VARIATION IN FORAGING STRATEGIES OF FOUR ANT SPECIES ASSOCIATED WITH *ACACIA DREPANOLOBIUM* IN LAIKIPIA, KENYA

by

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Mutualism is a widespread and fascinating type of species interaction. Ant-plant interactions, and those of tropical acacias and their inhabitants in particular, provide elegant examples of mutualisms. Much study has been devoted to the neotropical acacia-ants, but less attention has been given to those of East Africa. Mutualism theory states that ants should not need to forage away from their tree hosts, but few studies have addressed plant-ant foraging directly. This study, carried out in Kenya, examines the foraging habits of four acacia-ants with various relationships to their host plant, *Acacia drepanolobium*. The ants differ in their use of extra-floral nectar provided by the acacias, as well as in their recruitment to baits placed on the ground near their trees. Ants that display mutualistic behaviors are less likely to forage off of their host trees than are parasitic ants.

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CHAPTER I

INTRODUCTION

Standing in the Acacia savanna of the Kenyan highlands, one often hears a high, soft whistling. The sound is caused by wind rushing through thousands of small swellings on the branches of *Acacia drepanolobium*, or "whistling-thorn" acacias. Each of the swollen thorns is a dwelling for dozens of ants; the holes the ants make for entrances to these nests create perfect wind instruments. Every tree is covered in busy ants, and the ground beneath is riddled with foraging trails of the many species of ants that live in the savanna. *Acacia drepanolobium* is one of many species of plants known as myrmecophytes, which provide nest sites for ants in exchange for various benefits bestowed by the resident ants (Hocking 1970).

The relationships between myrmecophytes and their ant colonists are examples of mutualism, which is defined as the interaction of two or more species that is beneficial to all participants. Benefits to participants may include seed dispersal or pollination, protection from predators, shelter or nutrition (Boucher et al. 1982). Studies of pollination mutualisms such as that of the yucca and yucca moth make up a large portion of the mutualism literature (Addicott 1998) but studies of the relationships between ants and plants are equally useful and fascinating (Bronstein 1998).

Ant-plant relationships exist on every continent except Antarctica and have been found in every plant form (i.e., trees, shrubs, grasses)

(Hölldobler and Wilson 1990). Ant-plant associations have been well-documented, and are known to vary in form and intensity. The relationships range from facultative associations in which ants occasionally occupy plant cavities (Fiala et al. 1994; Fonseca and Ganade 1996) to obligate relationships in which the plants have evolved specialized structures to house ants and must associate with ants to survive (Janzen 1966, 1967). In addition to shelter, ant-plants (plants involved in interactions with ants) may provide nutrition for the ants in the form of extra-floral nectar or food bodies (specialized plant parts containing nutrients for ants); ants benefit plants by protecting them from mammalian or insect herbivores, pruning away competing vegetation, providing nutrients to the plants, or by various other measures (Hölldobler and Wilson 1990; Beattie 1985).

Possibly the most important service ants perform for their plant partners is protection against both herbivores and plant competitors. There are many well-documented cases of effective ant protection. In a study of the Amazonian canopy tree *Tachigali myrmecophila*, the associated ant *Pseudomyrmex concolor* reduced herbivory by 90% and increased leaf longevity to up to 2.6 times that of leaves not patrolled by the ants (Fonseca 1994). Similarly, Gaume et al. (1997) found that even the seemingly "timid" ants (*Petalomyrmex phylax*) on *Leonardoxa africana* in Cameroon reduced the number of insect herbivores and protected the plants from what could have been the significant loss of one-third of the leaf area.

Plants may have evolved ant attractants in response to the beneficial removal of insect herbivores by ants. Ants probably initially nested in plant tissues hollowed out by beetles or other herbivores, and as they protected their nests from other insects, the plants indirectly benefitted (Beattie 1985). Similarly, ants that fed on the honeydew excreted by scale insects or other Homopterans fiercely

protected these insect associates, and the protective benefits incurred by the plants may have outweighed the negative effects of the parasitic Homopterans (Beattie 1985). Plants that grew structures encouraging colonization by ants would have been less prone to herbivory, and thus would have had an evolutionary advantage.

The specialized structures evolved by plants to house ants are called domatia; the plants that bear them are called myrmecophytes. The growth of domatia is not induced by ant presence but instead occurs even in the absence of ants (Janzen 1967; Young et al. 1997). Plants bearing domatia are found in at least 37 families and are associated with ants from at least 25 genera (Hölldobler and Wilson 1990). The structures themselves occur in a broad range of plant tissues, including leaf pouches, hollow stems, and the swollen thorns of myrmecophytic acacias (Hölldobler and Wilson 1990; Janzen 1966; Hocking 1970). Interactions between ants and myrmecophytes are generally considered the most intense and highly evolved of the ant-plant associations (Davidson and McKey 1993; Janzen 1973; Hölldobler and Wilson 1990).

Plants that rely on ant protection may provide food for their protectors in addition to or instead of domatia. This food occurs in the form of extrafloral nectaries or food bodies. Extrafloral nectaries are nectar-producing structures unrelated to pollination and are found on various parts of plants including stems and leaves (Beattie 1985; Janzen 1966; Hocking 1970). The nectaries serve as a source of carbohydrates for ants and are often vigorously protected (Hölldobler and Wilson 1990). In many cases, plants produce more nectar for the ants at times of greatest vulnerability, such as times of rapid new growth or seasons when herbivores are most prevalent (Stanton, unpublished data; Beattie 1985). In this way plants can reduce the already low cost of nectar production (estimated by Beattie (1985) at 1%

that of leaf production) when protection is less crucial, while still keeping the ant guards on hand (Hölldobler and Wilson 1990).

Food bodies are particles of plant tissue that may contain other nutrients necessary to ants, such as lipids and proteins (Beattie 1985). They are found on a variety of plants; some of the most common examples are found in the genera *Acacia* (Janzen 1966, 1967), *Cecropia* (Sudd 1987), and *Macaranga* (Fiala et al. 1994). Little is known about the actual composition of food bodies, and thus the extents of both the cost to plants of their production and the benefit to ants of their consumption are unknown (Bronstein 1998).

Some of the best-known cases of obligate ant-plant association are the bull's-horn acacias of Central and South America and their ant mutualists. Janzen (1966, 1967, 1973) was the first to document the intricate mutualistic relationship between one of these acacia species and its ant inhabitant, *Pseudomyrmex ferruginea*. He determined by performing experiments in which he removed ants from some plants that *Acacia cornigera* is dependent on the ants it houses for protection from insect and vertebrate herbivore attacks and from encroachment by other plants, and that the ants, in return, receive nutrients from the plant in the form of extra-floral nectar and small proteinaceous food bodies called Beltian bodies (Janzen 1967).

Janzen (1966) suggests that the most beneficial aspect of mutualism for the ants is the increased accessibility of food: ants can forage on the tree for insect prey (thus also helping the plant) in addition to obtaining protein from food bodies or carbohydrates from the extra-floral nectaries. All of these food sources are located on the tree, making off-tree foraging unnecessary. Since foraging off the tree makes ants vulnerable to predation and requires a great deal of energy, the concentration of food on the acacia tree is of great benefit to the resident ants. Janzen (1966) states that if the ants had to depend on

food sources located off the acacias, the relationship would be weakened. This idea leads Janzen to the further conclusion that ant-acacia mutualisms can exist only if the trees always have leaves, and therefore nectaries, and a long dry season could lead to the breakdown of the mutualism. Davidson and McKey (1993) suggest that the longer dry seasons may explain the relative paucity of ant-plant mutualisms in Africa as opposed to the neotropics, where they are more abundant.

All ant-plant relationships have commonly been referred to as mutualistic and are frequently assumed to have coevolved (that is, to have reciprocally evolved traits that promote the specific association), but Janzen (1980) and Boucher *et al* (1982) point out that this is false in many cases. There are examples of ants that simply take advantage of their host plants without providing benefits to the plants (Stanton et al. 1999; Janzen 1967); such instances would be more accurately described as parasitism, if the ants harm the plants, or commensalism, if the ants neither benefit nor harm the plants (Boucher et al 1982). Yu and Pierce (1998) report an ant parasite of an ant-plant mutualism that sterilizes *Cordia nodosa* plants and takes advantage of the resulting increase in domatia production. These ants exploit the plant structures that evolved in response to beneficial ants, without themselves conferring benefits on their host plants; this makes the ants parasites of the mutualism, not merely parasites of the plants. While they use the plants, they also prevent mutualistic ants from colonizing those plants.

Just as parasitism may have been the starting point for the evolution of ant-plant mutualism, it may also indicate the dissolution of mutualism (Boucher et al. 1982). McKey and Davidson (1993) explain that competitively dominant ants may interfere with colonization by mutualistic ants, and that this is particularly true in cases when food

is scarce. These dominant ants out-compete subordinate ants for nest sites or food.

The ant-plant in this study, *Acacia drepanolobium*, is widespread throughout East Africa. It is similar to the neotropical bull's-horn acacias in many respects; however, rather than hosting a single obligate ant associate, it forms associations with several different ant species, four of which are certainly plant-ants (ants that nest only in myrmecophytic plants) (Hocking 1970). Every tree is colonized by a single ant species, and the resident ants defend their trees against invading ant colonies (Young et al. 1997). Three of the ant species that colonize *A. drepanolobium* are members of the large and highly diverse genus *Crematogaster* in the subfamily Myrmecinae; these are *C. sjostedti*, *C. mimosae*, and *C. nigriceps*. The fourth species is *Tetraponera penzigi*, which is a member of the subfamily Pseudomyrmecinae (the same subfamily as the *Pseudomyrmex* species of American acacia-ants). *A. drepanolobium* produces extrafloral nectaries on its petioles as does *A. cornigera* but differs from *A. cornigera* in that it does not produce food bodies (Hocking 1970).

In the Laikipia ecosystem in central Kenya, where this research was carried out, all four obligate acacia-ant species occur on *A. drepanolobium* trees. These trees make up over 97% of the overstory in many areas (Young et al. 1997). Thus the four species can be observed in the same habitat, and differences in their behaviors become strikingly clear. Young et al. (1997) noted that *Tetraponera penzigi* destroys the extra-floral nectaries on trees it inhabits, while the three *Crematogaster* species leave nectaries intact. Differences in rates of nectar production among trees occupied by the three *Crematogaster* species have also been recorded (Stanton et al., unpublished data). Both *C. sjostedti* and *C. mimosae* occasionally tend

scale insects (Homopterans), while the other species do not (Young et al. 1997).

Only *C. sjostedti* is known to occasionally nest off of *A. drepanolobium* trees; the other three ant species have obligate associations with *A. drepanolobium* (Hocking 1970). *C. sjostedti* generally nests in dead wood on the trees, and only occasionally takes advantage of the domatia, while the other three ants live only in *A. drepanolobium* swollen thorns (Hocking 1970; Young et al. 1997). *C. sjostedti* tends to live in larger, less healthy trees with less new growth and more damage by herbivores (Young et al. 1997). There is some question as to whether *C. sjostedti* is truly a mutualistic species. Trees colonized by *C. nigriceps* have a characteristic shape caused by the ants selectively pruning them to prevent the tree canopy from touching enemy colonies (Stanton et al. 1999). The pruning behavior also results in the sterilization of the tree, which indicates that *C. nigriceps* is a parasite of the mutualism (Young et al. 1997).

Palmer et al. (2000) report a competitive hierarchy in this system, with *C. sjostedti* dominant, followed by *C. mimosae*, *C. nigriceps*, and finally *T. penzigi*. This hierarchy was determined by staging conflicts between ant colonies of different species and observing the outcome. The ant species more likely to win a conflict is considered dominant.

Hocking (1970) made extensive observations of *A. drepanolobium* and its associated ant species throughout their range, but focused mainly on *C. mimosae*. He measured plant structures and nectar production, catalogued other insect and spider species found living on the acacias, and measured many behaviors of *C. mimosae*, including foraging rates, aggression, and nectar use. Measurements of these behaviors were not made for the other obligate acacia-ants, however, which suggests that

Hocking assumed the behaviors were either similar or of little consequence. Like Janzen, Hocking (1970) suggested that nectar probably accounted for most of the nutritional needs of the ants and that off-tree foraging was therefore unnecessary.

After observing the ants while working on other aspects of their biology, it became clear to me that *T. penzigi* never leaves its trees, while the other acacia-ants, particularly *C. mimosae* and *C. sjostedti*, apparently forage off their trees regularly. Despite the previous assumption that all acacia-ants must acquire their nutrition either directly or indirectly from the tree, it appeared that none of the *Crematogaster* species in this system depend exclusively on the acacias for food.

This disparity raises the question of whether foraging habits relate to varying levels of intensity in the associations between the four apparently obligate acacia-ants and their host tree. Perhaps mutualistic ants forage less off their host trees than do parasitic ants (e.g. *C. nigriceps*), which would reinforce the prevailing ideas put forth by Janzen. Similarly, *C. sjostedti* might also be more likely to forage off their trees if they are indeed non-mutualists. One would expect apparently mutualistic *C. mimosae* and *T. penzigi* ants to be more closely bound to their trees, although preliminary observations of *C. mimosae* suggest that they may actively forage off their trees as well. Alternatively, more intense off-tree foraging in these African species might simply be another aspect of the evolutionary disparity between Africa and the neotropics discussed by McKey and Davidson (1993). In either case, a comparison of off-tree foraging habits and nectar usage of the four competing ant species provides insight into the ecology of this deceptively simple ecosystem.

CHAPTER II

MATERIALS AND METHODS

This research was carried out in July- September of 1998 at Mpala Research Centre in the Laikipia District of Kenya. The study site was located at 36'50'E, 0'15'N, where the soil is predominantly Black Cotton vertisol and the average yearly rainfall is 500-600 mm/year (Young et al. 1997).

To determine the appropriate experimental approach, general observations of ant behavior were made, including notes about their presence on the ground, apparent food preferences and attendance at nectaries. Ant activity on randomly selected *A. drepanolobium* trees was observed for ten minute periods at various times during the day and night over a period of two days. These observations allowed me to rule out the possibility that one or more of the ant species had increased activity at night. To determine whether the ants would respond to baits, plastic dishes of diameter 15cm were filled with peanut butter and honey baits and were placed near the bases of *A. drepanolobium* trees occupied by *C. mimosae, C. nigriceps, C. sjostedti* or *T. penzigi*. These baits were checked in the morning and afternoon of the two following days. All visiting ants were noted, and attraction to baits was assumed when several ants of the resident species were found at the bait at a given time.

Foraging Counts

Study trees were selected at three sites within the study area. Trees with heights of 1.5-2m and generally healthy appearance were selected along a transect lying perpendicular to the access road, at approximately 10m from the road. Five trees occupied by each *Crematogaster* species (fifteen total trees) were selected at each site. The first tree encountered at 10m from the road was selected, and subsequent trees were chosen so that no two adjacent experimental trees were occupied by the same species. This prevented the counting of ants in transit from one tree of their colony to a neighboring tree. *T. penzigi* colonies were excluded from this experiment due to their lack of response to baits and aversion to ground-foraging.

Measurements of tree height and stem diameter (at 10cm) were made and used as covariates to ensure that differences in these characteristics were not related to differences in foraging activity.

Twenty-four hours before the foraging counts, all vegetation and other debris was cleared from an area of radius 0.25m around the base of each study tree. This improved visibility and eliminated the use of vegetation as ant "bridges," and provided a means to standardize the access available to ants travelling from the ground to the tree trunks. The twenty-four hour period between clearing the tree bases and the counts was intended to allow the ants to adjust to any disturbance caused by removal of surrounding vegetation.

All ants returning to their tree in a four minute period were counted. An ant was counted as "returning" when it moved from the ground to the tree base. Those ants which visibly carried food were noted, but all returning ants were counted as successful foragers, based on Gordon's (1993) finding that all harvester ants returning to colonies were laden, even when the food item was not immediately visible. Counts

were conducted once in the morning and again in mid-afternoon on two days, so that ants returning to each tree were counted a total of four times.

Nectary Occupancy

Nectary occupancy was determined by counting the number of nectaries on new growth occupied by ants at a given time. Observations were made at trees in the 1.5-2m height class along transects at three sites. Each transect consisted of 15 trees, with five trees occupied by each of the *Crematogaster* species. *T. penzigi* were again omitted due to their characteristic destruction of nectaries as described in the Introduction. One waist-high branch was selected in each cardinal direction on each tree. Waist height was chosen in order to standardize the measurements and because it corresponded to approximately two-thirds of the average tree height. It was noted whether one or more ants were present at each of the first ten nectaries from the distal end of the branch. Counts included only nectaries on new growth due to evidence that those nectaries accounted for the majority of nectar production (Palmer, unpublished data). If the new growth had fewer than ten nectaries, only the existing nectaries were included. Percentage occupancy was then calculated. When a branch had no new growth, the nearest branch was used as an alternate.

Bait Recruitment

To determine the extent of recruitment to baits by each species, series of baits were set up near study trees. The bait recruitment experiment provided a means of equalizing the off-tree food resources at each tree. Trees for this experiment ranged from 1.5-2.5m in height. The

bait consisted of unpasteurized acacia honey mixed with peanut butter, which had been determined to be attractive to all three *Crematogaster* species in the observations mentioned above. Bait was placed in hollow swollen thorns collected from the surrounding area. Only dried, fallen thorns were chosen in order to eliminate the possibility that lingering scents on the thorns would attract or deter ant visitors. Baits were placed in rows extending in each cardinal direction at 0.5, 1, and 1.5m from the tree base. When possible, the baits were placed on the ground, but occasionally vegetation was so thick that baits had to be placed on top of grasses, in which case they were placed on mats of vegetation no higher than 5cm from the ground. After periods of 0.5, 1, 2, 3 and 4 hours, the number of ants at each bait was recorded. All species of ants at baits were noted, including non-experimental ground-dwelling ants.

Analysis

All data were subjected to analysis of variance (ANOVA) using the Minitab general linear model (Ryan and Joiner 1994). Where significant effects were found, means were separated by the Tukey method.

Foraging Counts

ANOVAs were performed with respect to species and time of day. These tests demonstrate whether there is any statistically significant difference in foraging among the ant species, and whether the time of day has an effect on the number of ants foraging. Tree characteristics (i.e., height, stem diameter) were included as covariates to determine whether these factors affect foraging levels. Because they had no significant effect on foraging levels, the covariates were omitted from the final analysis. A square root transformation of values was used to obtain a normal distribution and homogeneous variance.

At many of the foraging count times, no actively foraging ants were present. The frequent lack of foraging ants makes it inappropriate to perform ANOVA because it skews the distribution and makes the variance heterogeneous. To correct this problem, the number of returning foragers at each time of day was averaged over the two observation days.

Nectar Occupancy

An ANOVA was performed with respect to ant species. Again, square root transformation of values was used.

Bait Recruitment

All observation times were combined to adjust for repeated sampling. A log transformation was used to obtain a normal distribution and homogeneity of variance. An ANOVA was then performed to separate the effects of ant species or distance of bait from the tree on ant attendance at bait. Only ants of the occupant species were used in this analysis.

CHAPTER III

RESULTS

All three *Crematogaster* species were found foraging off their trees, but *T. penzigi* was never observed on the ground. No significant difference in the number of ants returning to trees was found among *Crematogaster* species (Table 1, Fig. 1). Many trees occupied by *C. sjostedti* and *C. nigriceps* had few or no returning foragers (Fig 2., Fig. 3). There was also a high frequency of *C. mimosae* trees with few or no ants returning (Fig 4.). In several cases, however, more than 200 returning foragers were counted at *C. mimosae* trees (Fig. 4). There was a very highly significant difference in foraging activity in the morning and afternoon, with more ant activity recorded in the afternoon counts ($F= 2.83$, $p<0.001$)(Fig. 5). No significant interaction between time of day and ant species was found (Fig. 6).

The ant species did differ in their collection of nectar on the trees. *C. nigriceps* had a significantly higher extra-floral nectary occupancy than did the other two ant species ($F=55.71$, $p<0.001$), with a mean occupancy of 19.7% (Table 2, Fig. 7). *C. mimosae* and *C. sjostedti* did not differ significantly in their nectary visitation. Their mean occupancies were 1.9% and 0.8%, respectively. There was no effect of branch orientation on nectary occupancy.

All three *Crematogaster* species visited the bait dishes on the ground, indicating that the ants were attracted to the honey and peanut

butter mix. *Tetraponera penzigi* ants did not venture off their trees to visit the baits, which was consistent with previous observations that those ants do not forage off their trees.

The response to baits placed near the trees differed among ant species (Table 3). The numbers of *C. mimosae* found at baits were significantly lower than those of the other ant species ($T=3.779$, $p=0.0008$)(Fig. 8). Bait attendance by *C. nigriceps* and *C. sjostedti* did not differ significantly. The distance of the bait from the tree base had a very highly significant effect on bait attendance, with baits placed at 0.5m attracting more foragers than those at 1.0m ($T=5.026$, $p<0.0001$) or 1.5m ($T=6.575$, $p<0.0001$) (Fig. 9).

An interaction between species and distance was found, meaning that the three *Crematogaster* species differed in their responses to bait distance. *C. nigriceps* was more likely to be found at baits placed 0.5m from the tree than at farther baits ($T=4.321$, $p=0.0013$; $T=6.260$, $p<0.001$)(Fig. 10). Neither *C. sjostedti* nor *C. mimosae* was more likely to attend a closer bait than a farther one. While *C. sjostedti* had greater attendance than *C. mimosae* at all distances combined, the ants were no more abundant at their 0.5m baits than were *C. mimosae* at their corresponding baits.

Table 1. Analysis of variance for foraging of three ant species (*C. sjostedti, C. nigriceps, C. mimosae*). Data were averaged over two days and square-root transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Species	2	98.766	98.766	49.383	2.01	0.148
Time	1	63.579	63.579	63.579	20.83	<0.001
Species*Time	2	11.278	11.278	5.639	1.85	0.171
Tree(Species)	39	959.406	959.406	24.600	8.06	<0.001
Error	39	119.061	119.061	3.053		
Total	83	1252.091				

Table 2. Analysis of variance for nectary occupancy of three ant species (*C. sjostedti, C. mimosae*, and *C. nigriceps*). The values used for analysis were square roots of the percentage of occupied nectaries.

Source	DF	SS	MS	F	P
Species	2	135.50	67.75	55.71	<0.001
Error	42	51.07	1.22		
Total	44	186.58			

Table 3. Analysis of variance for bait recruitment of three ant species (*C. sjostedti, C. mimosae*, and *C. nigriceps*). The values used for analysis were log transformed.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Species	2	6.5023	6.5023	3.2512	5.95	0.007
Distance	2	13.8995	14.6969	7.3484	38.04	<0.001
Species*Distance	4	3.5188	3.5188	0.8797	4.55	0.003
Rep(Species)	30	16.3962	16.3962	0.5465	2.83	<0.001
Error	60	11.5910	11.5910	0.1932		
Total	98	51.9078				

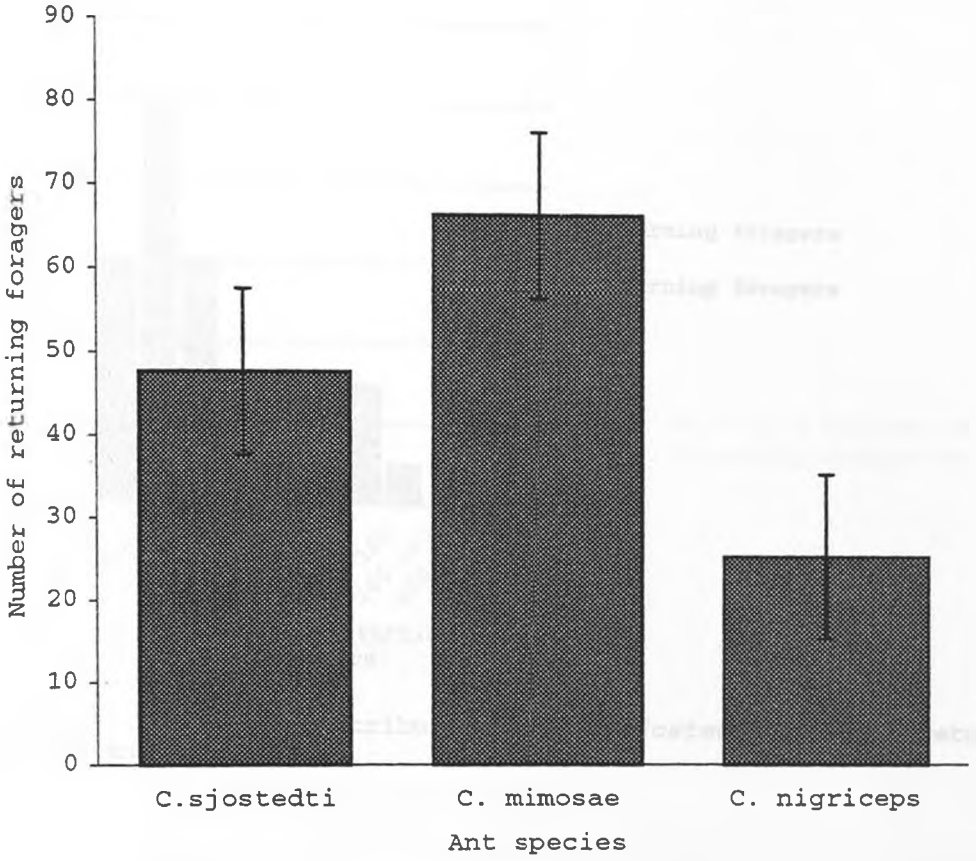

Figure 1. Mean number of *Crematogaster* ants returning from foraging. Analysis of variance showed no significant differences.

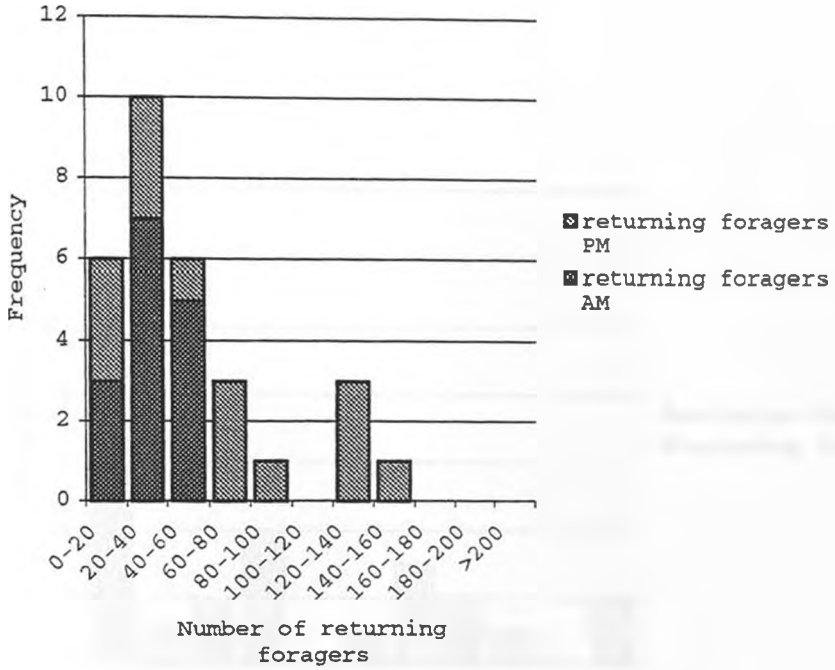

Figure 2. Frequency distribution for *C. sjostedti* foragers returning in morning and afternoon.

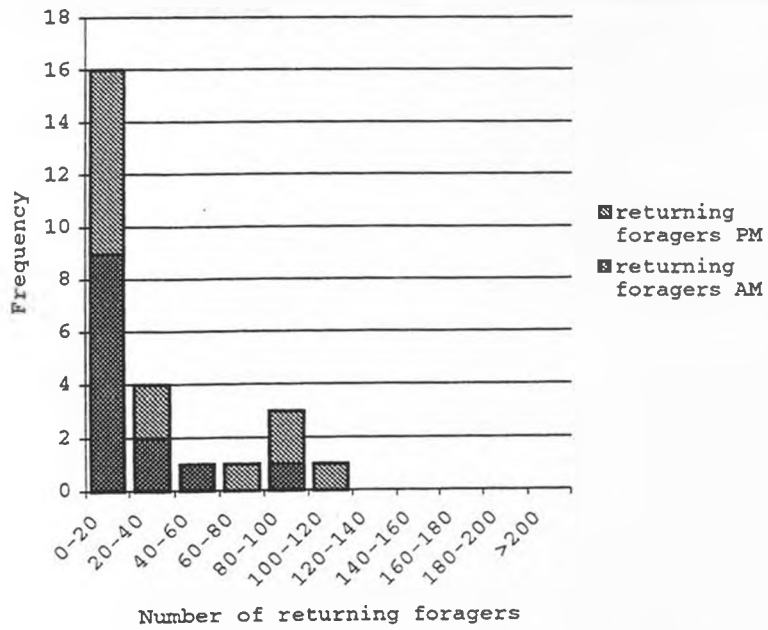

Figure 3. Frequency distribution for *C. nigriceps* foragers returning in morning and afternoon.

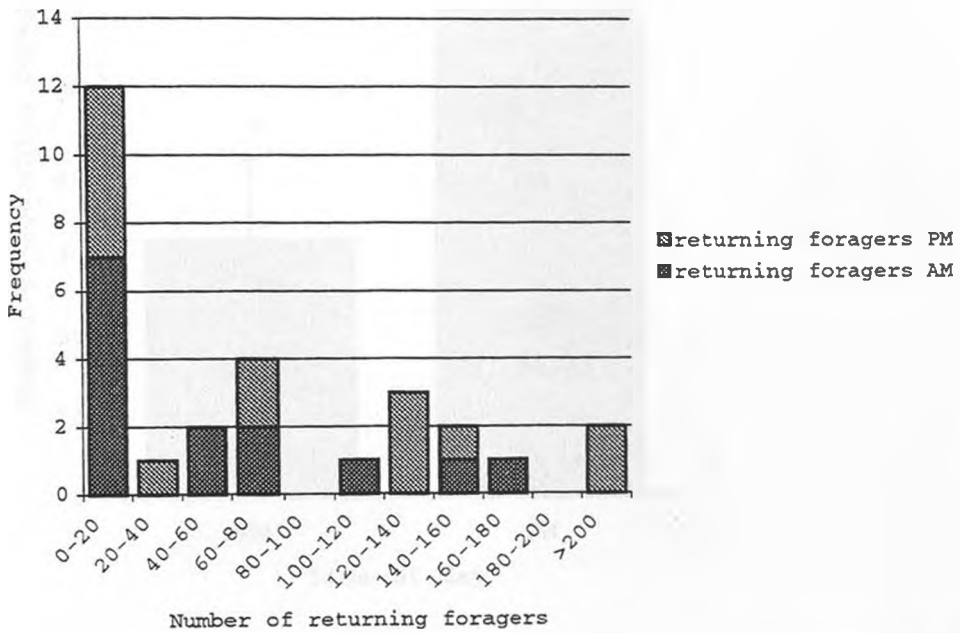

Figure 4. Frequency distribution for *C. mimosae* foragers returning in morning and afternoon.

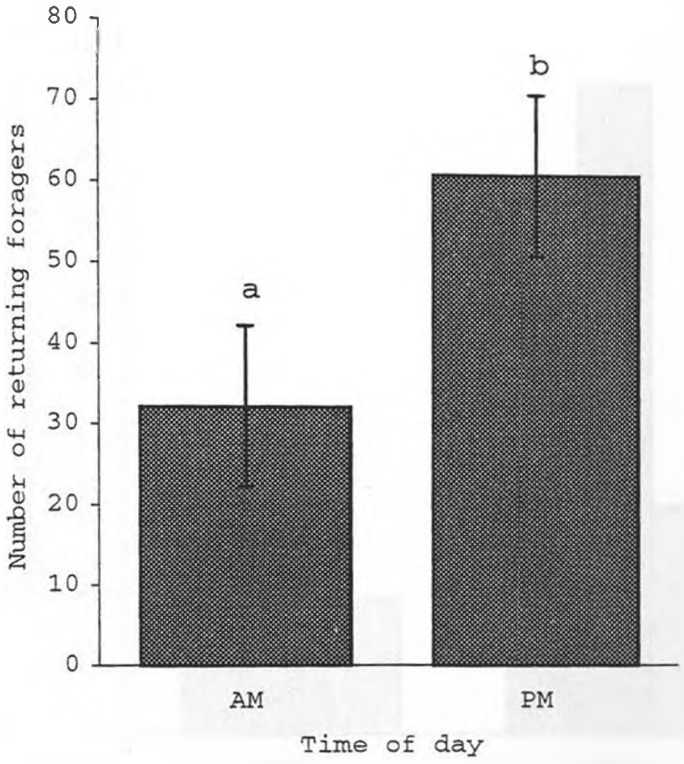

Figure 5. Mean number of returning *Crematogaster* foragers in morning and afternoon. Different letters above columns indicate significantly different means ($P<0.001$).

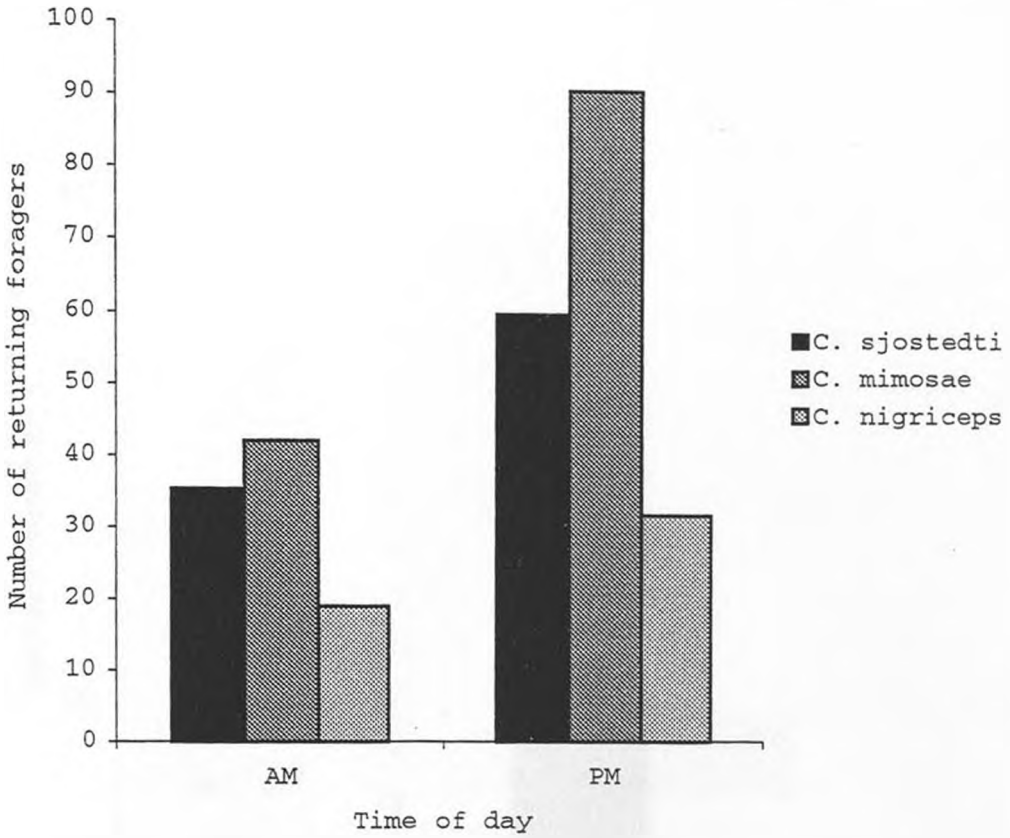

Figure 6. Mean number of returning *C. sjostedti, C. mimosae,* and *C. nigriceps* in morning and afternoon. Analysis of variance indicated no significant interaction between time of day and ant species.

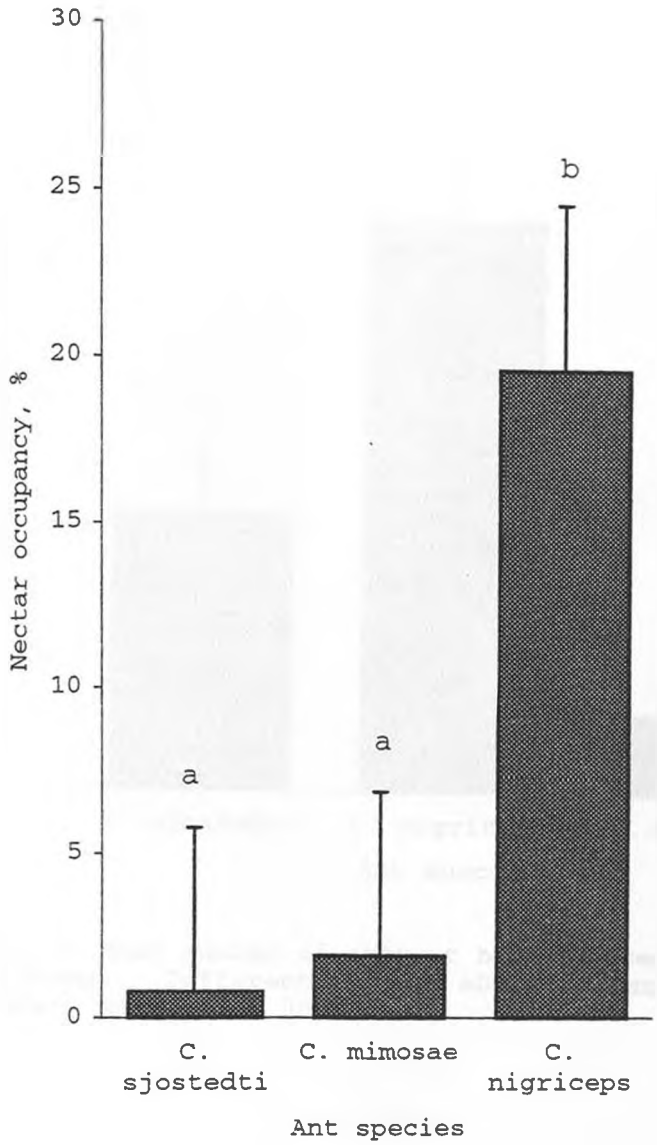

Figure 7. Nectar occupancy on new growth of *A. drepanolobium* by ant species. Different letters above columns indicate significantly different means ($P<0.001$).

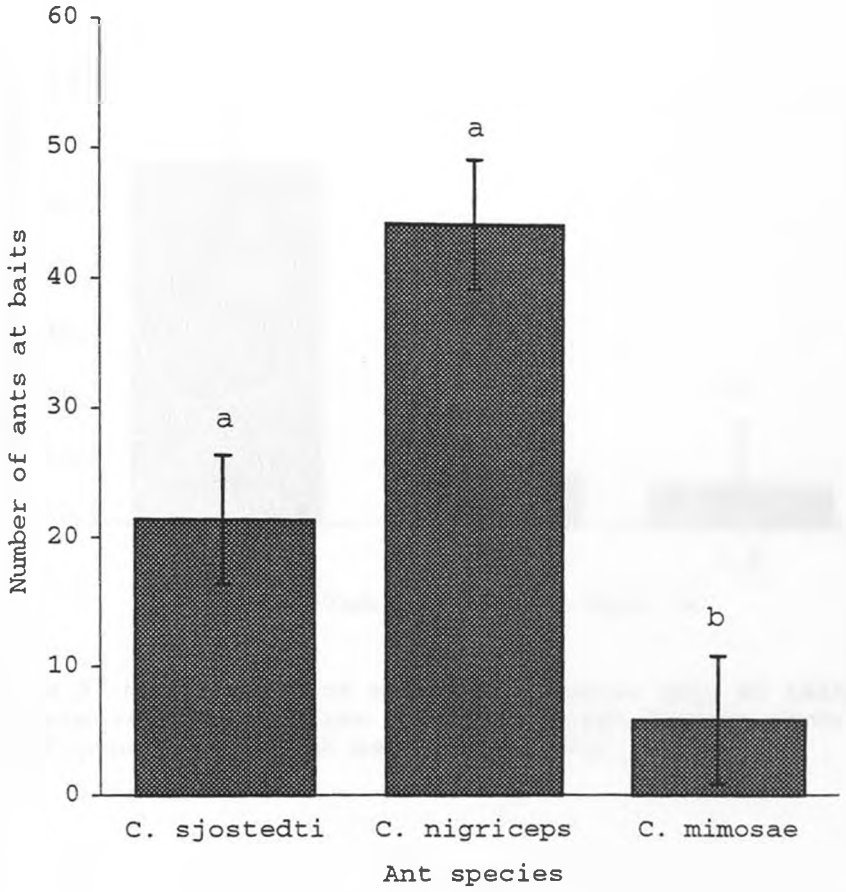

Figure 8. Mean number of ants at baits placed 0.5, 1.0, and 1.5 m from tree bases. Different letters above columns indicate significantly different means (P=0.0008).

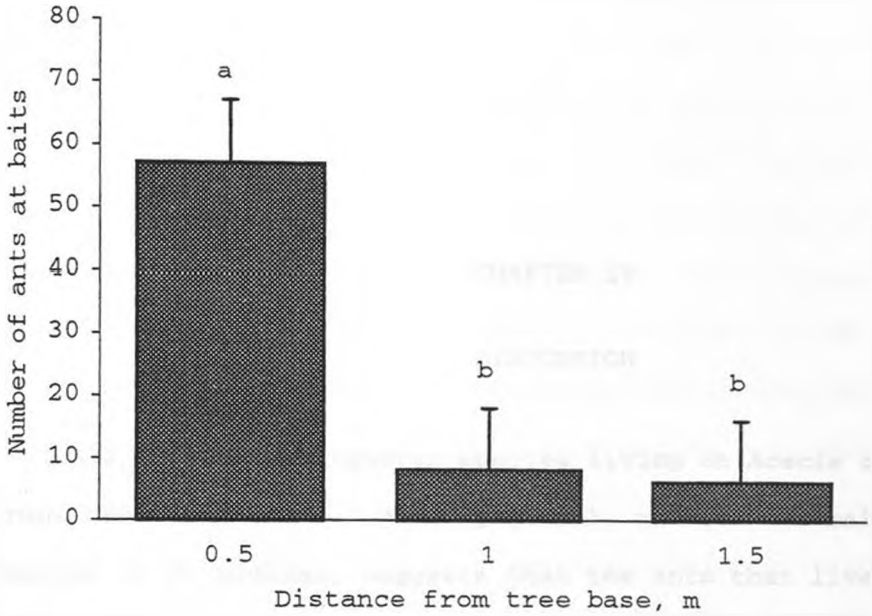

Figure 9. Mean number of all *Crematogaster* ants at baits as a function of distance from the tree base. Different letters above columns indicate significantly different means (P<0.0001).

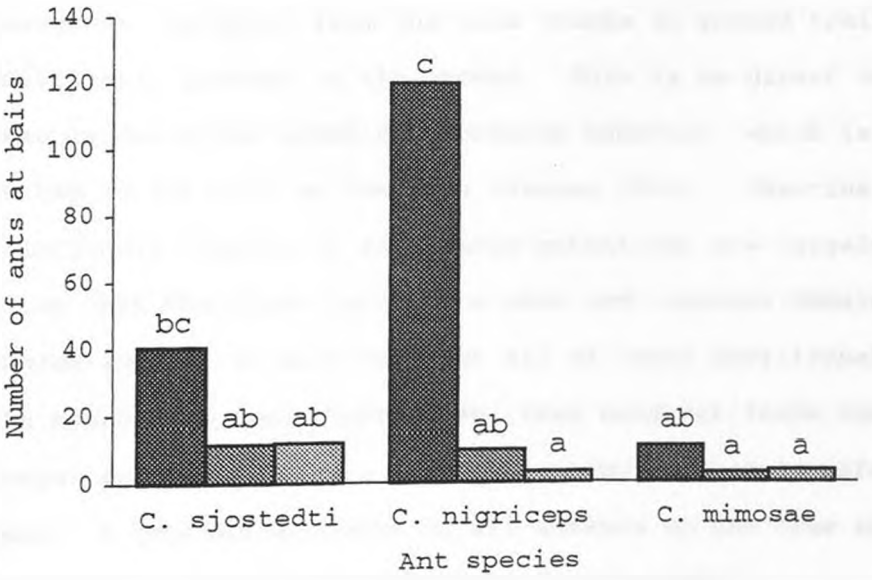

Figure 10. Effect of ant species and bait distance from tree base on bait recruitment. Different letters above columns indicate significantly different means (P<0.0001).

CHAPTER IV

DISCUSSION

All three *Crematogaster* species living on *Acacia drepanolobium* forage off their trees. Hocking (1970), who focuses mainly on the behavior of *C. mimosae*, suggests that the ants that live on *A. drepanolobium* rarely use ground trails and are strictly arboreal; however, ants of all three *Crematogaster* species were consistently observed on the ground during the day, sometimes on food sources such as cattle or lion dung, moss, or dead insects. While they frequently used grasses as "bridges" from the tree trunks to ground trails, they were unmistakably present on the ground. This is in direct contrast to more commonly described plant-ant foraging behavior, which is generally limited to activity on the tree (Janzen 1966). Theories of the evolutionary origins of ant-acacia mutualisms are largely based on the notion that the plants provide a safe and complete domain for the ants (Janzen 1966). If ants can meet all of their nutritional requirements with sources on their host trees, they need not leave the trees to forage, and can therefore use less energy and remain safely on the trees. A constant presence of all workers on the tree in turn provides greater protection from herbivores for the tree.

Clearly, the *Crematogaster* species living on *A. drepanolobium* either require or prefer off-tree food sources. *A. drepanolobium* trees produce no Beltian bodies or food bodies of any sort, so the only

protein sources on the trees are from insect visitors, fungi and other sources only indirectly provided by the trees. The large *Crematogaster* colonies might be unable to acquire enough protein from these sources, and thus forced to forage off their host trees. Although Hocking (1970) assumes that the need for the ants to forage off the trees is limited, he did make notes about returning foragers, which suggests that there was some number of workers leaving and returning to the trees.

Interestingly, *Tetraponera penzigi* ants do not leave their trees to forage or, apparently, for any other reason. *T. penzigi* must, therefore, be able to acquire sufficient food on the tree. This is striking because these ants have more limited sugar resources due to their habitual destruction of extrafloral nectaries, which might imply that they would be more likely to leave their trees to forage. Several factors may account for their truly arboreal behavior. First, they are Pseudomyrmecine ants, and are therefore more closely related to the *Pseudomyrmex ferruginea* that live on the bull's-horn acacias studied by Janzen (1966, 1967, 1973) in the neotropics. It is not surprising, then, that *T. penzigi* behavior more closely corresponds to that of the acacia-ants described by Janzen (1966, 1967) than do the behaviors of the three *Crematogaster* species studied.

Crematogaster species in general are widespread and tend to be dominant to Pseudomyrmecine species, out-competing them for nest sites, food resources and generally winning when colonies engage in conflicts (Janzen 1966, Hölldobler and Wilson 1990, Bronstein 1998). The species in this study system are consistent with this pattern, with all three *Crematogaster* species dominant to *T. penzigi* (Palmer et al. 2000). The second explanation for *T. penzigi*'s behavior is closely related to this order of dominance: remaining on the tree may reduce contact with enemy ants that would almost certainly defeat them in any confrontation.

In addition, other species in the *Tetraponera* genus are known to engage in a type of foraging behavior known as gleaning, which means that they scour all the surfaces of their host trees, picking up fungi, insect eggs, and other debris that prove nutritionally rich (Hölldobler and Wilson 1990). This behavior has not been observed in *Crematogaster* species. Gleaning, when combined with the nitrogen acquired from eating the nectaries themselves as well as insect intruders, evidently provides the small *Tetraponera* colonies with enough food to sustain themselves.

The lack of significant differences in foraging rates among the *Crematogaster* species came as a surprise because of preliminary observations. On first observation, *C. mimosae* and *C. sjostedti* seemed much more active on the ground and on tree trunks than did *C. nigriceps*. *T. penzigi* were entirely absent from the bases of their trees, and *C. nigriceps* were only occasionally very active on the ground or tree bases. However, there was no statistical difference in the number of workers returning to trees among the three *Crematogaster* species (Fig. 2). This is due in large part to the high level of variance in the data, particularly for *C. mimosae*.

While there were many *C. mimosae* trees with large numbers of returning foragers, there was also a surprising number of trees with no ants active near the base at all (Fig. 4). These ants are as a rule more active and aggressive than the other species, but apparently vary substantially in their activity levels. One possible explanation for the variable activity levels is that each colony forages only on some days, and does not venture from the trees on other days. Also, the size, age, or vigor of the colony could affect the capacity for off-tree foraging. Although tree height can be used to an extent as an estimate of ant population on the tree (Young et al. 1997), it is far from exact, and it cannot be determined how long a particular colony has inhabited a

tree. If a colony is weak or unhealthy, that could preclude foraging off the tree. If, on the other hand, a host tree is particularly vigorous or producing a large number of new swollen thorns, leaves, and nectaries, it might provide adequate resources for its resident ants.

There were significant differences in nectary visitation (Fig. 7), and the results were consistent with initial expectations. More nectaries on *C. nigriceps* trees were occupied than on trees occupied by the other *Crematogaster* species (Fig. 7). *C. sjostedti* nectary attendance was almost non-existent, which is not surprising given the typical lack of vigor and minimal new growth on their host trees (Young et al. 1997). The low level of nectary attendance by *C. mimosae* ants was more surprising, but may be explained by scale tending, which probably provides a great deal of carbohydrate (Hocking 1970). *C. sjostedti* also tend scales (Hocking 1970) and possibly Lycaenid butterfly larvae (personal observation), which presumably increases their ability to survive on unhealthy older trees.

As mentioned in the Introduction, ant-plant mutualisms are thought to have evolved in some cases when ants tended scales on plants and eventually started using the plants as homes as well (Beattie 1985). The ants probably provided protection to the host plants as a side effect of their presence on the plants, and this may have led to the coevolution of myrmecophytes and ants, and to the existence of extra-floral nectaries. Because *C. mimosae* seems in various ways to be the most beneficial to its host trees, it might follow that it would use the nectaries provided by the trees. It seems natural also that *C. nigriceps*, whose sterilization behavior at the study site indicates that it is likely a parasite of the mutualism between *A. drepanolobium* and another ant species, would make use of the nectaries (Stanton et al. 1999). *C. nigriceps'* overwhelmingly greater attendance at extrafloral

nectaries suggests that it requires more carbohydrate, which is consistent with it maintaining larger colonies on each tree.

Following the nectary attendance study, it seemed even more likely that *C. sjostedti* and *C. mimosae* were expending more energy on foraging away from their nests since they apparently were not using many resources on the trees. The results of the bait recruitment experiment are only partly consistent with this hypothesis. *C. nigriceps* and *C. sjostedti* recruited to baits placed within 1.5 m of their trees at significantly higher rates than *C. mimosae* (Fig. 8). This is consistent with predictions and informal observations of *C. sjostedti*. These ants depend on the trees for nest sites, but apparently not for their food. They were as likely to recruit to a farther bait as a closer one, perhaps indicating that they are less worried about encounters with other ground-foraging ants, although they were no more likely to be found at far baits than the other *Crematogaster* species.

C. mimosae was unlikely to be found at any bait (Fig. 10). The apparent lack of interest in baits placed on the ground is inconsistent with their presence at natural food sources such as dung, but can perhaps be explained by a preference for food sources other than the bait material. Also, their lower level of recruitment to baits is consistent with their clearly mutualistic relationship to the acacia trees. It confirms both Hocking's (1970) assumption that they need not venture much from the tree, and Janzen's (1966) idea that leaving the tree in some senses defeats the purpose of occupying it. Being aggressive ants, *C. mimosae* workers might have better luck capturing insect prey on the trees than would *C. nigriceps*, making off-tree foraging less of a necessity.

C. nigriceps recruited particularly heavily to the baits placed close to its trees (Fig. 10), indicating that those ants tend not to

venture as far from their nests. This is consistent with their low position in the competitive heirarchy. The results show that *C. nigriceps* workers scout for and recruit other workers to attractive food sources on the ground. Again, the density of the swollen thorns on their trees and the large populations on trees that can be inferred from this density may account for the high levels of food-acquiring behaviors observed in these ants.

The results support Janzen's (1966) hypothesis that truly mutualistic acacia-ants do not forage off their host trees. To a great extent, apparently mutualistic ants like *C. mimosae* and *T. penzigi* remain on their trees. This allows them to avoid encounters with competitors on the ground and to focus their energy on defending their colonies from intruders. In addition to confirming theories about plant-ant foraging behaviors, these findings reinforce the importance of determining the nature of each ant-plant relationship, rather than assuming all such relationships to be mutualistic (Davidson and McKey 1993). Further study of foraging habits, particularly quantitative studies of energy sources used by the various ant species, may prove useful. Stable isotope analysis is one method of quantifying nutrient exchange that has gained popularity in recent years (Sagers et al. 2000; Gaume and McKey 1999). This type of analysis might provide insights into the specific costs and benefits of ant-plant interactions in the Laikipia ecosystem.

Other directions of future research might include comparisons of foraging habits in different seasons. Although *A. drepanolobium* does not lose its leaves in the dry season (Young et al. 1997), new growth slows, which probably affects nectar production. Studies of changes in ant behavior from season to season might elucidate some of the differences between African and neotropical ant-plant interactions.

Mutualism has been receiving an increasing amount of attention in recent years. Although predator-prey interactions and parasitism have received much more attention in the scientific literature, Bronstein (1998) suggests that the study of mutualism is catching up, and what remains is to create a unified theory and standard approach. Boucher et al. (1982) suggest that studies of mutualism will contribute to the understanding of communities. Mutualism may also prove useful in studies of ecological disturbance and equilibria (Addicott 1984).

Because of its relative simplicity, the Laikipia ecosystem is ideal for studies of mutualism. As the behaviors of the four ant associates of *A. drepanolobium* are revealed, our understanding of the beautiful and complex nature of mutualism grows.

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