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Ant species confer different partner benefits on two neotropical myrmecophytes

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Abstract The dynamics of mutualistic interactions involving more than a single pair of species depend on the relative costs and benefits of interaction among alternative partners. The neotropical myrmecophytes *Cordia nodosa* and *Duroia hirsuta* associate with several species of obligately symbiotic ants. I compared the ant partners of *Cordia* and *Duroia* with respect to two benefits known to be important in ant-myrmecophyte interactions: protection against herbivores provided by ants, and protection against encroaching vegetation provided by ants. *Azteca* spp., *Myrmelachista schumanni*, and *Allomerus octoarticulatus demerarae* ants all provide the leaves of *Cordia* and *Duroia* some protection against herbivores. However, *Azteca* and *Allomerus* provide more protection than does *Myrmelachista* to the leaves of their host plants. Although *Allomerus* protects the leaves of its hosts, plants occupied by *Allomerus* suffer more attacks by herbivores to their stems than do plants occupied by other ants. Relative to *Azteca* or *Allomerus*, *Myrmelachista* ants provide better protection against encroaching vegetation, increasing canopy openness over their host plants. These differences in benefits among the ant partners of *Cordia* and *Duroia* are reflected in the effect of each ant species on host plant size, growth rate, and reproduction. The results of this study show how mutualistic ant partners can differ with respect to both the magnitude and type of benefits they provide to the same species of myrmecophytic host.

Keywords Ant-plant interactions · *Cordia* · *Duroia* · Mutualism · Partner benefits

Introduction

It is now widely recognized that mutualisms rarely involve a single pair of interacting species (Hoeksema and Bruna 2000; Stanton 2003). Studies have shown that mutualistic species can differ in the benefits they provide to a common partner species (Bristow 1984; Schemske and Horvitz 1984; Fraser et al. 2001). As a result, some species may be better mutualists than others. All species but the best mutualist species in a guild may impose an opportunity cost on their partners by preventing interaction with the best mutualist (Yu and Pierce 1998; Yu 2001; Stanton 2003). Seen this way, in a guild of mutualistic species, all species but the best mutualist species are actually parasites of the mutualism (Yu and Pierce 1998; Yu 2001).

However, species within a mutualist guild may not be easily ordered into a hierarchy of mutualist quality. Which species is the best mutualist in a guild may change depending on the abiotic environment or the biotic community in which the interaction takes place (Thompson and Pellmyr 1992; Bronstein 1994a; Thompson and Cunningham 2002; Stanton 2003; Gomulkiewicz et al. 2003). Shifts in mutualist quality can affect the population dynamics and coevolutionary trajectories of species in mutualist guilds (Thompson and Cunningham 2002). Several recent models explore the potential ecological and evolutionary consequences of differential partner benefits among species in mutualist guilds (Gomulkiewicz et al. 2003; Stanton 2003).

Mutualisms between myrmecophytic plants and ants have often been used as model systems for the study of mutualism (Bronstein 1998). The defining characteristic of myrmecophytes is their domatia—hollow, swollen structures (stems, thorns, petioles, or leaf pouches) in which ants nest (Heil and McKey 2003). In addition to nesting space, many myrmecophytes provide nutrition to their resident ants, either directly in the form of food bodies or extrafloral nectar, or indirectly via homopteran coccids. The obligate ant partners of

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myrmecophytes are usually mutualistic (Bronstein 1998; Heil and McKey 2003; but see Janzen 1975; Stanton et al. 1999; Yu and Pierce 1998). Ants benefit myrmecophytes by protecting them against herbivory or encroaching vegetation, or by providing nutrients (Davidson et al. 1988; Sagers et al. 2000; Heil and McKey 2003).

More than 100 genera of flowering plants include myrmecophytic species, drawing their mutualists from some 30 ant genera (Davidson and McKey 1993; Heil and McKey 2003). As with most mutualisms, there is seldom a one-to-one correspondence between the ant and plant species involved. Instead, most myrmecophytic plant species interact with a guild of ant species (Davidson et al. 1991; Bronstein 1994b, 1998; Vasconcelos and Davidson 2000; Feldhaar et al. 2003; Stanton 2003; Bruna et al. 2004).

It has recently become clear that ant species can vary in the benefits they provide to the same species of myrmecophyte. Specifically, studies have shown that ant species can differ in the quality of anti-herbivore defense provided to a common species of host (Suarez et al. 1998; Gaume and McKey 1999; Bruna et al. 2004; Tillberg 2004). Such differences among ant species in benefits provided to a common host may influence host fitness, especially if individual ant-myrmecophyte associations are long-lived.

In this study, I investigate whether the alternative ant partners of two Amazonian myrmecophytes benefit their host plants equally. Specifically, I ask whether ant species differ in the protection against herbivores and the protection against encroaching vegetation they provide to hosts. In evaluating both of these benefits simultaneously, I assess a broader range of benefits than have previously been considered in studies comparing multiple ant species partners of a myrmecophytic host. I also consider how unequal benefits among ant species affect host plant size, growth, and reproduction.

Methods

Study sites and system

Data for this study were collected at two study sites, Madre Selva Biological Station ("MSBS"; 3°37'S, 72°14'W) and Zona Reservada Allpahuayo-Mishana ("ZRAM"; 3°35'S, 73°26'W), both in the Department of Loreto, Peru. Separated by only 130 km, both sites have very similar climates. The sites receive 2,400–3,000 mm of rain a year and average daily max./min. temperatures of 31°C/23°C. Since no month at either site receives less than 100 mm of rain, the sites could be considered aseasonal (Whitmore 1998). However, the driest months of the year (June through October) average significantly lower rainfall than the wettest months (November through May), so there are dry and wet seasons.

The myrmecophytes *Cordia nodosa* Lam. (Boraginaceae) and *Duroia hirsuta* [Poeppig and Endl.] K. Schum

(Rubiaceae) occur at high densities in the primary *tierra firme* rainforest at both sites. Variation in plant density is greater within a site than between the two sites. Both *Cordia nodosa* and *Duroia hirsuta* produce hollow, swollen stem domatia whether or not ants are present. Both plant species produce one domatium per internode. As a result, the number of domatia on a plant is a good measure of plant size.

Cordia nodosa and *Duroia hirsuta* each associates with two or more species of symbiotic ant. The same species of ant symbionts occur at MSBS and ZRAM. The plants have two ant partners in common, *Azteca depilis* Emery (Dolichoderinae) and *Myrmelachista schumanni* Emery (Formicinae). These two species are the only ants that associate with *Duroia*. In addition to *A. depilis* and *M. schumanni*, *Cordia* associates with two undescribed species in the genus *Azteca* and *Allomerus octoarticulatus demerarae* Wheeler (Myrmicinae). In this study, the three species of *Azteca* that inhabit *Cordia* are treated as a single group because they are difficult to distinguish based on worker morphology alone.

Myrmelachista schumanni is unusual among plant-ants in being a polygynous and polydomous species (M.E. Frederickson, unpublished data). Colonies of *Myrmelachista* stretch over large stands of host plants, known locally as "supaychacras" or "devil's gardens." With the exception of *Myrmelachista*, the ant partners of *Cordia* and *Duroia* are all monogynous. In these monogynous species, a single colony of ants nests in a single host plant tree.

Census data

Censuses were conducted in a four-hectare plot at MSBS. The four-hectare plot was oriented along cardinal directions and divided into sixteen 50 m×50 m squares. The corners of each square were permanently marked with PVC tubes and stake flags. In July 2002 and again in July 2003, each square was exhaustively searched for all domatia-bearing individuals of *C. nodosa* and *D. hirsuta* by walking each square back and forth in a series of 2.5-m transects.

Each plant encountered was numbered and tagged. For each plant, the following data were recorded: plant species; plant height and number of domatia; species of ant occupant, if any; the presence of fruits and flowers; whether the plant had been girdled by the cerambycid beetle *Trachysomus* sp. (Yu and Pierce 1998); and whether the area around the base of the plant was devoid of vegetation. Plant height was measured as the distance from the ground to the highest leaf. In 2002, plant height was estimated visually; in 2003, it was measured using a telescopic measuring rod (Crain). The number of domatia on a tree was determined using binoculars when needed. The area around the base of a plant was considered devoid of vegetation if there were no stems within a 25-cm radius. Vegetation-free zones around trunks often extended much further than 25 cm, but this

radius was chosen so that the effect of neighboring conspecific trees would not be confounded with the effect of a focal tree. In July 2003, canopy openness was measured for each plant using a spherical crown densitometer (Forestry Suppliers, Lemmon 1956). Measurements of canopy openness do not include a plant's own canopy. Also in July 2003, trunk diameter at a height of 20 cm was measured for each plant.

In July 2003, data on standing levels of leaf herbivory were collected for plants in the plot. Between 16 and 34 host plants with each ant species were chosen at random and five leaves on each of these plants were examined. The lowermost five leaves for plants with mature leaves only, or the lowermost three mature leaves and two young leaves for plants bearing young leaves, were digitally photographed against a white background attached to the camera at a fixed distance. Leaf area loss was measured by comparing the actual leaf area in an image to the area encompassed by a digital trace of the inferred leaf margin in the absence of herbivory (Scion Image 4.0.2). Leaf area loss was converted to a percent of total leaf area.

Ant exclusion experiments

In April and May 2004, ant exclusion experiments were carried out at ZRAM. Twenty-one *Cordia* and 20 *Duroia*, each bearing at least two young leaves, were located along the trails and transects of the reserve. Eight *Cordia* were occupied by *Azteca*, seven by *Allomerus*, and six by *Myrmelachista*. Eleven *Duroia* were occupied by *Myrmelachista* and nine by *Azteca*. On each plant, one young leaf was randomly chosen to be the ant-excluded leaf, another young leaf to be the control leaf. In all cases, both leaves derived from the same domatium. The number of domatia on each plant was recorded.

Tanglefoot insect barrier was applied carefully around the petiole of the ant-excluded leaf. All worker ants present on the ant-excluded leaf were removed with forceps. The underside of the control leaf was marked with a permanent marker. Both leaves were photographed before treatment and again 12 days later. Percent herbivory was measured from the before and after digital photographs of the leaves as described above.

Statistical analyses

An arcsine square root transformation was performed on the percentage values of herbivory and canopy openness to improve normality (Sokal and Rohlf 1995). Differences among ant species in standing levels of host plant herbivory were analyzed using nested ANOVAs, with ant species as a fixed factor and individual plants nested within ant species. Differences among plants occupied by each ant species in levels of canopy openness were analyzed using univariate ANOVAs. Since domatia number and plant height are correlated

measures of plant size, differences in plant size among plants occupied by different ant species were analyzed using MANOVAs incorporating both response variables. Similarly, differences in growth among plants occupied by different ant species were analyzed using MANOVAs incorporating change in plant height and change in plant domatia number as response variables. When the MANOVAs were significant, univariate ANOVAs were then performed for each response variable separately (Zar 1999). Following all significant univariate ANOVAs, Tukey-Kramer post hoc tests for multiple comparisons were used to compare levels of herbivory, canopy openness, plant size, and plant growth among plants occupied by different ant species (Sokal and Rohlf 1995). Differences are reported as significant using an experiment-wide error rate of $\alpha=0.05$. Plant growth was measured as the change in host plant height or host plant domatia number over one year. Analyses were conducted on values for both absolute and percent growth. Percent growth was measured as final size minus initial size, divided by initial size. Only host plants that had the same ant species in 2002 as in 2003 were considered when making comparisons among ant species. Data on plants with no ants are not included in statistical analyses, but are presented in the figures for comparative purposes. Pearson correlations, χ^2 tests, and two-tailed *t* tests, were used to analyze correlations, frequencies, and mean differences between two groups, respectively. Herbivory rates between ant-excluded and control leaves were compared separately for each combination of ant and plant species. Sample sizes in the ant exclusion experiments were too small to reliably compare herbivory rates among plants with each ant species. Statistical analyses were carried out using Statview 5.0.1 (SAS Institute).

Results

Census data

Relative abundance

Ant species did not inhabit equal shares of the host plant population. In July 2003, there were 312 *Cordia* in the MSBS study plot. *Allomerus* inhabited 51% of these plants, *Azteca* inhabited 19%, and *Myrmelachista* inhabited 7%. For *Duroia*, *Myrmelachista* inhabited 65% and *Azteca* inhabited 11% of the 655 host plants in the plot. Three to four percent of host plants of both species were occupied by ants that are not obligately associated with *Cordia* or *Duroia* (*Crematogaster* sp. and *Pheidole* sp.). Twenty percent of host plants of both species were unoccupied by ants.

Turnover of ants on host plants

There was little evidence for turnover or succession of ant species on individual host plants. Host plants that

were occupied by ants in both 2002 and 2003 were observed to have the same species of ant occupants in both years. There was one exception: *Azteca* replaced *Myrmelachista* on seven host plants between 2002 and 2003. It was not possible to determine whether these plants were unoccupied before *Azteca* ants arrived, or whether *Azteca* displaced existing colonies of *Myrmelachista* on these trees. In all other instances of host plant colonization, new colonies of ants observed in 2003 established in host plants that had been unoccupied in 2002. For the most part, these plants were young saplings. In 2003, eighty percent of new ant colonies had established on plants that had fewer than five domatia and were less than a meter tall in 2002.

Herbivory

Leaf herbivory levels differed among plants occupied by different ant species. *Cordia* inhabited by *Azteca* or *Allomerus* had lower standing levels of leaf herbivory than *Cordia* inhabited by *Myrmelachista* (Table 1; Fig. 1a). For *Duroia*, *Azteca*-occupied plants exhibited lower standing levels of leaf herbivory than *Myrmelachista*-occupied plants (Table 1; Fig. 1b). There was no relationship between the standing level of herbivory and plant size for ant-occupied *Cordia* (domatia: $r=0.004$, NS; height: $r=0.049$, NS), nor for ant-occupied *Duroia* (domatia: $r=0.267$, NS; height: $r=0.148$, NS). This is not a result of comparing across ant species; considering each ant-plant pair separately did not yield any significant relationships between herbivory and plant size (results not shown).

The frequency of one type of stem herbivory, *Trachysomus* sp. girdling, also varied among plants occupied by different ant species. *Trachysomus* sp. is a cerambycid beetle that girdles *C. nodosa* stems (Yu and Pierce 1998). The portion of the plant above the girdle dies, rots, and eventually falls off, but the distinctive girdling ring around the stem remains visible for some time. In July 2003, twenty *Cordia* exhibited *Trachysomus* sp. girdling marks; 18 were occupied by *Allomerus*, one by *Azteca*, and one was unoccupied by ants. This represents significantly more attacks on *Allomerus*-occupied *Cordia* than expected by chance alone, given the

Table 1 Results of nested ANOVAs for the effect of ant species and individual plant (nested in ant species) on standing levels of leaf herbivory

Host plant species	Source	df	MS	F	P
<i>C. nodosa</i>	Ant species	2	0.278	3.886	0.03
	Plant (ant species)	68	0.072	3.860	<0.0001
	Error	284	0.019		
<i>D. hirsuta</i>	Ant species	1	0.649	10.939	<0.01
	Plant (ant species)	39	0.059	472.755	<0.0001
	Error	164	0.000		

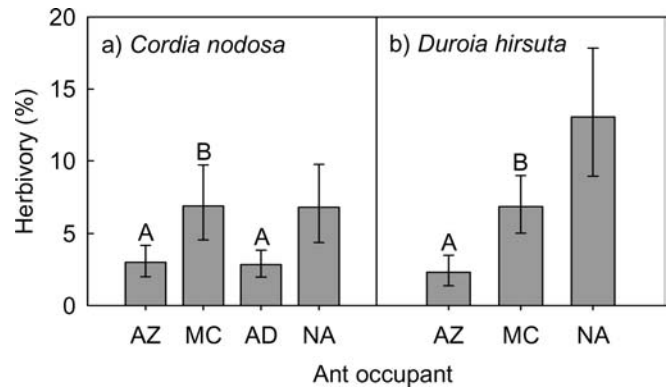


Fig. 1 Average standing level of leaf herbivory on **a** *C. nodosa* and **b** *D. hirsuta* as a function of ant occupant, if any (AZ *Azteca* spp., MC *M. schumanni*, AD *Allomerus octoarticulatus demerarae*, NA No ants). Bars marked by the same letter are not significantly different ($P>0.05$). Data on unoccupied *C. nodosa* and *D. hirsuta* are presented for comparative purposes, but were not included in statistical analyses. Error bars are back-transformed 95% confidence intervals

frequency of *Allomerus* in the plot (χ^2 test, $\chi^2_3=9.56$, $P=0.02$).

For *Cordia* with *Allomerus* ants, girdled plants were significantly larger than non-girdled plants in both height and diameter at 20 cm (height: t test, $t_{157}=2.838$, $P=0.005$; diameter: t test, $t_{157}=4.905$, $P<0.001$), but not higher in domatia number (t test, $t_{157}=0.695$, NS).

Canopy openness

The degree of canopy openness above host plants also varied significantly among plants occupied by different ant species. For both host plant species, *Myrmelachista*-occupied plants had significantly more open canopies than either *Allomerus*- or *Azteca*-occupied plants. On average (followed by 95% confidence intervals), *Cordia* plants had 10.7% (10.5–11.0%) canopy openness when occupied by *Allomerus*, 10.9% (10.4–11.5%) openness with *Azteca*, but 12.9% (11.7–14.2%) openness with *Myrmelachista* (ANOVA, $F_{2,184}=4.423$, $P=0.01$). Similarly, *Duroia* plants had 11.0% (10.6–11.4%) canopy openness when occupied by *Azteca*, but 11.9% (11.8–12.0%) openness with *Myrmelachista* (ANOVA, $F_{1,471}=4.076$, $P=0.04$).

Myrmelachista appears to increase canopy openness by pruning vegetation from around the base of its host tree. Host plants surrounded by areas devoid of vegetation had higher canopy openness than other host plants [*Cordia*: vegetated base = 10.9% (10.6–11.1%), no vegetation around base = 13.3% (11.4–15.3%); *Duroia*: vegetated base = 11.1% (10.4–11.9%), no vegetation around base = 11.9% (11.7–12.1%)]. The results of two-tailed t tests show that these differences, while small, are statistically significant (*Cordia*: $t_{199}=2.01$, $P=0.05$; *Duroia*: $t_{517}=2.44$, $P=0.02$). There was a strong association between ant species and the presence of areas devoid of vegetation around host plants. Three hundred

and sixty-seven out of 413 *Myrmelachista*-occupied *Duroia* were devoid of vegetation around the base, whereas 2 out of 60 *Azteca*-occupied plants were (χ^2 test, $\chi^2_1 = 223.42$, $P < 0.0001$). Similarly, for *Cordia*, 8 out of 17 *Myrmelachista*-occupied plants were devoid of vegetation around the base, compared to 1 out of 119 *Allomerus*-occupied plants and 0 out of 52 *Azteca*-occupied plants (χ^2 test, $\chi^2_2 = 73.33$, $P < 0.0001$).

Plant size and growth

Cordia plants occupied by *Azteca* were larger than plants occupied by *Allomerus* or *Myrmelachista* (Fig. 2a; MANOVA, Pillai trace = 0.393, $F_{4,370} = 22.653$, $P < 0.0001$; the results of separate univariate ANOVAs for plant height and plant domatia number are presented in Table 2).

Cordia plants occupied by *Azteca* also grew more than plants with *Allomerus*; growth with *Myrmelachista* was intermediate (Fig. 3a). The effect of ant species on host plant growth was significant in MANOVAs using increases in height and domatia number as response variables, regardless of whether increases were measured as absolute growth (Pillai trace = 0.158, $F_{4,348} = 7.472$, $P < 0.0001$) or as percent growth (Pillai trace = 0.066, $F_{4,348} = 2.950$, $P = 0.02$). Ant species was also a significant factor in univariate ANOVAs on absolute and percent growth in height and domatia number (Table 2). There was one exception: the univariate ANOVA on percent change in plant domatia number was non-significant.

Girdling of *Allomerus*-occupied *Cordia* was associated with low growth rate. Girdled *Allomerus*-occupied *Cordia* grew less in terms of both height (girdled -14.2 ± 16.4 cm, not girdled 17.3 ± 5.18 cm; t test, $t_{123} = 2.159$, $P = 0.03$) and domatia number (girdled -7.72 ± 3.72 , not girdled -0.06 ± 0.73 ; t test, $t_{125} = 3.298$, $P = 0.001$) than non-girdled *Allomerus*-occupied *Cordia*. Two significant outliers were detected in the height data using Grubbs test for outliers (Sokal and Rohlf 1995); these values were omitted from the analysis.

For *Duroia*, plants occupied by *Myrmelachista* were significantly larger, but did not grow significantly more than plants with *Azteca* (Figs. 2b, 3b). Ant species had a significant effect on host plant size (MANOVA, Pillai trace = 0.020, $F_{2,470} = 4.918$, $p = 0.01$; the results of separate univariate ANOVAs for plant height and plant domatia number are presented in Table 2), but not on host plant growth, whether measured as absolute growth (MANOVA, Pillai trace = 0.015, $F_{2,363} = 2.739$, NS) or as percent growth (MANOVA, Pillai trace = 0.015, $F_{2,362} = 2.785$, NS).

Fruiting and flowering

Only four *Cordia* plants (2%) were observed to be fruiting or flowering during censuses; censuses were conducted in the dry season, whereas *Cordia* flowers and fruits predominantly in the wet season. All four of these plants were occupied by *Azteca*. This is significantly more than expected by chance alone, given the relative abundance of *Azteca* in the plot (χ^2 test, $\chi^2_2 = 10.69$, $P < 0.005$).

One hundred and forty-six *Duroia* were fruiting or flowering during the censuses; six were occupied by *Azteca* and 140 by *Myrmelachista*. This represents significantly more fruiting or flowering *Myrmelachista*-occupied plants than *Azteca*-occupied plants, given the frequencies of *Myrmelachista* and *Azteca* in the plot (χ^2 test, $\chi^2_1 = 14.02$, $P < 0.001$).

Ant exclusion experiments

All ant species provided significant protection against herbivores to the young leaves of their host plants (Fig. 4a, b). Initial herbivory levels did not differ significantly between ant-excluded and control leaves, according to paired t tests (results not shown). In contrast, herbivory rates over the course of the experiment (i.e., final herbivory minus initial herbivory)

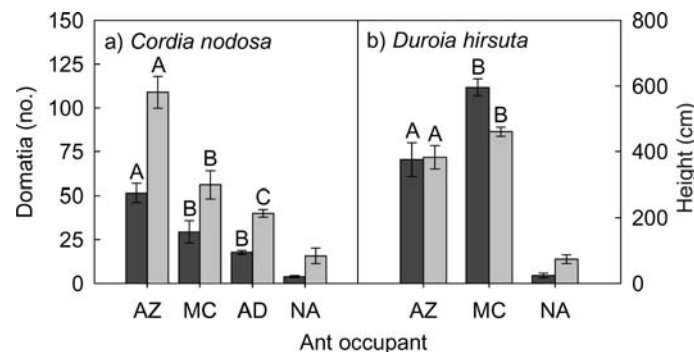


Fig. 2 Average (\pm SE) number of domatia (dark bars) and plant height (light bars) in 2003 for **a** *C. nodosa* and **b** *D. hirsuta*, as a function of ant occupant, if any (AZ *Azteca* spp., MC *M. schumanni*, AD *Allomerus octoarticulatus demerarae*, NA No ants).

Data on unoccupied *C. nodosa* and *D. hirsuta* are presented for comparative purposes, but were not included in statistical analyses. Bars marked by the same letter are not significantly different ($P > 0.05$).

Table 2 Results of univariate ANOVAs for the effect of ant species on host plant size, absolute growth, and percent growth

Host plant species	Analysis	Source	df	MS	F	P
<i>C. nodosa</i>	Size–domatia	Ant species	2	20553.558	36.726	<0.0001
		Error	185	559.640		
	Size–height	Ant species	2	2449204.362	52.992	<0.0001
		Error	185	46218.332		
	Absolute growth–domatia	Ant species	2	891.415	5.601	<0.01
		Error	174	159.142		
Absolute growth–height	Ant species	2	98943.155	15.116	<0.0001	
	Error	174	6545.745			
Percent growth–domatia	Ant species	2	1.147	2.274	0.11	
	Error	174	0.504			
Percent growth–height	Ant species	2	0.377	4.911	<0.01	
	Error	174	0.077			
<i>D. hirsuta</i>	Size–domatia	Ant species	1	89172.199	9.576	<0.01
		Error	471	9311.697		
	Size–height	Ant species	1	318286.331	3.900	0.05
		Error	471	81605.848		

differed significantly between ant-excluded and control leaves for all five combinations of ant and plant species (Table 3).

Host plant size was not significantly correlated with herbivory rate, with one exception. For plants occupied by *Allomerus*, there was a marginally significant correlation between domatia number and herbivory on ant-excluded leaves ($r=0.756$, $P=0.05$), and a marginally non-significant correlation between domatia number and herbivory on control leaves ($r=0.717$, $P=0.07$).

Discussion

This study compared the species in a guild of obligate, mutualistic plant-ants with respect to the two major benefits these ants provide to their myrmecophytic hosts: protection against herbivores and protection against encroaching vegetation. Species identity of ant occupants strongly influenced interaction outcomes between ants and the two myrmecophytic plant species in this study. There was a significant effect of ant identity on a suite of host plant variables, including (1) herbivory on

both leaves and stems, (2) presence or absence of adjacent vegetation, (3) degree of overhead canopy openness, (4) host plant size, (5) host plant growth, and (6) host plant flowering and fruiting. Ant influence on host plant leaf herbivory was confirmed experimentally.

My results demonstrate that although all ant species provide some protection against herbivory, ant species in this guild differ in the amount of protection they provide to their hosts. *Azteca* and *Allomerus* afforded more protection to the leaves of their host plants than did *Myrmelachista*. However, *Allomerus*-occupied *Cordia* suffered more attacks by the stem-girdling beetle, *Trachysomus* sp., than *Cordia* occupied by any of the other ant species.

Host plant size did not affect levels of herbivory on plants with ants other than *Allomerus*. In these cases, either plant size was not correlated with ant colony size, or ant colony size did not affect herbivory. For plants with *Allomerus*, girdling by *Trachysomus* sp. was more common on larger plants, perhaps because of a preference by the beetles for larger plants (Yu and Pierce 1998). In ant exclusion experiments, larger *Allomerus*-occupied plants tended to experience less leaf herbivory.

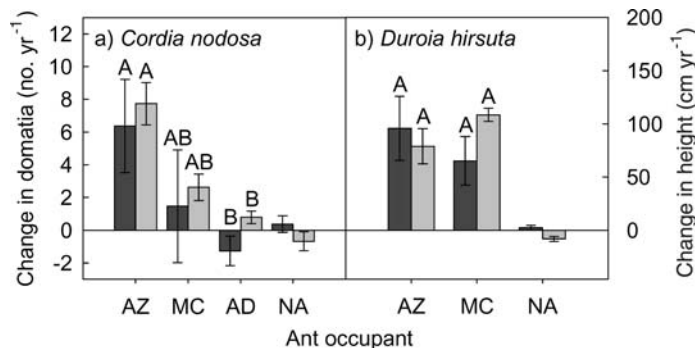


Fig. 3 Average change (\pm SE) in number of domatia (dark bars) and plant height (light bars) over one year for **a** *C. nodosa* and **b** *D. hirsuta*, as a function of ant occupant, if any (AZ *Azteca* spp., MC *M. schumanni*, AD *Allomerus octoarticulatus demerarae*, NA No

ants). Data on unoccupied *C. nodosa* and *D. hirsuta* are presented for comparative purposes, but were not included in statistical analyses. Bars marked by the same letter are not significantly different ($P>0.05$)

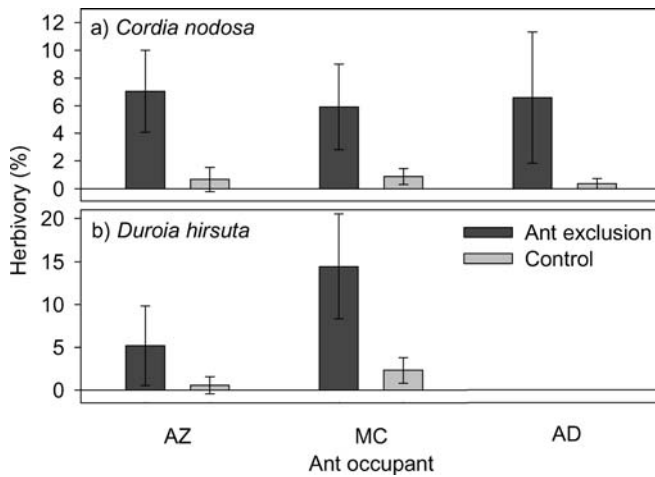


Fig. 4 Average herbivory rate over 12 days for ant-excluded leaves (dark bars) and control leaves (light bars) of **a** *C. nodosa* and **b** *D. hirsuta* as a function of ant occupant (AZ *Azteca* spp., MC *M. schumanni*, AD *Allomerus octoarticulatus demerarae*). In each case, herbivory was significantly greater on ant-excluded leaves than on control leaves ($P \leq 0.05$). Error bars are back-transformed 95% confidence intervals

Table 3 Results of paired *t* tests for differences in herbivory rate between ant-excluded and control leaves, by plant and ant species

Host plant species	Ant species	df	<i>t</i>	<i>P</i>
<i>C. nodosa</i>	<i>Azteca</i> spp.	7	3.376	0.01
	<i>Allomerus octoarticulatus demerarae</i>	6	2.438	0.05
	<i>M. schumanni</i>	5	2.956	0.03
<i>D. hirsuta</i>	<i>A. depilis</i>	8	3.319	0.01
	<i>M. schumanni</i>	10	2.784	0.02

For all ant species, ant-excluded leaves experienced significantly higher herbivory than control leaves

This suggests that, for plants with *Allomerus* ants only, larger plants (possibly with larger *Allomerus* colonies) are better defended against herbivory.

Several factors might explain why standing levels of host plant herbivory differed among plants occupied by different ant species. Some ant species are better able than others to find, attack, and remove herbivores from their hosts (Suarez et al. 1998; Gaume and McKey 1999; Di Giusto et al. 2001; Bruna et al. 2004). Recently, Davidson et al. (2003) proposed that one reason *Azteca* ants are such common plant-ants is that their highly carnivorous diet makes them very effective guards against plant herbivores. Bruna et al. (2004) linked behavioral differences among ant species to differences in levels of herbivore damage on host plants. My study expands on previous research by linking differences in herbivore damage with differences in host plant performance among plants occupied by different ant species.

Behavioral differences among the ant occupants of *Cordia* may explain the high frequency of *Trachysomus* attacks on *Allomerus*-occupied plants, relative to attacks

on *Azteca*- or *Myrmelachista*-occupied plants. Unlike *Azteca* or *Myrmelachista* workers, *Allomerus* workers do not actively patrol the trunks of their host plants, where *Trachysomus* beetles attack (Yu and Pierce 1998, Personal observation). Girdling by *Trachysomus* sp. had the expected result on *Cordia* plants; girdled *Cordia* grew less than non-girdled *Cordia*.

Differences among ant species in behavior may not account for all the observed differences among ant species in the levels of herbivory suffered by their host plants. While statistical tests of this difference were not reliable because of low power, herbivores consumed the ant-excluded leaves of *Myrmelachista*-occupied *Duroia* at more than twice the rate of ant-excluded *Azteca*-occupied *Duroia*. This suggests that high standing levels of herbivory on *Myrmelachista*-occupied plants may be the result of a greater herbivore load on these plants, rather than poorer protective efficacy of *Myrmelachista*, relative to *Azteca*. This would also explain the apparent contradiction between the high standing levels of herbivory on *Myrmelachista*-occupied plants and the significant anti-herbivore protection conferred by *Myrmelachista* in ant-exclusion experiments. The mechanisms responsible for the observed differences in herbivory levels among *Azteca*, *Allomerus*, and *Myrmelachista* require further investigation.

While *Myrmelachista* provides the least protection against leaf herbivory to *Cordia* and *Duroia*, this ant provides the most favorable light environment. Plants occupied by *Myrmelachista* had more open canopies above them than plants occupied by either *Allomerus* or *Azteca*. In tropical rainforests, canopy cover does not span the entire range between 0% and 100%. The range of canopy openness values is restricted to between approximately 2% and 18% (Aide and Zimmerman 1990; Nicotra et al. 1999). As a result, differences in canopy openness values of just a few percent can represent substantial changes in canopy cover and, as a corollary, light availability for plants living in the understory of a tropical rainforest. Thus, the few percent difference in absolute canopy openness between plants occupied by *Myrmelachista* and plants occupied by *Azteca* or *Allomerus* is likely to be biologically significant.

In addition to having more open canopies above them, *Myrmelachista*-occupied plants were also more frequently devoid of vegetation around the base than *Allomerus*- or *Azteca*-occupied plants. The significant three-way association between a lack of vegetation around the host plant tree base, increased canopy openness, and the presence of *Myrmelachista* strongly suggests that *Myrmelachista* improves the light environment of its host by clearing neighboring vegetation. However, *Myrmelachista*-occupied plants did not always have the highest growth rate relative to plants with other ants, possibly because the effect of increased light availability was tempered by the effect of high herbivory on the growth of *Myrmelachista*-occupied plants.

There has been some debate in the literature about whether *Myrmelachista* ants are responsible for clearing

vegetation from around *Duroia* and other myrmecophytes (Morawetz et al. 1992; Renner and Ricklefs 1998), or whether *Duroia* is an allelopathic tree (Campbell et al. 1989; Page et al. 1994; Pfannes and Baier 2002). My results are consistent with *Myrmelachista* clearing vegetation from around *Duroia*, since *Duroia* was free of nearby vegetation only when *Myrmelachista* ants were present and not when either *Azteca* or no ants were present. Furthermore, *Cordia* was also free of nearby vegetation when occupied by *Myrmelachista*.

Several other plant-ant species are known to prune encroaching vegetation from their host plants (Janzen 1966, 1967, 1969, 1972; Davidson et al. 1988). While ants may benefit indirectly from pruning via improved host plant vigor, Davidson et al. (1988) proposed that pruning behavior derives from the defensive needs of the colony. Davidson et al. (1988) demonstrated that pruning of encroaching vegetation by *Pseudomyrmex* ants living in *Triplaris americana* reduced invasions by aggressive *Crematogaster* ants. The benefits to *Myrmelachista* of pruning encroaching vegetation remain to be determined.

Variation among ant species in benefits provided to host plants was correlated with variation in host plant performance among ant species. All three measures of plant performance—size, growth, and presence of fruits or flowers—were broadly concordant. *Cordia* with *Azteca* ants were larger, grew more, and were more frequently observed fruiting and flowering than *Cordia* occupied by *Allomerus*. For the most part, *Cordia* occupied by *Myrmelachista* experienced intermediate host performance. *Duroia* occupied by *Myrmelachista* were larger and were more frequently observed fruiting and flowering than *Duroia* with *Azteca*, but did not grow more.

In this study, ant species turnover on individual host plants was never observed; ants colonized most host plants as saplings. This suggests that ant species identity affects host plant performance, rather than the reverse. An alternative explanation is that different ant species preferentially colonize host plants in different habitats, and habitat quality affects host plant performance. Some plant-ant species have been found to favor host plants in certain habitats—for example, host plants near rivers or termite mounds (Yu and Davidson 1997; Palmer 2003). However, in my study plot, there were no obvious associations between ant species and host plant habitat at the time of colonization. Yu and Pierce (1998) discard the possibility that *Allomerus* or *Azteca* colonize *Cordia* according to light regime. Studies are underway to determine how *Allomerus*, *Azteca*, and *Myrmelachista* find and choose host plants.

Plants with ants that provided either substantial protection against herbivores (i.e., *Azteca*) or increased canopy openness (i.e., *Myrmelachista*) grew relatively well, whereas plants with ants that provided neither (i.e., *Allomerus*), or plants without ants, did not. This pattern

was to a certain extent independent of host plant species, pointing to the importance of ant species identity.

Interestingly, no ant species provided superior protection against both herbivores and encroaching vegetation. From the plant's perspective, such an ant would be the best mutualist in this guild. Instead, no ant species was clearly the most beneficial partner for either host. This is especially evident in the case of *Duroia*, for which *Azteca* and *Myrmelachista* provided different benefits and resulted in similar host plant growth rates. Here, the extent to which one ant species or the other is most favorable to a host plant likely depends on context. *Azteca* may benefit plants that establish in high-herbivory environments more than *Myrmelachista*, whereas *Myrmelachista* may benefit plants that establish in low-light environments more than *Azteca*. Such context-dependence of interaction outcomes has been demonstrated in other mutualisms, particularly in plant-pollinator, plant-mycorrhizae, and facultative ant protection mutualisms (Bronstein 1994a; Johnson et al. 1997; Fenster and Dudash 2001). Future research will determine whether similar mechanisms are operating in these obligate ant-myrmecophyte mutualisms.

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