al. 2011). 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.

**Colony Growth**

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, Hobbs & Majer 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). Lab studies monitoring development time in *C. mimosae*, another Acacia-ant found on *A. drepanolobium*, show that larvae emerge as workers after 40-50 days (Kathleen Rudolph unpub. data). In the temperate ant *Crematogaster ashmeadi*, eggs become workers in about 50 days (Tschinkel 2002). Like many tropical ant species, *C. nigriceps* colonies appears to have homodynamous development (all developmental stages are present in nests at all times of year see Kipyatkov 1993, Isbell & Young 2007), suggesting 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-months 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 and 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 not likely constrained by nesting space (Fonseca 1999) since for half of all colonies (regardless of treatment), one out of 8 sampled domatia was nearly empty (i.e. contained < 5 immature ants (overall mean # immature ants per domatia = 42)). In contrast to (Grover et al. 2007), our (Carb-) colonies deprived of natural carbohydrate resources nonetheless continually produced brood, demonstrating an ability to compensate for the loss of tree-provisioned resources, perhaps by foraging more off of host tree.

**Implications**

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 vs. ant control 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 (Figure 2-2,2-3). 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 however precipitation was not an important explanatory variable in our foraging models. 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.
Table 2-1. Experimental design and colony demography (means ± SE) after 10-months of manipulation.

<table>
<thead>
<tr>
<th>Manipulations</th>
<th>EFN access</th>
<th>Artificial Nectary Contents</th>
<th>Colony Demography</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20% solution (sucrose + water)</td>
<td>Eggs</td>
</tr>
<tr>
<td>Supplemented (Carb+)</td>
<td>intact</td>
<td>98(47)</td>
<td>159(22)</td>
</tr>
<tr>
<td>Removal (Carb-)</td>
<td>removed</td>
<td>92(30)</td>
<td>183(23)</td>
</tr>
<tr>
<td>Control</td>
<td>intact</td>
<td>94(50)</td>
<td>142(23)</td>
</tr>
<tr>
<td>Source</td>
<td>df</td>
<td>dfDen</td>
<td>F-ratio</td>
</tr>
<tr>
<td>---------------------</td>
<td>----</td>
<td>-------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Resource defense</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>5.16</td>
</tr>
<tr>
<td>rainfall</td>
<td>1</td>
<td>177</td>
<td>63.04</td>
</tr>
<tr>
<td>tr x rainfall</td>
<td>2</td>
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<td>1.12</td>
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<tr>
<td><strong>Recruitment to</strong></td>
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<td></td>
</tr>
<tr>
<td>baits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>treatment (tr)</td>
<td>2</td>
<td>21</td>
<td>0.14</td>
</tr>
<tr>
<td>distance (0.5 or 1m)</td>
<td>1</td>
<td>69</td>
<td>3.36</td>
</tr>
<tr>
<td>tr x distance</td>
<td>2</td>
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<td>tr x distance x rainfall</td>
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</table>
Figure 2-1. Artificial Nectaries. A) Adding nectar to artificial nectary; B) *Crematogaster nigriceps* ants feeding. Photos courtesy of Kathleen Rudolph.
Figure 2-2. Effect of carbohydrate manipulation on ants’ aggressive defense of host plant resources and total monthly rainfall. Aggressive defense is reported as the mean (± SE) number of C. nigricpes 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) not significantly different between groups (F2, 33 =1.35; P=0.27). Grey histogram bars report total monthly rainfall in mm.
Figure 2-3. Ant discovery of baits located 0.5 and 1.0 m from colony focal trees. Tuna baits were ‘found’ if one or more *C. nigriceps* 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).
Figure 2-4. Carbon and nitrogen stable isotope ratios for *C. nigriceps* workers compared to natural (extrafloral nectar - EFN) and artificial (sucrose) carbohydrate sources. Mean (± SD) of δ^{13}C and δ^{15}N by treatment: Carbohydrates supplemented (Carb+), removal (Carb-), and control (C). Asterisk indicates that (Carb+) was significantly different from removals and controls in δ^{13}C values. Mean for δ^{13}C, δ^{15}N of Sucrose [-12.61, 0]. Mean (± SD) for δ^{13}C, δ^{15}N of EFN [-26.96 (±1.62), 2.38 (± 1.55)].
CHAPTER 3
INTRASPECIFIC SLAVERY OR TRUCES FOLLOW NON-KIN CONFLICT OVER
NEST SITES IN AN AFRICAN ACACIA-ANT

Introduction

Intraspecific conflict over resources can be costly. The evolution of assessment systems enables many adult competitors to settle disputes with minimal physical or energetic escalation (Huntingford & Turner 1987). Still, intense combat often between evenly matched opponents does occur over particularly limited and valuable resources (mates, nesting sites, territory, but rarely food) (Enquist & Leimar 1987, 1990, Innocent et al. 2011). Victory in such high stakes battles can be decided through elaborate displays or violent contests (Huntingford & Turner 1987) that continue until one opponent concedes from exhaustion or injury, or dies (Maynard Smith 1974). Winners that outlast or dispatch losers pay costs for success (depleted energetic reserves and accumulated wounds) and as a result may experience a diminished capacity to defend themselves (from predators and parasites) and their hard won gains in subsequent contests (Riechert 1988, Marden & Waage 1990).

Empirical studies widely document a positive feedback between successful fighting experiences and victory in future contests termed ‘the winner effect’ (Hsu et al. 2006). The converse pattern where winning conflicts increases vulnerability to future defeat or ‘pyrrhic victory’ has been suggested as a factor affecting potential costs and benefits of persistence in fights (Hammerstein & Parker 1982) but experimental tests are rare (Murray 1987, Innocent et al. 2011). Furthermore, whether and how winners respond behaviorally to cope with the costs of fighting and facilitate recovery remains largely unexplored. Consequently we ask, do winners experience a window of
vulnerability following costly fights, and if so how do they respond to speed recovery from conflict?

Ants are compelling model organisms for the investigation of these questions for two main reasons. First, violent fights over territory are common (Hölldobler & Wilson 1990). Territorial intruders are grasped or stung by defending workers, which often leads to dismemberment or death for both combatants. Second, unlike for unitary organisms (Neat et al. 1998), costs of these colony conflicts to winners can be quantified in discrete units (i.e. individual workers killed). In ant colonies, sterile workers forgo reproduction to raise new nest mates and future reproductives that are produced by a fertile queen(s). As a consequence of this ‘superorganism’ arrangement, loss of the queen(s) ultimately results in colony death (Hölldobler & Wilson 2009). Loss of individual workers can be viewed more as costs by reducing colony size and thus the ability of the colony to acquire and defend resources and raise young (Adams 1990, Palmer 2004).

In East African savannas, colonies of the obligate mutualist Acacia ant Crematogaster mimosae attack large mammals, insect herbivores, and other plant-ant competitors (C. nigriceps, C. sjostedti, and Tetraponera penzigi) to protect and maintain sole control of host Acacia drepanolobium trees (Palmer 2004). Swollen thorn domatia provided by these trees are required for the successful rearing of immature ants (brood). As colonies grow, more nesting space is required but cannot be built by workers. Consequently, aggressive inter- and intra-specific wars are waged to displace neighboring colonies from their host plants (Palmer 2004, J. Lemboi pers com). Because A. drepanolobium trees occur in high-density, monospecific stands with ~ 99% of trees > 1m inhabited by ants (Palmer et al. 2000), violent territorial battles are often
initiated when expanding tree canopies contact one another or when elephants knock trees into one another (Stanton et al. 1999a). Resident colonies may lose tree territory to other ant species at rates as high as ~ 7.5% over 6 months (Palmer et al. 2000). *Crematogaster mimosae* colonies are numerically dominant in the system (inhabiting 52% trees (Palmer et al. 2008)), but rates of intraspecific turnover on host plants are unknown.

During territorial battles, individual workers from each colony engage in fights to the death. Takeover success has been positively correlated with larger colony size (Palmer 2004), but expansion also involves potentially heavy casualties for winners. Newly acquired territory (as well as original host trees) may be precariously defended by a diminished worker force, and at risk from attack by other space-limited neighbors.

Defeated colonies leave behind not only valuable living space in the form of swollen thorns, but also many of their immature workers. If eaten, these protein-rich brood could serve as a nutritional subsidy to aid recovery of victorious colonies. Other insects such as cannibalistic spiders exhibit this behavior following costly territorial conflict (Moya-Laraño et al. 2002). An alternative strategy, with the potential to provide more immediate benefit and uniquely available to ‘superorganisms’, would be for victor colonies to enslave abandoned brood (Fouks et al. 2011). In some ant species, newly eclosed workers can both associate with and be accepted by unrelated workers. For example, immature ants either raided from heterospecific nests in the wild (D’Ettorre & Heinze 2001) or experimentally added to foreign nests in the lab (Krag et al. 2010, Fouks et al. 2011) can become a functioning part of host colonies. Yet in more than a decade of intensive research on *A. drepanolobium/C. mimosae*, there have been no reports of mixed species *Crematogaster* colonies forming after interspecific fighting.
Striking morphological between species would make the presence of such colonies easy to observe. By contrast, in conspecific conflicts, the identity of winners and losers cannot be visually detected. Therefore it is unknown if intraspecific *C. mimosae* conflicts result in complete takeovers (with the destruction of the opponent colony’s queen) and if so whether sterile individuals from the losing colony are treated as ‘spoils of war’ and enslaved by winning colonies.

Through a series of field manipulations of *C. mimosae* colonies we investigate 1) the costs of intraspecific conflict to winners and 2) the existence of a post-fight window of vulnerability. We hypothesized that fighting over tree territories produces significant casualties and that coincident with these losses, winner colonies are less able to defend hosts against mammal herbivores and other ant competitors. We also examine 3) genetic relatedness within and between colonies (both before and after fights) using microsatellite markers. We hypothesize that like interspecific takeovers, territorial battles between conspecifics result in complete colony takeovers. We further predict that post-conflict winner workforces are built back through the enslavement of non-kin enemies.

**Methods**

**Experimental Colony Selection**

Colonies of *Crematogaster mimosae* range in size from small single tree incipient colonies to large multi-queen, multi-tree clusters containing hundreds of thousands of individuals (Palmer *et al*. 2000). We restricted our search to single tree colonies 1.2-2.0m in height (33-67mm diameter) to ensure that colonies would fight conspecifics in experimental battles and not retreat to nearby host trees. Colonies on this size tree are estimated to contain 3,500-6000 workers (Palmer 2004). Focal colony trees were all located close enough together that the canopies could be physically conjoined. We
predicted that a benefit of using these small colonies would be simplified colony genetic structure (colonies with single or few queens).

Colony boundaries were mapped using reciprocal transplants of individual workers and watching for aggressive interactions with resident ant (Hölldobler 1979, Palmer 2004). Latex gloves were worn when handling ants and washed with 95% ethanol between individual trials to avoid contaminating subsequent individuals with colony specific chemical signatures. To aid the observation of fast moving transplanted workers, we applied a small dusting of florescent powder (Day-Glo (Day-Glo Color Corp., Cleveland, OH) to the thorax of the test individuals and also an individual from the resident colony as procedural control. If transplanted ants were quickly attacked (but nest mate controls were not) we considered trees to belong to separate colonies. We repeated this method on all nearest neighbor trees up to 5m away to select suitable colonies. Each colony within the pair was identified with metal tags as either A or B with a shared Fight ID number (Table 3-1).

**Manipulations and Analysis**

Between 14-July and 04-August 2011, we induced fights between pairs by tying the canopies of A and B trees together with wire. *Acacia drepanolobium* stems are flexible and tolerated bending. Canopies remained connected for 8 months after fights.

Immediately prior to fights we collected three healthy swollen thorns filled with live workers and brood from each tree into labeled containers rimmed on the interior with Fluon (Bioquip, Rancho Dominguez, CA) and covered with mesh cloth to prevent ants from escaping. These ants were kept contained and isolated in the lab on a diet of sugar water and tuna for ~ 30 days. Live individuals ($n=2-4$) were returned to the field and placed on the main stems of Colony A and B trees approximately 6 and 30 days
after fights and observed in the manner of reciprocal transplants described above. Behavioral winner and loser colonies from each experimental fight pairing were unambiguously identified if individuals from one container were not attacked by resident workers when placed on or near each main stem of the joined trees. This indicates that their original colony was victorious. Additionally individuals from the other container must also be attacked – indicating that their original colony lost. Fight outcomes were deemed ambiguous if neither or only one of the above results were observed.

**Costs of fighting to winners**

Large plastic tarps secured at ground level between paired fight trees (n=6) were employed to collect injured and dead workers that fell from host plants when canopies were experimentally joined and fighting commenced. Dead and injured workers on tarps were collected every 24 hours (if fights lasted more than one day), dried and weighted. Average worker weight was estimated by (dry weight 10 fully intact workers /10) and total fight casualties by (total tarp content dry weight/ individual worker dry weight). Our casualty values are likely an underestimate as windy conditions blew ant body parts and some dead workers off tarps in the field.

Whether intraspecific contest are decided as wars of attrition where individuals from each colony fight to the death in dyads (Palmer 2004) could not be ascertained from observations during the chaos of induced battles. We attempted to determine the casualty ratio experimentally in the lab. To do this we introduced different ratios of non-nest mate workers into sterile arenas to fight. Interestingly these experimental trials often did not induce aggressive responses or fatal fighting. Workers may need contextual cues from many nest mates or host trees to initiate defensive responses. Therefore, to parse winner and loser colony contribution to total casualties, we collected
dead workers from tarps into 70% EtOH. We genotyped 13-16 individuals from each staged conflict and matched them to their respective colonies (following protocols in molecular methods and analysis).

**Vulnerability associated with fighting**

Singletree colonies similar in size and vigor to experimental fight trees were used as controls (n = 10 for control and experimental fight). Surveys for vulnerability were conducted on both control and experimental trees ~3-5 days before and ~6 days after fights.

We examined changes in colony response to large mammalian herbivore browsing using established methods modified from (Palmer & Brody 2007). Two observers carefully approached trees and each visually identified an isolated branch with new growth and one swollen thorn 15 cm from the tip. Disposable ‘mitts’ crafted prior to fieldwork (two sheets of paper towel 11”x6” folded over by 1 inch and taped along 3 sides) were placed on surveyors’ right hands. With a leather-gloved left hand, focal branches were simultaneously raked 3 times and then enveloped by the mitt. Worker ants that attacked and were present on mitts after 30 second were collected along with the mitt into labeled Ziplock bag. Bags were sealed and placed in a -20 freezer where workers died and could later be counted. Surveys occurred on the 3 days before experimental fights were induced (within tree replication n=6, 2 surveyors x 3 days) and then again 6 days after paired fights concluded.

To detect changes in colony defense of main stems, an important access point for invasion by competitors (Hölldobler 1979, Davidson et al. 1988), we took macro digital photos of tree trunks (same trees as above) each including the trunk and nearby ruler size standard. Every tree stem was photographed 5 times (2 mornings and 3 afternoons
immediately preceding fights) and again beginning ~6 days after fights concluded. Photos were cropped to show the area of the stem from ground level up to 10 cm. We counted the number of ant heads visible within this frame.

Change in average response within colonies (post-fight – pre-fight values) between controls and experimental fights were analyzed using t-tests (JMP 8.0)

**Genetic Relatedness Within and Between Colonies.**

**Sampling / collection methods**

Before trees were tied together we collected ~ 30 workers from Colony A and B by tapping swollen thorns with a pencil and funnelling falling ants into a collecting vial (70% ethanol). All collecting devices were carefully inspected between collections to make sure no escapees accidentally contaminated subsequent samples.

For post-fight collections on Sept 15, 2011 (hereafter 2mo) and March 22, 2012 (hereafter 8mo) we clipped 5 swollen thorns from branches that were attached to either Colony A or Colony B’s main stem depending on which was the definitive behavioral loser of the fight or in the case of unclear behavioral results, the smaller tree. Swollen thorns were sealed into individual Ziplock bags and frozen to kill ants. Contents of each trees’ galls (mature and immature ants) were pooled onto labeled vials with 70% ethanol.

For the Colony A and B pre-fight samples as well as 2mo after samples, only whole fully developed workers were genotyped. Individuals were inspected under a dissecting microscope to ensure they were whole with no legs missing (indicating they were alive at the time of collection and not cached casualties or emerged workers that were recently killed) and to be sure no other individuals DNA (e.g. entangled leg) may be contaminating the focal individual. For 8mo samples we analyzed only immature ants
(nearly all worker pupae, but for trees with < 24 pupae we extracted large larvae or male pupae). We have no field data indicating the life expectancy of workers and analyzing immatures allowed us to determine whether offspring produced 8 months after fights belonged exclusively to one colony (suggesting complete takeover), or whether both pre-fight genotypes remained (indicating colony fusion with both colony A and B queens present). If new maternal genotypes were revealed we could infer that new queens were present within the colony. This would indicate either secondary takeover by non-relatives or possibly the emergence of reproductive daughter queens. For both Colony A and B in each Fight ID sample sizes are listed in Table 1 but roughly follow: 8 workers Pre-Fight (PF), 48 workers and 24 immature ants from purported loser colony side of combined fight pair after 2 months, and 8 months respectively.

Lab protocols

Individuals were extracted either using Qiagen DNA easy kits or Qiagen Puregene extraction techniques. All PCRs were performed in 15 μL volumes using a GenAmp®9600 Thermalcycler (Applied Biosystems, Carlsbad, CA, USA). PCR cocktails of 5μL Qiagen master mix and 3 microliters of primer soup with either 10 (Set 1 - (Rubin et al. 2009) ) or 7 (Set 2) labeled primers multiplexed in 10 μmolar mix (see sup for dilution recipe) were used for amplification of samples from PF and 8mo. For 2mo samples where 48 individuals from each fight pair were analyzed, we first determined which loci contained alleles that could distinguish Colony A from B (from PF analysis results). We selected the 3-5 most diagnostic loci and included only primers from those loci in the PCR reaction. If individuals could not be definitively assigned with the subset of primers, they were re-run with more loci (Set 1 and/or Set 2). The cycling protocol for primer Set 1 consisted of an initial denaturation step of 15 min at 95 °C,
followed by 35 cycles of 94 °C denaturation for 45 s, annealing at 62 °C for 90 s and extension at 72 °C for 60 s and a final 10 min at 72 °C. For primer Set 2 an initial denaturation step of 15 min at 95 °C, followed by 30 cycles of 94 °C denaturation for 45 s, annealing at 62 °C for 90 s and extension at 72 °C for 60 s (with the final 20 cycles dropping the annealing temp to 61 °C) and a final 10 min at 68 °C. PCR products were run on a Capillary Electrophoresis Genetic Analyzer (an ABI Prism 3130) at UCDNA Sequencing Facility at UC Davis and analyzed using the GeneScan software (Applied Biosystems, Carlsbad, CA, USA). Fragment data were visualized and scored using STRand Version 2.3.69 (Toonen & Hughes 2001).

Analysis of genetic data

Sibship reconstruction - Parentage for each worker was reconstructed using max likelihood approach implemented in COLONY v2.0.1.8 (Wang 2008). Null alleles and scoring errors were accounted for using 0.05 default error rate at all loci and no a priori relationships were assumed. Data for all individuals (from all fight pairs at all times n=765) were combined for analysis. Maximum likelihood methods separate individuals into full sibling or half sibling families with associated probability of inclusion and exclusion (Table 3-1). Mother and Father ID’s were also inferred for each individual allowing reconstruction of queen number and the identity of the maternal and paternal lineages producing the workers present at each sampling point.

Relatedness - Allele frequencies obtained from COLONY analysis (above) were used to calculate relatedness in COANCESTRY v 1.0.0.1 (Wang 2008). Relatedness values between winner and loser colonies PF were based on all genetic data from 17 loci. Because 2mo individuals were genotyped at a reduced number of loci, relatedness estimates within individual winning colonies across time all time points (Figure 3-2) were
based only on data from those restricted 3-5 diagnostic loci. For colonies where genetic
reconstruction did not reveal a distinct winner (393ID and 359ID), the PF relatedness
(Figure 3-2) used for statistical analysis is the mean of average within colony
relatedness for both Colony A and B. For each fight pair, individuals were grouped by
their sampling origin, Colony A PF, Colony B PF, 2mo, 8mo, and compared to all others
within the group to produce average within colony relatedness at each time period.

From the 7 relatedness estimators offered by COANCESTRY, we selected Triadic
Maximum Likelihood estimator of relatedness (TrioML) because it restricts values to 0-1
making interpretation intuitive. In contrast to other pairwise methods, this measure uses
a third reference individual to help minimize error (Wang 2007).

To test for effects of fighting on relatedness within colonies through time, we
compared TrioML values across PF, 2mo and 9mo sampling periods using standard
least squares regression with Fight ID as a random effect.

Results

Identifying Winners

Behavioral field assays performed six days after the conclusion of physical
aggression identified a definitive winner and loser colony for fight dyads in 78% of cases
(7/9). Molecular analysis of the two ambiguous behavioral outcomes determined a clear
winner (348ID) but no clear winner for (393ID) (Table 3-1). Notably, behavioral assays
identified a definitive winner for (359ID) however molecular results were not consistent
with that conclusion (Table 3-1, Figure 3-2). Field assays repeated after ~30 days
corroborated our earlier identification of the victor colony demonstrating an ability by
four colonies to discriminate between kin and non-kin up to 1-month after battles.
Costs of Fighting for Winners

Estimated worker losses in fights between colonies ranged from 390-10,073 individuals (n = 6, mean 5,816 ± 1,576). A subset of these casualties were genotyped and matched as full or half siblings to Colony A or B Pre-Fight (PF) samples, all with a probability of assignment >0.94 in maximum likelihood sibship configurations from COLONY (Wang 2008). Winning colonies (as determined by genetic data and/or behavioral assays) experienced high costs to fighting, with 19-56% (n = 4, mean 33% ± SE 8) of the dead or injured belonging to the victors. This means that on average almost 2,000 winner workers perish in battles (0.33 x 5,816). If experimental colonies contained between 3,290-6,580 individuals before fights (Palmer 2004) then successful colonies could expect to lose between 1/3 and 2/3 of their pre-fight workforce as a result of fighting.

In one case where fusion followed fighting instead of a single colony winning, losses mounted to ~10,000 total individuals. We infer from pupae genotypes that both PF queens remained alive and producing brood inside a combined colony after 8mo. This suggests a truce between previous enemies was achieved. Casualties in this instance were shared approximately equally between fighting partners (7:8).

Vulnerability

Following fights, winner defense of host trees declined significantly. Canopy defense dropped by more than half compared to PF levels as fewer ants from winner colonies responded to simulated branch herbivory than from control colonies (P = 0.0059, Fig 1A). Winner protection of stem access points also fell, with a marginally significant difference between treatment and controls (P = 0.0667, Figure 3-1B). Simulated browsing incited no defense on 35% of surveyed branches after fights (21 vs. 6
undefended before fights). Importantly, in the days after conflict, winners were 2.2x less likely than controls to have any workers attempt to repel threats to branches on a given day (mean # days with no ants attacking mitts – treatment 2.2±0.5, control 1.0±0.5) and 3x less likely to have workers patrolling host tree trunks on a given day (mean # days with no ants present in stem photos – treatment 3.4±0.6, control 1.1±0.5).

**Colony Genetic Structure**

Pre-fight relatedness between colonies was low for all nine fight pairs analyzed using molecular markers (TrioML estimate of r <.08 for all pairs, Table 3-1). Most colonies were determined to contain full and half sib workers produced by a single queen (Table 3-1). However two PF colonies (351ID Colony B? and 393ID Colony B) were determined to include workers produced by multiple queens (Table 3-1).

Post-fight relatedness suggests three different outcomes, complete rejection of non-kin, slavery, and fusion. For fight pairs (286ID and 360ID), all post-fight individuals (n= 68-70 from 2mo and 8mo) were assigned as full siblings with individuals from only one PF colony (complete rejection of non-kin). The remaining 7 pairs contained workers 2months after fights that were matched as full siblings with individuals from both PF Colony A and B. Consistent with slavery, 6 months later (8mo after fights) all genotyped larvae (n=23-24) from 5 of these 7 pairs were assigned to only one of the PF colonies. For these colonies, molecular data suggests a single queen-right colony succeeded in conflict and subsequently included unrelated workers but confirmed that loser queen(s) were either no longer present or no longer contributing larvae to the colony at 8 months. Surprisingly for fights (359ID and 393ID), genotyped larvae at 8mo were full siblings with either PF Colony A and B individuals, indicating that one colony did not completely overtake the other and that both PF queens were present and producing offspring.
Furthermore, 16 individuals (76%) of 8mo samples from fight 359 were classified by COLONY as full sibs with each other (probability inclusion /exclusion >0.99) but were inferred to be the offspring of a novel maternal genotype. Overall within-colony worker relatedness decreased significantly from a PF average of 0.72±0.03 to 0.52±0.08 after 2 months (P=0.002). Average relatedness for larvae at 8 months after fights, however, was similar to PF worker relatedness (.68±0.08), indicative of restoration of pre-fight relatedness conditions at a colony level (Figure 3-2).

Discussion

Worker Losses and Vulnerability

While territorial fights between C. mimosae colonies can be fatal to losing colonies, they also can be costly to winning colonies. Battle casualties reduce winner colony size by as much as 2/3, and remaining individuals are spread over a larger territory (their former host plus the newly gained tree canopy).

In contrast to recent investigations highlighting advantages to combat victory beyond resource acquisition (e.g. future victory (Davidson et al. 1988, Hölldobler 1979) and better health (Archie et al. 2012)), we find that after successful fights, winning ants may suffer from a window of vulnerability. This window of vulnerability is likely to make colonies more susceptible to attack from other ants, and to loss of territorial value (e.g. removal of extrafloral nectaries, domatia, and reduced tree growth) as a consequence of browsing. Less than one week after experimentally induced wars, colony defense of host tree canopies dropped by 66% and tree trunk protection fell by 62% compared to pre-fight levels (Figure 3-1). Manipulations of ant abundances on A. drepanolobium trees reveal a negative relationship between colony size and branch browsing by elephants (Goheen & Palmer 2010). The actively growing shoot tips favored by large
herbivores are also the site of carbohydrate-rich extrafloral nectar production, which colonies rely on to fuel activity and feed developing larvae (Palmer et al. 2008). Simulated browsing of these resources incited no defense on 35% of surveyed branches after fights (21 vs. 6 undefended before fights). Inability to protect tree-based energy sources may diminish the value of newly won territory, and likely temporarily hinders colonies’ ability to produce and sustain workers.

Fewer ants patrolling tree trunks (and complete absence of defenders in 33 survey periods post-fight compared to only 7 before) suggests greater winner vulnerability to secondary takeover from nest-limited neighbors. In other taxa, individuals pursuing new territories (e.g. neighbors seeking to upgrade, subordinates, floaters) survey residents’ activities and condition and also eavesdrop on conflicts (Johnstone 2001). Non-combatants then apply information gained through monitoring to target weakened competitors (Clutton-Brock et al. 1979). During C. mimosae battles and when host trees are disturbed by herbivores, pungent alarm pheromones are released (Wood et al. 2002) and can be easily detected by humans and flies (K. Rudolph per.obs.). Alarm pheromones can be specific and induce a variety of different stereotyped behaviors including attraction to source or repulsion, increased movement, heightened aggression (Verheggen et al. 2010). Particular chemical components of pheromones signal threats across great distances and may widely communicate that colonies are under attack. It seems probable that unique chemical signals are used to indicate the threat type (ant competitors vs. browsers) and thus induce the appropriate response within colonies. If true, then conflict between C. mimosae colonies (and perhaps the resultant diminished resource holding capacity) may be announced to neighbors. In a system where colony size clearly underlies competitive success, we
infer that public battles fought to gain territory may subject winning colonies to increased risk of attack. These findings represent a rare experimental quantification of the costs and consequences of escalated fighting for winners in their natural environment.

**Colony Relatedness Following Conflict**

Our further investigation of how vulnerable victors respond behaviorally generated a surprising finding. Following high casualties in fighting, five winner colonies enslaved non-kin enemies. Despite fatal aggression between non-nest mates in these experimentally induced fights, winner colonies two months after conflicts contained live workers that were full siblings with the loser colony (Table 3-1). Losers represented an estimated 4-44% of post-conflict workforce. This integration of losers is not consistent with queen-right colony fusion (both queens present) because no brood matched 'loser' genotypes at 8mo (n=22-24 individuals per fight). We conclude that loser queens were either killed or escaped during fights, and infer that their undeveloped offspring are reared by winners, acting as a captive and temporary work force within the winner colony. Genetic similarity between winner and loser colonies does not explain the incorporation of non-colony members, as average pairwise relatedness values between fighting colonies was invariably low (TrioML<.08, mean 0.02±0.06, cousins should exhibit r values of ~.25) (Table 3-1). We cannot rule out the possibility that loser workers present at the time of conflicts gave up to join the victors, but we do not expect this happens given the extreme levels of aggression that characterize fights (discussion below). Yet regardless of the route into winner colonies, non-kin sterile workers laboring for the fitness benefit of an unrelated queen/colony represents one of the few natural examples of intraspecific slavery in ants (Hölldobler & Wilson 1990). Our study also
presents the first case where intra-specific slavery has been triggered experimentally in the wild via forced territorial conflict.

We expect that after our experimental takeovers 1700-5550 loser immatures could remain within domatia. This is estimate is based average number of brood per domatia (37.3±2.9)(Isbell & Young 2007) and the number of domatia counted on experimental trees (48-150). Winner adoption of loser brood could potentially double the number of emerging worker in a matter of days with no additional inputs. Pupae isolated from workers in the lab do not require tending to emerge as fully formed workers (K. Rudolph unpublished data). Additionally, we found that larvae develop into workers in 40-50 days, and production of eggs to become workers would take even longer. Retaining loser brood may further boost colony size by stimulating the surviving queen's egg production (Offenberg et al. 2012). Enslavement of abundant non-kin brood is a more efficient way (in terms of time and energy) of converting loser brood into valuable workers than a potential alternative – cannibalism.

**Intraspecific Slavery and Colony Fusion in Context**

Previous descriptions of intraspecific slavery in ants comes from species that are close relatives to facultative and obligate interspecific slave-making taxa (Kronauer et al. 2003). *Crematogaster*, while a species-rich genus (476 known species (AntWeb), has no known obligate slavemakers (Hölldobler & Wilson 1990) and diverged from known obligate slave-making species > 80 mya (Moreau et al. 2006). Like many other obligate plant ants and cavity nesters, *Crematogaster* sp. are known to compete strongly with conspecifics for nest space (Fiala & Maschwitz 1990) and invade the domatia of heterospecific neighbors (Davidson et al. 1988). Conspecific usurpation, though difficult to detect, is also predicted (Foitzik & Heinze 1998). Our findings suggest
that slavery could be an overlooked phenomenon in these circumstances, and potentially widespread in ground-nesting ants that also engage in territorial conflicts.

Interspecific slavery, social parasitism and brood raiding/pleiometrosis in colony founding (Bartz & Hölldobler 1982, Bernasconi & Strassmann 1999) are other cases of non-kin association in ants, but importantly are rarely associated with high worker-worker aggression or fatal fighting. In other cases of intraspecific integration, ‘victims’ allow invaders into nests with little resistance, and if escalation does occur, few casualties result (Gadau et al. 2003, Kellner et al. 2009, Foitzik & Heinze 1998). In other cases of ant wars where fighting kills scores of workers, aggression is a mechanism of colony rejuvenation and not a major cost ((dead workers are fed to developing larvae in the absence of protein sources (Mabelis 1978)). High density of conspecifics is expected to favor less costly conflict (ritualized fighting (Hölldobler 1976) or reduced conflict via low inter-neighbor aggression (Steiner 2011). *Crematogaster mimosae* natural history is in stark contrast to these patterns because high aggression typifies interactions between large neighboring colonies, resulting in violent conflict indicative of a strong ability to discriminate nest mates from non-nestmates. Clear and lasting markers of colony affiliation do not seem easily overcome. Colonies in the field retain ‘self’ identity even when polydomous nests span several host trees separated by many meters. Furthermore individual workers that have been isolated in the lab from original nests for at least 30 days retain signals of nest affiliation. Our data show that incorporation of non-kin is not a result of permanent lost recognition (Holway et al. 1998), an obligate way of life (Buschinger 2009), or a proactive means for colony growth (Nonacs 1993) found in other systems. Instead, enslavement behavior follows losses
and suggests a defensive strategy that provides victors a potentially accelerated means to colony size recovery.

Our other outcome – the unexpected fusion of colonies after costly fighting - does not appear as advantageous. In fact for another well-studied colonial organisms, the cnidarian *Hydractinia*, contact between two groups produces one of three responses, immediate aggression, amicable fusion, or transitory fusion (fusion followed by rejection, Tsutsui 2004). The converse, rejection followed by fusion that we find, is not observed. For two fight pairs we found wars were followed by colony truces with both queens surviving. In one staged conflict, an estimated 10,000 workers died before mergers occurred. It is possible that for colonies with low asymmetry, and social insects generally, assessment of fighting ability is difficult. Fights may escalate either because the superior competitor cannot be readily determined or contested resources (host trees) are essential for colony survival, perhaps both. However cues indicating growing cost of conflict (e.g. fight duration) may induce de-escalation behavior in workers and protect against Pyrrhic victory. Reciprocal de-escalation in both colonies could then result in truce. Queen cooperation at times of vulnerability has precedent in ants (joint colony founding) but not known for mature colonies following conflict.

In conclusion, we demonstrate that costly fighting increases vulnerability, which is associated with a significant, temporary decrease in within colony relatedness for *C. mimosae* (Figure 3-2). Different routes (enslavement and queen/colony truce) lead to similar post-fight (2mo) outcomes, and each appears to overcome previously strong colony self-non-self recognition systems. Decades of research in ants have focused on the importance of shared ancestry for colony cohesion and cooperation. Our study contributes to a growing understanding that colony composition and recognition can be
complex, and environmental context may be an important and underappreciated factor in this complexity. Moreover, we identify a system where inter-individual behaviors among non-kin swing from one extreme interaction (fatal fighting) to another (coexistence of non-kin within a single nest). Further exploration will help determine how signaling mechanisms and community conditions mediate conflict escalation and resolution.
Table 3-1. Sampling design, colony relationships, and experimental fight outcomes for *Crematogaster mimosae*.

<table>
<thead>
<tr>
<th>Fight pair ID</th>
<th>sample n (A, B, 2mo, 8mo)</th>
<th># loci (A, B, 2mo, 8mo)</th>
<th># queens (A, B, 2mo, 8mo)</th>
<th>Winner (behav assay)</th>
<th>Winner (molecular data)</th>
<th>Slaves?</th>
<th>Prop Slaves (at 2mo)</th>
<th>Between colony relatedness (A vs B)</th>
<th>A and B genotypes present after 8mo?</th>
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<td>360</td>
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<td>17, 17, 4, 17</td>
<td>1,1,1,1</td>
<td>A*</td>
<td>A</td>
<td>no</td>
<td>0</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>386</td>
<td>8, 8, 44, 24</td>
<td>17, 17, 5, 17</td>
<td>1,1,1,1</td>
<td>B*</td>
<td>B</td>
<td>no</td>
<td>0</td>
<td>0</td>
<td>no</td>
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<tr>
<td>348</td>
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<td>17, 17, 4, 17</td>
<td>1,1,2,1</td>
<td>unclear</td>
<td>B</td>
<td>yes</td>
<td>0.21</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>351</td>
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<td>17, 17, 4, 17</td>
<td>1,2,3,1</td>
<td>A</td>
<td>A</td>
<td>yes ★</td>
<td>0.17</td>
<td>0.08</td>
<td>no</td>
</tr>
<tr>
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<td>0.04</td>
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<td>1,1,2,1</td>
<td>A</td>
<td>A</td>
<td>yes</td>
<td>0.04</td>
<td>0</td>
<td>no</td>
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<td>17, 17, 4, 17</td>
<td>1,1,2,1</td>
<td>B</td>
<td>B</td>
<td>yes</td>
<td>0.04</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
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<td>17, 17, 5, 17</td>
<td>1,2,3,2</td>
<td>unclear</td>
<td>?</td>
<td>unclear ° fusion</td>
<td>0.04</td>
<td>yes</td>
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<tr>
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<td>8, 8, 46, 21</td>
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<td>1,1,2,3</td>
<td>A</td>
<td>?</td>
<td>unclear ° fusion</td>
<td>0.03</td>
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<td></td>
</tr>
</tbody>
</table>

A = individuals collected from A tree (Pre-fight)
B = individuals collected from B tree (Pre-fight)
2mo = individuals from joined A+B trees (2 months after fight)
8mo = individuals from joined A+B (8 months after fight)

a n - number of individuals (indv) genotyped from each period (A, B, 2mo all indv = workers; 8mo indv = pupae or larvae)
b 2mo loci restricted to those with diagnostic alleles differentiating A and B genotypes
c Winner of behavioral assays (★) only one tree of the pair showed definitive winner and loser
d 2mo samples assigned as full sibs with loser colony genotype with (inclusion/exclusion probability > .99) for all except ,
  ★ 351 - half sibs with B(1) = 7 indv (p >.95), full sib with B(2) = 1 indv(p>.99)
  ° 393 - full or half sib with B(1) (10 indv) p>.85 , full sib with B(2) (10 indv) p>.99, full sib with A (28 indv) p >.99
  # 359 - full sib with B (24 indv), full sib with A (22 indv) p >.99
e average pairwise relatedness (TrioML estimator in COANCESTRY (Wang 2008))
f 8mo samples excluded as full sibs from loser genotype at p>.99
  393 - A=12 indv, B(1)= 11 indv;  359 - A=4 indv, B=1 indv, new queen=16 indv
Figure 3-1. Vulnerability following conspecific conflict. Winner colonies decreased defense after battles (mean ± SE change in the number of ants guarding host trees) in canopies (A, -66%) and on main stems (B, -62%) relative to pre-fight levels. Un-manipulated controls did not show a decrease in defense over the same period.
Figure 3-2. Average within colony relatedness over time for fight manipulations. Pre-fight relatedness values are from winner colonies (except for 393 and 359). For these two colonies where no definitive loser could be identified, values are an average of PF Colony A and B relatedness. Workers collected 2 months after fights were significantly less related on average to their nestmate contemporaries than workers sampled before fights and larvae sampled 8 months after (P=0.002). Fight pairs 360 and 386 (open symbols) contained no loser genotypes 2mo after. Solid symbols = colonies with slaves (loser genotype present at 2mo but not at 8mo). Line symbols = colonies with no definitive winner (both fight pair genotypes present in workers at 2mo and larvae at 8mo), inferred queen truce and fusion.
Figure 3. Images of experimental fights. Conflict induced by joining host tree canopies of separate colonies (A) with a tarp to catch casualties. (B) *Crematogaster mimosae* battle in progress on the surface of a contested domatia. Photos courtesy of Kathleen Rudolph.
CHAPTER 4
CONTEXT DEPENDENCE AND THE RELATIVE STRENGTH OF POSITIVE AND NEGATIVE INTERACTIONS

Introduction

To move community ecology toward a more predictive science, ecologists have been tasked with advancing beyond pair-wise studies of species interactions and exploring how multiple species interactions and environmental variability influence life history traits and ecological processes (Agrawal et al. 2007). Increasing evidence also suggests that explicit consideration of positive interactions is necessary for a more complete understanding of ecological community dynamics (Stachowicz 2001, Hay et al. 2004). This latter point may seem unsurprising since the distribution and abundance of countless plant species (that form the base of many terrestrial food webs) are influenced by classic mutualistic (i.e. positive) interactions (Boucher et al. 1982). For example, survival of individual plants can depend on nutrient transfer from bacterial and fungal symbionts or defense by ants (Heil & McKey 2003, Klironomos et al. 2011). Similarly, pollinator and seed dispersal services can determine a plant’s success or failure in reproducing (Jordano 1987).

Another important reason for incorporating positive interactions in studies of community dynamics is that they are not simply unconditionally beneficial or without cost (Bronstein 2001). On the contrary, plants must produce rewards to maintain their partnerships and such offerings are inferred to be expensive for several reasons (Heil & McKey 2003). First, the extreme lock and key nature of some partner phenotypes (e.g. long tubular flowers and hummingbird pollinators) indicates strong selection for filters that exclude all but a specific partner or suit of partners (Fenster et al. 2004, Heil et al. 2005). Second, many rewards are inducible, only produced at times when partners are
present or services are required (Stone et al. 1996, Heil et al. 1997). Third, some plant partners have evolved an ability to sanction or punish cheaters that do not provide appropriate goods or services (Kiers et al. 2011, Jandér et al. 2012, Heil & McKey 2003). The fraction of a plant’s resource budget located to pay these expenses should vary from species to species but also through time and space depending on biotic and abiotic context (Heil & McKey 2003). Consequently to anticipate the effects of these interactions on individual partners, the wider community, and perhaps even on long-term selection pressures, it is important to evaluate the costs of partnership maintenance. Costs have been quantified in absolute terms (e.g. % total assimilated resources used to produce food bodies for ant defenders (Heil et al. 1997)) but what has not been attempted is to quantify costs in a comparative framework (as in food web ecology (Berlow et al. 1999)). In other words, how do the net effects of ‘positive’ interactions on plant performance compare to effects of other interactions, particularly those predicted to be ‘strong’ (Wootton & Emmerson 2005).

Across savannas in East Africa, *Acacia drepanolobium* trees host ant species in return for defense against mammal and insect herbivores (Hocking 1970). These ants use and protect trees in various ways (Palmer et al. 2010). At Mpala Research Centre in Laikipa, Kenya, workers of the most aggressive and numerically dominant species *Crematogaster mimosae* act as bodyguards in exchange for domatia housing and carbohydrate-rich nectar secreted from extrafloral nectaries (Palmer & Brody 2007). This species extracts additional liquid carbohydrates from trees by tending sucking insects that process phloem and excrete honeydew which the ants consume (Hocking 1970, Young et al. 1997). Colonies also boost the number of workers and brood
sheltered within canopy branches by building carton inside swollen thorn domatia to increase habitable surface area. Supporting greater numbers of ants can benefit plants (Rocha & Bergallo 1992, Pringle et al. 2012) however more is not always better for hosts (Izzo & Vasconcelos 2002, Fonseca 1993). While other studies report that the net effects of ant defense are positive (Chamberlain & Holland 2009, Rosumek et al. 2009, Trager et al. 2010) even when ants behave in seemingly exploitative ways (e.g. tending phloem feeding insects, see Styrsky & Eubanks 2007, Pringle et al. 2010), in this system supporting ants via offered and purloined ‘rewards’ appears to be taxing to host trees. When protected from mammal herbivores over multiple years, trees relax defense investment and produce fewer food and housing structures (Young & Okello 1998, Huntzinger et al. 2004, Palmer et al. 2008). Whole colony removals also show that at least over a fraction of a tree’s lifetime (54 months) individuals without ants experience a slight increase in growth and reproduction (Stanton & Palmer 2011).

*Acacia drepanolobium* trees expending resources on ant defenders are simultaneously interacting strongly with grass to acquire resources. The competitive (i.e. negative) interaction between trees and grass for water and nutrients can be so strong that it underlies shifts from grassland to forest ecosystems (Scholes & Archer 1997, Sankaran et al. 2004). Landscape level manipulations conducted by humans’ intensively grazing livestock show that reduction in grass cover can favor growth by trees and lead to bush encroachment (Moleele et al. 2002). As with absence of ant defenders, experimental removal of grass beneath *A. drepanolobium* significantly increases growth of trees of all size classes (Riginos 2009).
In this study we ask two questions; what are the costs of mutualism relative to competition for *A. drepanolobium* and do they vary across environmental gradients? We conducted a factorial experiment manipulating tree interactions with grass (presence/absence) and ants (presence/absence), and repeated this experiment in two habitats one of apparently high growth and productivity and one with more limited acacia growth. The same plant performance measures (diameter and height growth and reproduction in the Short site) could be collected for each treatment group enabling direct comparison of the magnitude and direction of interaction effects. I predicted that removal of ants would increase plant growth (as in Stanton & Palmer 2011) over the span of our study but this increased growth would be comparatively less than that seen in trees with grass removed (Riginos 2009). I further expected that site-specific conditions would shape the strength and direction of interactions, with shrub sites trees benefiting more from absence of both interactors. This hypothesis is based on the suggestion that Short site trees are resource limited and therefore likely competing more strongly with grass and allocating relatively more to ants than Tall site trees.

**Methods**

**Study System**

*Acaica drepanolobium* 'the Whistling Thorn Acacia' occurs in monodominant stands throughout East Africa from Ethiopia to the Congo and is strongly associated with seasonally waterlogged “black-cotton” vertisol soils (Coe & Beentje 1991). This species has been focus of decades of research both ecological (concerning its mutualistic association with four species of acacia ants and its importance for endangered rhinos (Stanton *et al.* 1999b, Palmer *et al.* 2000, Wahungu *et al.* 2010, Palmer *et al.* 2010) and economic (as a valuable charcoal wood and documented

**Tall and Short sites**

At Mpala Research Centre, both tall and short forms of *A. drepanolobium* occur with an abrupt but contiguous transition between the two over a landscape with no visible changes in topography (slopes <5%) (Okello et al. 2001). Extensive stands of trees up to 6 m tall often occur less than one hundred meters from stands of the same species that are no more than 3m tall (Palmer 2004) hereafter referred to as Tall sites and Short sites (Figure 4-1, 4-2). Hypotheses to explain short forms include fire frequency (Pratt 1971), succession, herbivory (Coe & Beentje 1991, Milewski et al. 1991), soil composition or depth, and waterlogging yet I am aware of no focused investigations of these differences. Some common mechanisms underlying patterns of ‘bush’ forms within tree species appear unlikely to operate in this system. I can exclude fire as an important factor maintaining differences in morphs at Mpala because burns have been suppressed since the early 1900’s (Goheen et al. 2010). While harvesting trees for charcoal is common in the area, bush morphs also cannot be explained as a single successional or resprouting cohort because felling trees has been restricted for more than 50 years (T. Young pers. com).

Genetic differentiation between the morphs is another unlikely explanation for several reasons. Although seeds are adapted for wind dispersal over short distances, mammal herbivores do consume branch tissue and attached pods (Goheen et al. 2007).
Elephants and giraffes, the most common mammal browsers of *A. drepanolobium* move freely between Tall and Short sites and are expected to transport seeds from one site to the other. Seeds are robust and germinate after experimental treatments of HCl acid, beetle damage and burning, making them likely to also survive digestion (Okello & Young 2000). Finally, trees from both sites set flowers synchronously (K.P. Rudolph pers. obs.) and given short distances between sites I found no barriers to cross pollination by bees or other insect pollinators (Stone *et al.* 1996).

Other classical explanations for ‘stunted’ forms of characteristically large trees include site specific differences in wind intensity, precipitation, altitude, light levels and litterfall (Busby & Motzkin 2009), none of which are observed to differ strongly between Tall and Short sites.

Potentially more relevant factors all involve productivity. To investigate underlying differences in standing grass biomass and productivity (in our case re-generation of biomass), we selected 8 trees in both Tall and Short sites with characteristics matching experimental focal trees (see below). Grass growing within 60 cm radius of main stem were clipped down to 2 cm, collected, dried, and weighed to the nearest gram with Pesola® scales in Aug 2010. At 4-6 month intervals (Feb 2011, May 2011 and Dec 2011) we repeated this procedure. To establish differences in conspecific competitive environment I first measured the distance between focal trees in each site and their nearest neighbor and the neighbor’s diameter at 10 cm. We also counted the number of healthy (not dry or damaged) swollen thorn domatia produced by focal trees to further understand how forms vary by site.
Focal tree selection

Between Oct and November 2009 at the end of prolonged and severe drought (92mm over 10 months, < 20% of average for the period), 3 teams of 2 people searched an area of ~ 15 hectares in each site for experimental trees. To make the results of the present study comparable to two previous studies (Riginos 2009, Stanton & Palmer 2011) we restricted our experimental trees to between 110 and 170 cm tall. Trees of this size are expected to contain 3,000-6,000 workers (Palmer 2004). We know from more general surveys of both areas that trees grow differently in height relative to diameter in the two sites (K. Rudolph unpub. data). To control for those effects we only included trees with diameter 25-45 mm and that fit a height to diameter ratio of 18:1. We measured height as the elevation of the highest living branch tissue using telescoping tree poles. We recorded tree diameters using digital calipers on tree stems 10 cm above ground level.

Tree age was not a factor we were able to control. While 1-2 m trees in Tall Sites are predicted to be 20-40 years old (Palmer et al. 2010), I expect that similarly sized trees in short sites are even older. Unfortunately, weakly tri-modal wet and dry seasons and equatorial location of the study site limit the regularity of tree ring production and make determining tree age very difficult and outside the scope of this study (Gourlay 1995).

Because ant inhabitants can modify trees in ways likely to affect subsequent growth (especially *C. nigriceps* which prunes axillary shoots and limits branch growth), we selected only *C. mimosaee* trees with no recent history of residency by alternate species. To do this we scanned branches from tips back toward main stems and checked for signs of axillary shoot damage. If no removal was noted then trees were not
likely to have harbored *C. nigriceps* recently. For each focal tree in both sites we counted the number of healthy (not dry or damaged) swollen thorn domatia produced and also measured the distance between focal trees and their nearest tree neighbor and the neighbor’s diameter at 10 cm to further understand how forms vary by site.

**Ant and Grass Manipulations**

A total of 176 trees meeting these specific requirements (88 in each site) were marked with metal tags and all fruits attached to trees were removed. This allowed us to track treatment effects on reproduction in Short Sites. In each site, 22 trees were assigned to each of the following four treatments (Figure 4-3):

- **ants** (ants absent, grass present) - To initially exclude ants we followed methods in Stanton and Palmer (2011). We sprayed tree canopies with pyrethrin-based insecticide (0.6% alpha-cypermethrine) to eliminate resident ants (Stanton & Palmer 2011, Frederickson *et al.* 2012). To ensure all workers had died we revisited trees and injected small amount of the same insecticide into swollen thorns where ants still appeared active. Colonization by neighboring colonies was prevented by construction of sticky barriers. To do this we first wrapped stems with duct tape to shield tree bark and then applied a coat of Tanglefoot (The Tanglefoot Company, Grand Rapids, Michigan, USA). Following initial ant removal, trees were visited on intervals of two – four weeks to refresh Tanglefoot barrier as needed and to check for colonization by winged queens. We exterminated incipient colonies using surgical syringes to inject ~ 2 cc of a 3% dilution of Dawn Original (phosphate-free) dishwashing soap (Procter and Gamble, Cincinnati Ohio USA) and water into swollen thorns (Drees & others 2002).

- **grass** (ants present, grass absent) - All grass plants growing under trees at the time of ant removal treatment initiation were dry and apparently dead due to severe
drought conditions in 2009. We clipped and discarded all above ground biomass within a 60cm radius under trees assigned to -grass and –ants/-grass treatment groups. When new grass shoots began to grow following rains in January 2010, we permanently eliminated them following methods in Riginos (2009). All new shoots were sprayed with glyphosate (MAMBA, Dow AgroSciences Indianapolis, Indiana USA) and this cleared area was maintained as bare for the remainder of the study with reapplication approximately once per year thereafter.

-ants/-grass (ants absent, grass absent) - These combined treatments received both above manipulations.

control (ants present, grass present) - These trees were agitated at the same time other treatments were implemented but otherwise remained un-manipulated.

**Treatment Responses**

**Growth** - Height and diameter of focal trees (our proxy for fitness responses) were re-measured in July 2010, May 2011 and Dec 2011. To arrive at stable, averaged growth metrics, we calculated best-fit lines for each tree using height and diameter values from July 2010, May 2011, and Dec 2011. We used the slope of these lines (average growth per month) as response variables in our analysis.

**Reproduction** - Newly produced fruits are green and mature to reddish brown at which point they dry and crack and 1-7 seeds dangle from open pods until detached by strong winds (Coe & Beentje 1991). Beginning in July 2010 we collected all mature fruits and pods from experimental trees every 2 months until the study terminated in Dec 2011 (18 months). Seeds and pods were completely dried and then weighed to nearest 0.001g.
Herbivory - Mammalian and wood boring beetle herbivores can greatly impact tree growth and survival (Birkett 2002, Palmer et al. 2008, Stanton & Palmer 2011). To determine how probability of herbivory and tissue loss varies by site and to account for its effect in our model of treatment differences, we recorded damaged accrued at each resurvey period (July 2010, May 2011, Dec 2011). Mammalian herbivores can remove or snap shoot tips or whole branch systems. We quantified this loss by measuring the diameter of damage branches just below the point loss with calipers. Beetle damage can occur in the same way with chewed branches dangling and dead, or by boring into stems and killing tissue but leaving dead branches attached. To measure loss in this latter case we followed dead branch systems to the base of healthy tissue and measured diameter there. We also made visual estimates of the % of canopy lost to browsing. Total canopy damage from July 2010-Dec 2011 was assigned a level on a scale of 0-6. No browsing=0, <10 % of canopy damaged=1, 10-20%=2, 21-30%=3, 31-40%=4, 41-50%=5, >50=6. We used these categorical levels of herbivory intensity as a random effects in our whole model analysis of tree growth and reproduction.

Data Analysis

Of the 176 trees marked in Oct 2009, 167 were included in analyses. In the Tall Site, 5 trees could not be relocated after initial tagging. Two trees were excluded because of elephant damage resulting in tree mortality (one a (-grass) treatment, the other a (-ants/-grass) treatment) and one other tree (-grass) appeared very unhealthy at the time of the first resurvey and was not re-measured. In the Short site, one (ants/-grass) tree was also killed by elephants. Herbivores did not seem to preferentially destroy trees without ants.
All analyses and tests for normality of residuals were performed in (JMP, version 8.0.2; SAS Institute 2009). Diameter growth rate, fruit mass, total mass of fruits and total tissue lost to herbivory were log$_{10}$-transformed and height growth rate data was cube-root transformed to fit assumptions of normality (Shapiro Wilk W test > 0.90).

We modeled diameter and height responses with a general linear mixed model (GLMM) using restricted maximal likelihood (REML) to include herbivory intensity as a categorical random effect. The REML procedure is robust to deviations from normality and unbalanced design (Lele & Taper 2002). Our whole model tested for fixed effects of 3 factors each with two levels; ants (present/absent), grass (present/absent) and site (Tall or Short). We included all two-way interactions and the three-way interaction between all fixed effects. We used a reduced model excluding site as a fixed factor to determine ant and grass effects on fruiting in Short site alone because only a single tree in the Tall site fruited. Logistic regression with ant and grass as fixed effects (same as reduced model above) was used to assess probability of fruiting.

Because we expected intensity of herbivory to be an important explanatory variable in our whole model, we further tested how the probability of tree damage was associated with ant or grass with Chi Square. Whether trees without ants or grass were browsed more across sites was assessed with Fisher’s exact tests. If herbivory occurred, we examined how ants and grass affected the total amount of tissue lost total tissue lost with a two fixed factor general linear model.

To test for site differences on tree characteristics we included neighbor distance and size, and number of focal tree swollen thorns as multiple responses in a MANOVA model. Total biomass lost to herbivory and initial grass biomass was analyzed with
one-way ANOVA. Spericity tests were significant for total biomass regrowth measures because variance between measurements changed dramatically through time. Therefore we analyzed site effects on regrowth through time using the more conservative multivariate repeated measures ANOVA. We used Fisher’s exact test to analyze whether trees in the Short site had a greater probability of being browsed than those in the Tall Site.

Results

Site differences

Initial standing biomass of grass collected in Aug 2010 was similar between sites \((F_{1,14}=1.024; P=0.32)\) (Table 1). Over 15 months, the Short site produced 31% less regrowth compared to Tall Site \((F_{1,15}=11.10; P=0.0049)\) (Figure 4-5). We found that focal trees at the Short site contained ~ 50% more swollen thorns \((F_{1,165}=40.83; p<0.0001)\) and had neighbors that were 1.22x closer \((F_{1,165}=17.15; p<0.0001)\) but 32% smaller \((F_{1,165}=58.60; p<0.0001)\) than in the Tall Site. The probability of *Acacia* branches being damaged by mammals or beetles over the study was greater in the Short site than Tall \((Fisher’s Exact Test P=0.044)\) however if damaged, the total amount of tissue lost was larger in Tall sites with marginal significance \((F_{1,104} = 3.08 P=0.082)\).

Treatment Responses

Growth

Tall site trees grew more quickly, adding diameter at more than twice the rate on average \((F_{1,165} = 42.76; p<.0001.)\) and gaining height almost 4.5 times faster compared to short sites \((F_{1,165} = 40.12 p<.0001)\) (Table 4-2, Figure 4-4). Despite the microhabitat and tree trait differences in Tall and Short sites, we found that grass removal increased tree growth rates and ant removals decreased growth in both sites (Figure 4-4). In the
Short site, vertical growth is strongly suppressed and neither ant nor grass manipulation was able to release trees from this restriction. When growth was compared to control trees instead of considered in absolute terms, Short site treatment trees grew (in height and diameter) relatively more than those in the Tall site (Table 4-2). Individual –ant and –grass treatments created nearly equal but opposite patterns of growth across sites and the effects were additive in the combined treatment (Fig 4-4, Table 4-2).

The full model examining effects of ants (present/absent), grass (present/absent), and site (Tall and Short) revealed a significant influence of each on diameter growth (Table 3). Interactions did not help explain the growth responses. Herbivory was modeled as a random effect and explained ~10% of variation in diameter growth. Ants and site, but not grass, also significantly affected rates of change in height. The significant interaction between ant and site is linked to Tall site trees growing differentially in the presence versus absence of mutualist defenders, whereas stunted Short site trees grew similarly under both conditions. Herbivory intensity was an even more important explanatory factor in the height model, explaining 24% of the variation in the data.

**Reproduction**

71% of trees in the Short site fruited at least once over 18 months. Only a single individual in the Tall site did. In the Short site, neither ant nor grass manipulation (presence/absence) significantly affected the total mass of reproductive tissue produced (Table 4-1) nor whether trees fruited or not (ants $\chi^2 = 2.379; P=0.12$), (grass $\chi^2 = .337; P=0.56$). At the Short site we observed reproduction in 25% of –grass, 45% of control, and ~75% of both –ant and –ant /–grass trees. Regression analysis including all
treatments shows a negative but non-significant trend between diameter growth rate and total mass of fruits (log transformed) ($F_{1,61}=3.22; P=0.0776$)

**Herbivory**

Combining data from Short and Tall sites, we found the probability of branch tissue loss due to beetles or mammals from July 2010 to Dec 2011 was significantly greater for trees with no grass than trees with grass (Right-tail Fisher Exact Test $P=0.0007$). Trees with no ants were marginally more likely to be damaged by herbivores than trees with ants (Right-tail Fisher Exact Test $p =0.09$). For trees where herbivory was detected, the absence of ant defenders resulted in a 71% increase in total tissue loss in the Short site ($F_{1,58}=6.0798; P=0.0167$) and a 190% increase in Tall sites ($F_{1,43}= 9.36 P=0.0038$) compared with trees with ants. Trees without grass experienced < 1% more herbivory than trees with grass ($F_{1,103}=40: P=0.69$). Overall, the absence of ants significantly increased the total amount of branch herbivory ($P=0.0002$, Figure 4-6).

**Discussion**

**Context Dependence**

Ant defenders have an overall beneficial effect on host plant performance. This result from our study echoes conclusions made in three recent meta-analysis (Chamberlain & Holland 2009, Rosumek et al. 2009, Trager et al. 2010). Explicitly testing for the context dependence of beneficial effects proposed in earlier work (Bronstein 1994, Holland & Bronstein 2008), Chamberlain et al (2009) found the majority of empirical studies (largely measuring herbivory reduction) support the idea that ants act as true mutualist partners. More focused analysis by Trager et al (2010) on studies including plant reproductive responses similarly determined that ants have a net beneficial effect on host plants. Our replicated treatments across two sites that varied in
productivity, tree density, fruiting probability etc. (Table 4-1) produced no support for spatial context dependence of ant defender effects. Both height and diameter growth were consistently greater in trees with defenders as compared to those without (Figure 4-1, 4-2).

While our findings appear in line with the growing consensus about plant-ant interaction, another study conducted prior to ours in the Tall site with the same tree and ant species produced a qualitatively different picture, one where the presence of ants decreased plant performance (though non-significantly) over 53 months of manipulation (Stanton & Palmer 2011). Interestingly both Stanton and Palmer’s (2011) study and ours show that *C. mimosae* colonies significantly reduce herbivory by mammal herbivores and beetles ($F_{1,103} = 14.82; P = 0.0002$). It appears then that despite reliable defensive services, trees do not experience the benefit of ants consistently through time. This may be due to ‘year effects’ which are known to modify outcomes of replicated ecological experiments depending on the unique suite of conditions present at treatment initiation (Vaughn & Young 2010). The *A. drepanolobium* manipulations presented in this work began at the end of a very severe drought (October 2009) while data in Stanton and Palmer (2011) were collected before and during that period (March 2005-July 2009). Rainfall during our experiment was above average such that 67% of months exceeded long-term averages compared to only 33% of months included in Stanton and Palmer (2011) (T. Young unpublished data). Reduced water availability may limit whole tree energy production (King & Caylor 2010) and make supporting ant defenders a larger expense in dry than in wet years. For ants to retain their positive effect on plant performance, their defensive activity must save more plant
carbohydrates (in the form of synthesized tissue) than they use. If herbivory is absent or intensity is low (Frederickson et al. 2012) and/or hosts lose the ability to regulate rewards (Rico-Gray & Thien 1989) then ants may be a net cost instead of benefit. Stanton and Palmer (2011) discuss how costs of maintaining security forces should be borne in the short term because ants ultimately ward against rare but catastrophic herbivory from elephants. Our findings indicate that even protection from minor herbivory can create a measurable (albeit temporally contingent) benefit to hosts.

**Strength and Direction of Interactions**

**Effects on growth**

Effect sizes for ants and grass varied strongly in their absolute and relative magnitude depending on site (Table 4-2 Figure 4-3). In the Short site, -ant and -grass manipulations had larger relative effects on tree growth rate compared to controls yet represented a smaller total change than in the Tall site trees (Table 4-2). Notably 1-2m focal trees grew very little vertically over the course of our monitoring regardless of treatment in the Short site. It is known that hydraulic constraints can drive changes in tree allometry along gradients of increasing stress (e.g. aridity or waterlogging) creating “miniature” tree forms (Ryan & Yoder 1997, Rodríguez-González et al. 2010, Lines et al. 2012). Short distances between Tall and Sort sites in our system point to local edaphic factors such as soil clay content (Williams et al. 1996, Furley 2010) or shallow, impenetrable subsoil layer (Scholes & Archer 1997) instead of variation in rainfall as important contributors to the stress affecting tree growth (J. E. Herrick pers.com). Further investigations are needed to pinpoint the specific causes of reduced productivity but our work shows both trees and grass display limited biomass production in Short
Sites (Table 4-1) and that releasing trees from competition does not remove the limits on tree height.

Pooling growth data for diameter and height across sites we discovered nearly equal but opposite effects of ants and grass on growth with ant (presence/absence) explaining more variation in growth rate than grass (presence/absence) (Table 4-3). Combined treatments also show near perfect additive effects of ant and grass on the expansion rate of stem diameter. This diameter response measure seems most indicative of whole tree growth because it does not plateau like height (Figure 4-2) and is less susceptible to sharp decreases due to herbivory (e.g. when slender vertical branches are browsed resulting in an inaccurate interpretation of large growth effect when in reality damage is slight). From the observed additivity of effects, we conclude that tree uptake of resources does not interact in unpredicted ways with allocation to defense. In fact our experiment reveals that from a trees’ perspective, paying ants for defensive service appears to save more resources (branch and leaf tissue that remains functional and does not need to be regrown) than could be recouped by not competing with surrounding grass.

**Effects on reproduction**

Focal tree fruiting was restricted to the Short site, save for one tree in the Tall site, and as with height restriction at this site we are not certain why similarly sized trees of the same species display divergent reproductive life histories. Some studies find that age and not size determine onset of reproduction in plants (Thomas 2011). Given the slower rates of diameter growth in the Short site it we strongly suspect that those trees are older for a given diameter than trees in Tall sites and thus may reproduce at the same time in both sites but at very different sizes. Alternatively, the same stress
expected to limit tree growth may also induce early fruiting. Long-lived organisms often delay reproduction in favor of juvenile period of prolonged growth because pay off in fitness is greater with this strategy. If the likelihood of survival to this future state is low, individuals are expected to shift to an earlier reproduction (Thomas 2011). One reason for a reduced lifespan and thus an adaptation to fruit at small sizes may be threat of fire. *Acacia drepanolobium* is expected to be a fire adapted species that maintains its foliage in crowns several meters above ground and protected from flames burning grass layer (Okello & Young 2000, Okello *et al*. 2008). If trees in the Short site and many other parts of Kenya are physiologically unable to escape above the fire zone of vulnerability, then fruiting early and within the fire return interval may ensure some degree of fitness (Wakeling *et al*. 2011).

Plant growth is widely used and viewed as a surrogate for fitness especially for perennial and long-lived species where documenting production of offspring and their survival is impractical. It is notable then that documented changes to growth rate (proxy for fitness) in response ant and grass manipulation were not mirrored in our more direct measure of fitness, total seed production over 18 months (Table 4-3). It is possible that the period over which we collected fruits (~ one year less than growth data) was not sufficient to accrue observable treatment effects. It is also possible that while we know treatments did affect growth we do not know the exact amount of resources manipulations freed up or consumed. For example trees that benefited from grass removal could invest all their savings in aboveground growth, shunt some to belowground root stores (Poorter 2011), or some to reproduction depending on current demands and predicted future benefits. Likewise trees suffering from loss of ants could
divert limited resources to storage to improve survival capability or, at the other extreme, invest heavily in last gasp reproduction (Brody et al. 2010). Therefore allocation patterns and associated tradeoffs may act on trees individually leading to an inconsistent reproductive response to treatments.

**Implications**

In savannas it is well known that competition influences species dynamics and resultant community structure (Scholes & Archer 1997, Sankaran *et al.* 2004). This study and others in this system (Riginos & Young 2007, Riginos 2009) show that competition with grass decreases tree growth, a pattern that remains predictable through time, over space and across demographic stages. The novel finding of this work is that positive interactions can impact growth as much or more than negative interactions and that these effects are consistent over a heterogeneous landscape. Yet when compared with results from other work (Goheen & Palmer 2010, Stanton & Palmer 2011) we see that both the strength and direction of effects can be temporally variable. *Acacia drepanolobium* trees occur in some regions without *C. mimosae* (K. Rudolph *pers. obs.*). Understanding when and where these ants benefit trees vs. impose costs may help predict the spatial distribution of the partnership. Furthermore, exploring the long term temporal changes in costs and benefits of particular ant partners may help explain why trees hosting multiple ant species over a lifetime are predicted to experience increased fitness (Palmer *et al.* 2010).
Table 4-1. Characteristics of similarly sized *Acacia drepanolobium* trees growing in Short and Tall sites.

<table>
<thead>
<tr>
<th></th>
<th>TALL SITE</th>
<th></th>
<th>SHORT SITE</th>
<th></th>
<th></th>
<th>F ratio</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>se</td>
<td>mean</td>
<td>se</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biomass grass within a 60 cm</td>
<td>233</td>
<td>21.44</td>
<td>202</td>
<td>21.44</td>
<td>1.02</td>
<td>0.3286</td>
<td></td>
</tr>
<tr>
<td>radius of main stems (g) initial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biomass grass regrowth (g)</td>
<td>577</td>
<td>35.12</td>
<td>396</td>
<td>41.20</td>
<td>11.10</td>
<td>0.0049</td>
<td>*</td>
</tr>
<tr>
<td>Total over 18 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neighbor tree distance (cm)</td>
<td>285</td>
<td>11.30</td>
<td>224</td>
<td>10.77</td>
<td>15.39</td>
<td>&lt;.0001*</td>
<td>*</td>
</tr>
<tr>
<td>Neighbor tree size (diam in mm)</td>
<td>68</td>
<td>2.16</td>
<td>45</td>
<td>2.07</td>
<td>57.06</td>
<td>&lt;.0001*</td>
<td>*</td>
</tr>
<tr>
<td>Swollen thorn #</td>
<td>41</td>
<td>2.18</td>
<td>60</td>
<td>2.08</td>
<td>40.83</td>
<td>&lt;.0001*</td>
<td>*</td>
</tr>
<tr>
<td>% trees fruiting</td>
<td>0.01%</td>
<td></td>
<td>71%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbivory (yes/no, over 18 months)</td>
<td>n = 44/36</td>
<td></td>
<td>n = 60/27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbivory - total biomass lost</td>
<td>41</td>
<td>4.97</td>
<td>34</td>
<td>4.25</td>
<td>t-Ratio = -1.84</td>
<td>0.082</td>
<td></td>
</tr>
<tr>
<td>(branch diam in mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>assuming unequal variance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Fisher's exact test prob herb > for SS vs TS 0.044*
Table 4-2. *Acacia drepanolobium* responses to factorial removal of ants and grass in Tall and Short sites. Diameter and height growth reported as change per month. Fruit mass is reported as total amount over 18 months.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Diameter (mm) Mean±SEM</th>
<th>% growth vs. control</th>
<th>Height (cm) Mean±SEM</th>
<th>% growth vs. control</th>
<th>Fruit mass (g) Mean±SEM</th>
<th>% Repro vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tall Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>20</td>
<td>0.35 ± 0.04</td>
<td>-</td>
<td>1.96 ± 0.39</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- ants</td>
<td>22</td>
<td>0.26 ± 0.04</td>
<td>-26</td>
<td>1.23 ± 0.28</td>
<td>-37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- grass</td>
<td>19</td>
<td>0.41 ± 0.05</td>
<td>+17</td>
<td>2.68 ± 0.41</td>
<td>+37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- ants/-grass</td>
<td>19</td>
<td>0.34 ± 0.05</td>
<td>-3</td>
<td>1.31 ± 0.37</td>
<td>-33</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Short Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>22</td>
<td>0.17 ± 0.03</td>
<td>-</td>
<td>0.57 ± 0.18</td>
<td>-</td>
<td>8.72 ± 3.30</td>
<td>-</td>
</tr>
<tr>
<td>- ants</td>
<td>22</td>
<td>0.08 ± 0.03</td>
<td>-53</td>
<td>0.29 ± 0.17</td>
<td>-49</td>
<td>11.28 ± 3.21</td>
<td>+29</td>
</tr>
<tr>
<td>- grass</td>
<td>22</td>
<td>0.25 ± 0.03</td>
<td>+47</td>
<td>0.45 ± 0.32</td>
<td>-21</td>
<td>8.65 ± 3.82</td>
<td>-1</td>
</tr>
<tr>
<td>-ants/-grass</td>
<td>21</td>
<td>0.15 ± 0.03</td>
<td>-12</td>
<td>0.33 ± 0.17</td>
<td>-42</td>
<td>13.2 ± 3.21</td>
<td>+51</td>
</tr>
</tbody>
</table>
Table 4-3. Effects of ants, grass, and site on tree growth and reproduction

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>df</th>
<th>dfDen</th>
<th>F Ratio</th>
<th>prob&gt;F</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>diameter (mm/mo)</strong></td>
<td>167</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.36</td>
</tr>
<tr>
<td>ants</td>
<td>1</td>
<td>157</td>
<td></td>
<td>8.624</td>
<td>0.0038</td>
<td>*</td>
</tr>
<tr>
<td>grass</td>
<td>1</td>
<td>157</td>
<td></td>
<td>7.497</td>
<td>0.0069</td>
<td>*</td>
</tr>
<tr>
<td>site</td>
<td>1</td>
<td>156</td>
<td></td>
<td>54.755</td>
<td>&lt;0.0001</td>
<td>*</td>
</tr>
<tr>
<td>ant x grass interaction</td>
<td>1</td>
<td>154</td>
<td></td>
<td>0.135</td>
<td>0.7135</td>
<td></td>
</tr>
<tr>
<td>ant x site</td>
<td>1</td>
<td>155</td>
<td></td>
<td>0.223</td>
<td>0.6371</td>
<td></td>
</tr>
<tr>
<td>grass x site</td>
<td>1</td>
<td>155</td>
<td></td>
<td>0.002</td>
<td>0.9663</td>
<td></td>
</tr>
<tr>
<td>ant x grass x site</td>
<td>1</td>
<td>157</td>
<td></td>
<td>0.004</td>
<td>0.9520</td>
<td></td>
</tr>
<tr>
<td>herbivory - random effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% variation contributed by RE = 10.39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **height (cm/mo)** | 167 |    |       |         |        | .39 |
| ants              | 1   | 156|       | 6.631   | 0.0110 | *   |
| grass             | 1   | 155|       | 0.269   | 0.6051 |    |
| site              | 1   | 154|       | 60.538  | <0.0001| *   |
| ant x grass interaction | 1   | 154|       | 0.220   | 0.6398 |    |
| ant x site        | 1   | 154|       | 4.030   | 0.0465 | *   |
| grass x site      | 1   | 154|       | 1.670   | 0.1981 |    |
| ant x grass x site | 1   | 155|       | 2.669   | 0.1043 |    |
| herbivory - random effect | |    |       |         |        |    |
| % variation contributed by RE = 24.15 | |

| **fruiting (g) - Short Site only** | 62  |    |       |         |        | .07 |
| ants             | 1   | 51 |       | 0.006   | 0.9381 |    |
| grass            | 1   | 58 |       | 0.249   | 0.6196 |    |
| ant x grass interaction | 1   | 58 |       | 0.110   | 0.7418 |    |
| herbivory - random effect | |    |       |         |        |    |
| % variation contributed by RE = 5.64 | |
Figure 4-1. Variation in *Acacia drepanolobium* growth form. At the Tall site (A) trees reach heights up to 7m however at the Short site (B) trees do not exceed 3m. Photos courtesy of Kathleen Rudolph.
Figure 4-2. Diameter and height relationships for *Acacia drepanolobium* trees in Tall and Short sites. Non-linear data fit with polynomial curves (grey) best fit for trees in Short sites (black) best fit for trees in Tall sites.
Figure 4-3. Factorial experimental design. For *grass* treatments (B), all vegetation within a 60 cm radius of focal tree main stems, grey circle, was clipped, sprayed with herbicide and remained absent from Nov 2009 – Dec 2011. Resident ants were removed (D) with insecticide and neighboring colonies were prevented from recolonizing through the application of sticky barrier of Tanglefoot, red cuff. The combined treatment - *ants* / *grass* received both manipulations (C) and controls were unmanipulated (A). Photos courtesy of Kathleen Rudolph.
Figure 4-4. Tree growth rates under factorial manipulation of ants and grass (mean ± SE). A) average change in stem diameter (at 10 cm above the ground) per month. B) average monthly height change. Each point represents an average of ~22 focal trees.
Figure 4-5. Standing grass biomass, regrowth and rainfall across sites. Means (± SE) of grass clipped from within a 60cm radius of A. drepanolobium trees in Tall (dark circles) and Short (open circles) sites. Initial collection in Jul 2010 removed all vegetation down to 2cm. Regrowth period is indicated by horizontal bar above dates with breaks where repeated clipping events occurred in Feb, May and Dec 2011. Rainfall per month is plotted along the x-axis.
Figure 4-6. Effect of grass and ants on tree branch herbivory. Total branch diameter lost per tree due to mammal or beetle herbivores (Means ± SE). Neither the presence nor absence of grass significantly affected herbivory ($P=0.6177$). The absence of ants significantly increased herbivory ($P=0.0002$).
CHAPTER 5
NEW METHODS FOR ACACIA-ANT MANIPULATION

Introduction

Studies of the interaction between plants and their ant bodyguards have been hugely influential in shaping our understanding of coevolution, plant defense theory, social insect behavior and mutualism. Swollen-thorn Acacias have been the focus of much of this work and most prominent among these may be the semi-arid savanna tree Acacia drepanolobium. A database search on BIOSIS using the key words “Acacia drepanolobium” returned 104 scientific journal articles. Searches for the two ant species targeted in my research “Crematogaster mimosae” and “Crematogaster nigriceps” yield 23 and 27 studies respectively (though not mutually exclusive from the first search). Well over half of these studies include data collected at Mpala Research Centre.

From the beginning, work on the ‘Whistling-thorn Acacia’ has included in situ and ex situ manipulation in addition to keen natural history observation. Hocking (1970) reported exhaustively on host plant characteristics and detailed ant defender behavior in the field. He also determined by growing seeds in Canada that domatia could be produced in the absence of ant defenders. More recently at Mpala, landscape level and whole tree manipulations have been used to understand the ecology of ant-plant dynamics. KLEE (Kenya Long Term Enclosure Experiment) established in 1995 uses electric fences to exclude different combinations of wildlife and cattle (Young et al. 1998). Work inside the fences has revealed how mammal species individually and in combination influence savanna landscapes via changes in ant partner species and tree performance (Goheen et al. 2007, Palmer et al. 2008, King & Caylor 2010). Controlled burns in large parts of the A. drepanolobium black cotton habitat have shown how trees
respond to fire, an important and long suppressed feature of savanna woodlands (Okello et al. 2008). Decreasing tree density through the removal of whole trees, researchers have examined the effect of tree-tree completion on growth and also test how modification of the 'landscape of fear' alters herbivore habitat use (Riginos & Grace 2008). Tying trees together to induce fights between ant colonies has also revealed dominance hierarchies mediated by colony sized that contribute to coexistence between the four ant species in the system (Palmer 2004). Finally removal of whole colonies (Chapter 3, Stanton & Palmer 2011) or reduction in colony size (Goheen & Palmer 2010) has helped detail the costs and benefits of ant partners.

Digging deeper into the biology of these organisms necessitates the development of manipulation methods with a bit more finesse. Here I describe several tools created during my fieldwork to conduct manipulations within individual A. drepanolobium trees (artificial nectaries, baboon-proof food dispensers and non-stick field barriers). I also include a description of how to maintain colonies of C. nigriceps and C. mimosae colonies away from their host plants. These methods may be of assistance to experimentalists in other ant-plant systems.

**Methods**

**Artificial Nectaries ~ Nectalators**

Attempts to manipulate sugar access over > 1 day (as needed in Chapter 2) proved quite challenging. Acacia ants readily consume artificial nectar sources. Unfortunately, the intense equatorial sun in Kenya quickly evaporates all moisture from liquid carbohydrates. If open containers filled with artificial nectar are placed in trees, ants gather at the edge to feed and are frequently pushed or fall in and drown in the
liquid. After many prototypes, I found an economical and non-lethal solution for dispensing artificial nectar (Figure 2-1R).

**Materials**

- 35mm film canisters (black)
- Drill and bits
- Water wick cord
- Large tapered thorns
- Thin wire

Two 4 mm diameter holes were drilled into one side of the cup about 1.5 cm from the top edge. One large hole was drilled into the center of the removable cap. Two smaller holes were poked with a heavy pin into the cup near the top edge offset by 180 degrees from each other and 90 degrees from the initial two drilled holes. A 30 cm length of wire was slid through these holes (~ 15 cm extending out each side). Wrapping this wire around branches allowed containers to be firmly attached to trees. Water wick cord (Pepperell Braiding Co.) about 9 cm long was threaded thorough the hole in the cap so only 1 cm of frayed tip is exposed from the top. When the container is filled with liquid and the cap was pressed into place the wick dangles into the container and ‘wicks’ moisture up from the bottom of the cup and out to the external environment. The diameter of the wick and the cap hole must be similar so ants can feed off the exposed wicking strands but cannot fall inside. This device can be refilled from the two holes drilled holes in the side with a squeeze wash bottle with a narrow tip (Figure 2-1L). These are plugged with large, clipped *Acacia etbaica* thorns after filling to prevent ants climbing inside and drowning. Both thorns must be removed to fill, one for the nozzle and one to let displaced air out as the liquid enters.
There were some flaws in the design. Wicks needed to be replaced periodically as they would grow moldy and get clogged with sugar crystals that hindered the wicking action over time. I also discovered that sunbirds could either poke wicks down into the container or pull them out and then feed off the sugar water inside (Nectalator = hummingbird feeder). Knots in both top and bottom ends of the wick around the lid could have prevented this but may affect wicking action. After using Nectalators for the study in Chapter 2, I discovered an alternate, seemingly effective design (Rowles & Silverman 2009) that may be quite useful but was not tested on A. drepanolobium trees.

**Baboon-proof Food Dispensers**

Previous attempts to offer arboreal protein rewards to ants in the field involved packing tuna in 1.5mL centrifuge tubes and wiring them to host trees. This method failed because jackals discovered and removed the tubes (T.M. Palmer pers. com). In Summer 2011 I needed to augment colony protein in an area that received daily visits from a large troop of baboons. Baboons crack open swollen thorns to eat proteinaceous ants and brood inside (Isbell & Young 2007)(Figure 5-1A). To successfully prevent their access to the tuna protein I was offering ant colonies, I used castoff PVC devices used by researchers studying termites. Figure 5-1B shows the major components.

**Materials**

- PVC pipe
- Thin wire
- Wire mesh

Small ~25cm sections of 5cm diameter PVC were drilled with two holes about 12 cm apart along the side of the pipe. Wire was thread through this and allowed the pipe to be attached to trees. Another two holes (180 degrees offset from each other) were
drilled near each end of the pipe. Squares of wire mesh were cut and bent to fit inside each end as a protective cap. By threading small gauge wire though one of these holes, weaving it through the mesh and out the other hole, and bending the ends, we effectively locked caps into place. We added film canisters filled with tuna to these chambers before locking the last cap into place and this allowed ants to access protein through the mesh but did not permit baboon to remove tuna from trees.

**Non-stick Field Barriers**

The same characteristics that make Acacia-ants good defenders make them very difficult to manipulate and control. Workers respond aggressively to any disturbance and very thoroughly cover all parts of their host tree (Figure 5-2D). Simply brushing up against trees creates a swarming mass of ants determined to thwart the attacker. A quote from Hocking (1970) sums up the experience perfectly, “As every browsing herbivore knows, “collecting” in the center of trees bristling with three inch thorns and carrying up to 20,000 adult ants is no picnic.” Consequently, easy experimental modification of branches had been prohibited. Yet, to collect extra floral nectar for the isotope analysis in Chapter 2 I needed to exclude ants from active (and extremely well defended see Palmer & Brody 2007) nectaries (Figure 5-2A). To date the only suitable method of ant exclusion from branches had been to create a smaller version of the sticky barriers used in Chapter 4. To do this tape is first applied to plant tissue then coated with a layer of Tanglefoot. This creates an impenetrable barrier that workers cannot cross. This works relatively well to keep ants off main stems, however Tanglefoot is not an easy substance to work with. A problematic consequence of this method is that ants run into the barriers during and after the application process and become imbedded and die in the Tanglefoot. Over time, so many individuals can
become stuck that the colony can actually use the carcasses of dead workers as a bridge across the barrier. Once the barrier is crossed, colonies are able to regain access to access to restricted areas. Tanglefoot is by design a long-lasting, tacky substance, difficult to contain and extremely difficult/ impossible to remove if spread to other surfaces, e.g. leaves, fingers, hair etc.

I developed a less messy method for keeping ants off of branch tissue. This technique causes no serious branch damage and can keep ants away from branch tips in the field for days to weeks. The method also allows branches to be wrapped in mesh to prevent flying insects from accessing undefended tissue (Figure 5-2C).

**Materials**

- Lids from 2lb Dannon yogurt containers
- Drill and various sized bits
- Utility knife
- Insect-a-slip (a liquid Fluon coating from Bioquip, Rancho Dominguez)
- Fan shaped paint brush
- Micro binder clips

To construct, first drill hole in the center of the lid using a drill bit only slightly larger than the diameter of the branch that ants will be excluded from. If creating lids without knowing the exact branch size, it is a good idea to make several versions with different sized holes. From the center hole, slit the radius of the lid through the outer edge with a utility knife (new sharp blade is best). Holding the rim of the lid near each side of the new cut it should be possible to bend the lid to create a wide opening (that, with some twisting should enable it deform to slip onto a branch in the field and then spring back into place once released). After making the cut, use an artist’s fan paintbrush to coat the outside surface of the lid with a thin layer of Insect-A-Slip. Paint from the center all the way to edge careful to coat the newly cut seam. Let this dry overnight. The dry coating
can be easily scratched and will flake if bent roughly which will compromise its effectiveness – gentle manipulation is important. In the field, locate a branch internode with wide spacing between thorns. This will facilitate lid deployment. Use shears to remove any long thorns or leaves that may touch the Fluon surface (Figure 5-2B). If other branches are likely to touch the lid from the sides, consider bending and tying these away from the focal branch. It is also important to consider the wind and whether branches could be blown into one another. Make sure the Fluon-coated surface is facing away from the branch tip. Immediately after the lid is slipped onto the branch, make sure the cut seam in the lid is perfectly flush to create a ‘seamless’ surface. Clip the seam in with a micro binder clip that straddles the seam on the lid lip to hold the seam together. It is best if the seam is pointing straight at the ground (Figure 5-2B). You will see that ants immediately try to scale the new barrier. If positioned properly ants will be unable to easily climb lids and retreat, or slip and fall to the ground. Any ants trapped on the terminal end of the branch can be flicked off. Voila! Ants on one side, none on the other (Figure 5-2B). It is best to observe the barrier for several minutes to make sure it cannot be breached.

This technique is ideal for use on horizontal branches. Branches positioned parallel to the ground make lids sit upright. This maximizes the effect of gravity in preventing ants from crossing over. Branch tips can be ‘bagged’ with mesh fabric to keep flying insects away. For this it helps to roll the mesh edges over each other and clip them with more mini binder clips. Take care with using these lids on branches with very new tissue as the plastic edge on the center hole can cut/girdle into the soft bark.
Maintaining *Crematogaster* Colonies in the Lab

Ants have been kept contained in homes and laboratories for centuries. Typically housing is provided in “enclosures” consisting of fully sealed containers or open containers with water or Fluon barriers to prevent escape. For terrestrial ant species not particularly well adapted to climbing vertical surfaces, this appears to work well. Unfortunately, *C. mimosae* and *C. nigriceps* are very effective climbers and attempts to keep them in shallow, stackable plastic containers rimmed with Fluon were initially unsuccessful. Containers with straight or tapered sides (wider at the top, narrower at the bottom) allowed ants to gain purchase on the Fluon coating. With the help of gravity ants could eventually scale up and out of the container if the lid was not securely attached. Because we needed to feed and manipulate colonies regularly, keeping the colonies sealed up 100% of the time was not an option. One solution was to find deeper containers with taller sides and increase the depth of the Fluon barrier. This would increase the lab space required to house the containers. In the end we discovered that simply inverting the containers (Figure 5-3A,B) solved the problem. Lids were now on the bottom and a large hole was cut in the bottom of the container (now the top). Cuts for the opening did not extend to the very edge of the container where the sides started to turn downward. For ants to escape from this enclosure they must scale the sides of the container (with gravity pulling against them) and be completely upside down right before exiting through the top. This is very easy for them to accomplish without the Fluon coating but impossible with it. The inverted setup is also very easy to clean. All colony debris and pheromone trials that were laid down on the lid (bottom) could be washed off by detaching the lid when necessary and cleaning it separate from the top part containing the Fluon coating.
Ants that nest in the ground, in cavities or in carton castles all rear their brood in dark spaces. For Acacia ants, nest space comes in the form of hollow, swollen thorn domatia. We found that small containers with lids (artificial domatia) were taken to quite readily by both *C. mimosae* and *C. nigriceps*. Clear sides of the cups wrapped with aluminum foil kept out sunlight and a small hole drilled into the opaque lid allowed worker access to the inside (Figure 5-3A).

To transfer ants and brood from field-collected domatia into new nests in the lab, we first placed several of these artificial domatia (foil wrapped cups) inside a plastic container enclosure. We then cracked open domatia and dumped contents (including carton in the case of *C. mimosae*) into the artificial domatia. Any brood that spilled out of the cups during this chaotic process were rounded up by attentive workers and returned to the artificial domatia (Figure 5-3C). When ant activity became less frenzied, lids were snapped into place.

Colonies can survive in the lab using these methods for at least 2 months when fed a diet of sugar water absorbed into cotton balls and a protein source (e.g. canned tuna).
Figure 5-1. Excluding Baboons. A) A baboon opening *A. drepanolobium* domatia to feed on the ants inside. B) A sturdy PVC “Baboon-proof food dispenser” prevents animals from stealing protein (tuna) baits. Photos courtesy of Kathleen Rudolph.
Figure 5-2. Non-stick field barriers. A) Collecting extrafloral nectar (EFN) from nectaries after 24hrs of ant exclusion. B) Plastic yogurt lids with visible surface coated with Fluon. The cut seam that allows the lid to be fitted over branches extends from the center hole directly down to the fingers in the photo. The micro binder clip near fingers holds the two sides of the seam flush and eliminates any ant footholds. C) Green mesh surrounding branch tips additionally excludes flying insects from consuming the EFN. D) Image showing what branch tips would look like without the barrier in place. Photos courtesy of Kathleen Rudolph.
Figure 5-3. Lab enclosures for *Crematogaster* colonies. A) View from above a colony in an enclosure. The red bottom is the lid of the Rubbermaid container. Holes cut into the container bottom (now top) do not extend to the natural edge. This prevents ants from escaping over Fluon-coated sides. B) Image of access to colonies through the top of the enclosure and containers stacked to save space in the lab. C) Artificial domatia cups with ants, carton and brood inside. Aluminum foil around the sides creates a light free environment when opaque lids are in place. Holes drilled into lids allows ants to move freely in and out. Photos courtesy of Kathleen Rudolph.
CHAPTER 6
CONCLUSIONS AND FURTHER RESEARCH

Carbohydrates as Fuel

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 the 10-month field study described in Chapter 2, I 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, I did not find that supplemented colonies increased protein foraging. Instead colonies with reduced carbohydrate access discovered a greater proportion of protein baits, suggesting carbohydrate deprivation increases foraging intensity. I found no significant effect of carbohydrate manipulation on brood or alate production. My 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. I predict that these factors may be essential to understanding the consequences of carbohydrate access in natural plant-ant systems.

**Water is Key?**

The study presented in Chapter 4, also reveals a role for abiotic factors in shaping community ecology and species interactions. Results from both my research and that of Riginos (2009) suggest that the interaction between *Acacia drepanolobium* and grass is consistently negative across time and space. Interestingly, the effect of ants on trees appears to be temporally variable. Results from Chapter 4 identify a positive effect of *Crematogaster mimosae* ants on tree growth while Stanton & Palmer (2011) found that ants had a net negative effect. One untested factor that may explain this change in the direction and magnitude of interaction effects is water availability. Average monthly rainfall during my experiment was significantly greater than during either previous study (P=0.009). When water (a limiting resource in many savannas, see Scholes & Archer 1997) is abundant, tradeoffs between producing rewards and growing or reproducing may be minimized. Host tree allocation to EFN and domatia production for ant defenders may therefore be less costly during dry periods. Additionally, if trees are producing abundant and vulnerable new tissue during wet periods, then the benefits of ant protection may also be increased. In short, when water is readily available, the cost of supporting ants may be outweighed by the benefits of their defense.

**Ant’s Eye View**

In Chapter 3 I transitioned to examining costs from the ant’s perspective. I found that territorial conflict can result in significant worker loss in Winner colonies. Concurrent with these losses I found that Winner colonies are less able to defend their host trees.
against simulated attack by herbivores or neighboring ant colonies. During recovery from such costly conflict I found that colonies exhibit a wide range of behavioral responses. Some Winner colonies appear to reject all non-kin that could help rebuild their worker force. In other Winner colonies however I found evidence for incorporation of such individuals – loser (or enemy) genotypes being alive and present within the winner colonies. In two cases I also found following violent conflict, colonies appear to fuse with both queens remaining to produce offspring.

The extreme plasticity in responses toward non-kin that I discovered suggests that examining the mechanisms underlying recognition/discrimination may be fruitful new areas of research in this Acacia ant system. It particular it would be interesting to investigate whether adoption of non-kin is deliberate or passive due to leaky recognition systems and inability of individual workers to discriminate against non-relatives. Further, whether developmental stage (egg, larvae, pupae or worker) or caste (worker vs. reproductive) affects an individual’s probability of being incorporated or rejected is an open question in Crematogaster ants. Finally, no examinations of the cuticular hydrocarbons of these ants have been attempted. These chemicals are expected to signal colony affiliation and may be altered in unknown ways to allow non-related individuals or colonies to cooperate. Going forward, I hope to combine field and laboratory observations, and genetic and chemical analysis get a better ‘ant’s eye’ view of the environment and improve our understanding of social insect behavior and ecology.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Kathleen Perello Rudolph was born in Santa Paula, CA in 1980. She grew up in a family of ranchers and spent countless hours as a youngster exploring in the outdoors. Both her parents and grandparents inspired her interest in natural history and biology. She attended the University of California, Davis and received a B.S in Wildlife and Conservation Biology in 2002. As an undergraduate she worked at the Bohart Museum of Entomology and saw first hand that the amazing assortment of insects found in the pages of National Geographic are in fact real. A study abroad trip to Costa Rica in 2001 furthered her interest in tropical biology and sparked a particular fascination with ants.

After graduation, Kathleen worked as a research technician on Barro Colorado Island in Panama. For over a year she worked for Dr. Phyllis Coley researching the interactions between plants and insect herbivores. Two more field jobs followed, one working on Anchialine pool ecology in Hawaii and one studying Western Bluebird breeding behavior in Carmel Valley, California. It was during the latter that she met fellow biologist, Jay McEntee. In 2004, she was hired by George Roderick to be the manager of his molecular and evolutionary biology lab at UC Berkeley.

Determined to return to the field and this time focus on ant ecology, Kathleen began graduate work with Todd Palmer at the University of Florida in 2006. She served as a teaching assistant for several introductory biology labs, ecology labs, and an online biology course. For 5 summers she conducted research at Mpala Research Centre in Laikipia, Kenya. To fund this work she received grants from various funding agencies including National Geographic, the very organization that helped galvanize her career in biology.