

Revisiting the *Cecropia-Azteca* mutualism: ant effects on the fitness of juveniles

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Participants: Stephen Ironside, Scott Drucker
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Abstract

Mutualisms between plants and animals are consumer-resource interactions in which one or both individuals experience net benefits. In ant-plant mutualisms, plants exchange food and nesting sites for protection from the ants against herbivory and epiphytic growths. Mutualisms are conditional and range from obligate to facultative interactions, and can be spatially or temporally variable. The *Cecropia-Azteca* relationship, one of the most conspicuous ant-plant associations in the Neotropics, has been well-studied. However, it has also been doubted; some consider it an obligate mutualism while others deem the association neutral or even parasitic. In this study we assessed the importance of ants to *Cecropia* by assessing the levels of herbivory on young leaves on trees with and without active ant colonies. We also assessed the effects of light availability, tree height, and herbivory type on the percent herbivory of the leaves, and whether the presence of ants affected the growth of epiphytes. The availability of light, tree height, and type of herbivory had no significant effect on percent herbivory. The presence of active ant colonies on *C. obtusifolia* significantly reduced levels of herbivory and the growth of epiphytes. The species of ant present made no difference to herbivory levels.

Introduction

Mutualisms are interspecific consumer-resource interactions in which one or both individuals experience a net benefit. In the case of ant-plant associations, plants provide food and oftentimes nesting sites to the ants; the associated ants in turn provide protection from both herbivores and encroaching epiphytic growth. Defense by symbiotic ants is an effective form of protection from herbivores; ants are highly mobile and tend to defend younger, more vulnerable leaves, while fulfilling a goal usually fulfilled by chemical

defenses (Heil et al. 2000). Historically, two views regarding plants' necessity of their associated ants have arisen. Over a century ago, Belt (1874) and Müller (1874, 1876) hypothesized that the saplings of many tree species benefit from protective ant interactions. The suggestion of a mutualistic relationship between ants and plants provoked a flurry of debate. Indeed, many prominent naturalists such as W.M. Wheeler described these relationships as neutral or even parasitic (Wheeler 1942). Detailed field studies and experimental work was lacking, however, until the pioneering work of Janzen reignited interest in ant-plant associations and established that plants may benefit from associations with ants (e.g. Janzen 1966, 1967, 1975). It is now understood that plants may benefit in numerous ways from ant associations: ants protect plants from herbivores and other predators, they help disperse seeds and fruits, they can pollinate flowers, and they can feed plants essential nutrients (Beattie 1985).

Mutualistic ant-plant interactions range from species-specific, co-evolved obligate relationships to generalized, facultative associations in which one or both partners can live successfully without the other (Bronstein 1994b). In some cases, such as the obligate *Acacia-Pseudomyrmex* mutualism, neither the ants nor the *Acacia* can survive without the other. The *Pseudomyrmex* aggressively defend their host tree against herbivores and epiphytes in exchange for nesting sites in the tree's specialized, hollow thorns and food from its Beltian bodies (Janzen 1966). Ant-*Acacia* species have even lost their chemical defenses, implying that they rely heavily, if not exclusively, on their resident ants (Janzen 1966, Gonzalez-Sulser and Thompson 2004). In other cases, the association becomes more facultative and ambiguous. Wild cotton (*Gossypium thurberi*), for example, attracts various generalized ant species to its extrafloral nectaries (EFNs) in order to gain protection from herbivores, but when EFN production wanes, so does the aid of the ants (Rudgers 2004). This facultative system also occurs in *Ipomea carnea*; generalized ants are attracted to EFNs in order to gain protection from herbivores, but the ants are not associated with the plant in other ways that are common in obligate systems (Keeler 1977).

Mutualistic interactions between species can be temporally or spatially variable, creating a gradient in conferred benefits for participants from strong mutualists to antagonists (Bronstein 1994b). *Leonardoxa africana*, an understory tree in the rainforests

of Cameroon, can be inhabited by either *Petalomyrmex phylax* or *Cataulacus mckeyi* ants. They both benefit from the extrafloral nectaries and the swollen, hollow internodes on the tree. The distributions of these relationships are mutually exclusive. *P. phylax* patrol the tree day and night and significantly reduce herbivory on young leaves; however, *C. mckeyi* only patrol when the EFNs are producing more nectar, and fail to attack herbivores consistently, resulting in increased herbivory on their host (Gaume and McKey 1999).

The *Cecropia-Azteca* association is one of the most conspicuous ant-plant mutualisms in the tropics (Longino 1989). The *Cecropia*'s hollow internodes provide domatia for *Azteca*, and Müllerian bodies on trichilia at the base of the petioles provide food. *Azteca* are known to protect the *Cecropia* from herbivores and encroaching epiphytes (Janzen 1969, Shupp 1986, Heil et al. 2000). Some species of *Azteca* are more aggressive than others; in many cases, a slight shake will cause the ants to swarm up and down the tree looking for potential threats, while other species will not come to tuna baits or react to violent blows to the tree (Longino 1991, pers. obs.). Effects of spatial conditionality on mutualisms can be seen in some *Cecropia* species in Puerto Rico and most other Caribbean islands. These *Cecropia* are not occupied by ants, and the plants no longer produce the trichilia that make the glycogen-rich Müllerian bodies which feed the ants, as is common with *Cecropia* on the Neotropical mainland (Janzen 1973). Habitat-related variables may also influence herbivory levels and the success of *Cecropia-Azteca* interactions once they have been established (Bronstein 1998).

Abiotic factors such as light availability have been shown to condition the outcome of ant-plant interactions (Kersch and Fonseca 2005), and in *Cecropia*, higher light intensity increases the production of Müllerian bodies and the levels of condensed tannins and total phenolics (Folgarait and Davidson 1994). Other factors, such as the type of herbivory on the leaves, also helps reveal the effectiveness of resident ant colonies; if leaves are devoured by *Atta*, it would suggest that the resident colony failed to expel large numbers of herbivores feeding at one time. If the leaves have been eaten mainly by generalist herbivores, it might show that the ants were strong enough to fend off the overwhelming *Atta* (Erika Deinert, pers. comm.). Young *Cecropia* can be colonized when they are less than one meter tall; multiple queens may start colonies on a single tree

until only one colony is successful and can begin to protect the plant from herbivory (Cindy Sagers, pers. comm.). As a *Cecropia* gets taller, it is more likely to be inhabited by ants (Bonato et al. 2003). All of these factors have effects on the fitness of juvenile trees. More important, however, is that the fitness of juvenile *Cecropias* will increase if they are experiencing lower levels of herbivory and have less of a chance of being damaged by vines or mosses (Schupp 1986, Maron 1998).

There has been evidence indicating that the *Cecropia-Azteca* relationship is a neutral association. Healthy *Cecropias* without active ant colonies are relatively easy to find, and many *Cecropias* with active colonies have been found with extensive herbivory (Janzen 1969). *Cecropia* may suffer low incidence of attack by herbivores even when *Azteca* are absent (Fáveri and Vasconcelos 2004), and herbivores have been observed on trees that are occupied by ants (de Andrade and Carauta 1982). It has been shown in Malaysia that introduced *Cecropia* grow well even though the associated ants are absent from the entire region (Putz and Holbrook 1988).

In this study we examined the effects of ants on the levels of herbivory on leaves of two *Cecropia* species. We also examined whether ant colonies had an effect on the growth of vines and mosses on the trees. The presence and species of ants, the height of the trees, the relative light availability, and the type of herbivory present were analyzed in order to better understand the importance of ants to the fitness levels of *Cecropia*.

Methods

This study was conducted from 27 to 30 April 2009 at the La Selva Biological Station, Heredia Province, Costa Rica (10° 26' N, 83° 59' W). La Selva is classified as lowland tropical wet forest (*sensu* Holdridge 1947) and receives approximately 4000 mm of rain annually (Roberts 1994). Juvenile *Cecropia* trees and their affiliated ants were sampled from secondary forest in and around the abandoned Huertos project plots along the Sendero Tres Rios, Sendero Occidental, and Sendero Las Vegas trails of the La Selva station. The Huertos project was a long-term agro-forestry project in which chemical supplements, particularly Nitrogen, were added in different concentrations to each plot (Tillberg 2004). The project was ended from 2002-2003, and since then *Cecropia* and other pioneer species have colonized the plots. *Cecropias* in these plots were oftentimes clustered in groups of 2-4, and were usually located between two and thirty meters from

the cleared trails.

To determine whether the presence or absence of ants has fitness consequences for *Cecropia*, 41 juvenile *Cecropias* between 1 m and 6 m tall, 34 of which were *C. obtusifolia* and 7 of which were *C. insignis*, were sampled. For each individual, the height to the top of the youngest node was calculated using a Suunto® clinometer and an open reel meter tape. The presence or absence of vines and mosses was recorded. A qualitative assessment of sunlight in the immediate area of the individual (sunny, semi-shady, and shady) was made. The presence or absence of ant colonies on each tree was determined by extensive shaking and tapping of the tree and was recorded. If ants were present on a tree, samples were taken in Eppendorf tubes containing 95% ethanol for later identification by R. Vargas. Four randomly selected juvenile *C. obtusifolias* that were determined to not have ants were cut open to make sure there was no colony present. To assess herbivory, the second and third leaves from the top of each tree were collected and taken back to the La Selva laboratory. The type of herbivory on each leaf, either generalist herbivory or *Atta* herbivory, was noted.

Color JPEG photographs were taken of the leaves against a white background using a Nikon D200 DSLR camera, with manual settings of 1/125, f/3.5, ISO-320, and on-board flash, under fluorescent lighting from a distance of approximately 1.75 m. ImageJ 1.41o software (National Institutes of Health 2009) was used to trace the leaves at 4x magnification to measure the total area of each leaf as well as the total leaf area eaten. The total areas from leaves two and three were combined, as were the total areas after herbivory had been accounted for, to provide a more accurate assessment of herbivory on each tree. Percent herbivory was then calculated.

A Stepwise Multiple Regression in the forward direction with a probability to enter of 0.25 and a probability to leave of 0.1 was used to evaluate the presence/absence of ants, tree height, the type of herbivory, and the relative light levels as potential determinates of percent herbivory for *C. obtusifolia* individuals. As a result, a Mixed Model ANOVA was constructed with ant presence/absence and tree height as explanatory variables. A post-hoc Wilcoxon Sign-Rank test and a simple linear regression were used to evaluate the effect of each variable separately. A Wilcoxon Sign-Rank test was used to determine whether the species of ants inhabiting the trees had an

effect on the percentage of herbivory on the *C. obtusifolia* leaves. A Contingency Table Analysis was used to determine the effects of ant presence/absence on the number of vines and/or mosses on the *C. obtusifolia* trees.

The effect of ant presence/absence on *C. insignis* was evaluated subjectively rather than statistically due to low sample sizes in both categories.

All statistical analyses were conducted using JMP v. 5.1.2 software (SAS Institute 2004).

Results

Of 34 *C. obtusifolia*, fourteen had active ant colonies: four had *Crematogaster* spp., four had *Azteca xanthochroa*, four had *A. alfari*, and one had *A. constructor*. Of seven *C. insignis*, one was colonized by *A. constructor*. Two of the four juvenile *C. obtusifolias* that were cut open were each found to have one *A. constructor* queen, one tree had one *A. xanthochroa* queen, and one tree had two *A. xanthochroa* queens. None of these trees had workers present.

Only ant presence/absence ($p = 0.158$) and tree height ($p = 0.087$) entered the ANOVA model generated by the Stepwise Multiple Regression. Type of herbivory and light environment did not. When all *C. obtusifolia* trees were included in the analysis, both ant presence/absence and tree height were significant determinants of percent herbivory (ANOVA: $F = 3.739$, $df = 2$, $p = 0.036$; $R^2 = 0.205$; Effects test: ant presence/absence $p = 0.054$, height $p = 0.032$). A post-hoc Wilcoxon Sign-Rank test showed that ant presence/absence was having an effect on herbivory levels ($Z = -3.1$, $p = 0.002$). A post-hoc linear regression on percent herbivory versus height showed that height was having a slight effect on herbivory levels ($F = 3.13$, $df = 1$, $p = 0.087$; $R^2 = 0.095$).

After one outlier, a tall tree with high herbivory, was excluded, tree height ceased to be a predictor of percent herbivory ($p = 0.346$; Figure 2), while ant presence continued to be associated with reduced levels of herbivory ($p = 0.001$; Whole Model: $F = 6.848$, $df = 2$, $p = 0.0038$; $R^2 = 0.33$). Height did not affect the level of herbivory (Figure 1), but the herbivory levels were significantly lower when ant colonies were present (Figure 2).

The species of ant inhabiting *C. obtusifolia* trees with active colonies did not have

a significant effect on percent herbivory, with or without the outlier (Wilcoxon -Sign-Rank test including outlier: $\chi^2 = 2.72$, $df = 3$, $p = 0.438$. Wilcoxon Sign-Rank test excluding outlier: $\chi^2 = 3.59$, $df = 3$, $p = 0.31$. Table 1). Ant presence/absence was found to have a significant effect on the presence or absence of vines and mosses on *C. obtusifolia* (Chi-squared, Likelihood Ratio: $\chi^2 = 6.64$, $df = 2$, $p = 0.036$; $R^2 = 0.108$. Table 2). Trees with ant colonies had significantly less vines and mosses than did trees without ant colonies.

The one *C. insignis* tree with an active ant colony had much less herbivory than did the six trees where ants were absent (Mean of percent herbivory with ants = 0.116% ; mean of percent herbivory without ants = 7.09% \pm 7.55%).

Discussion

Interspecific mutualisms are interactions in which two species experience higher fitness when they occur together than when they occur independently (Bronstein 1998). Protective ant-plant interactions have been shown to benefit both the myrmecophytic plant and its specialized ants. In this study we examined the importance of *Azteca* ants to *Cecropia* at the La Selva Biological Station. *C. obtusifolia* with active ant colonies had significantly lower levels of herbivory than trees without ants, and the presence of ants significantly reduced the growth of vines and mosses on trees.

Contrary to our predictions, the species of ant present did not have an effect on herbivory levels in *C. obtusifolia*. However, we did find four trees inhabited by *Crematogaster spp.*, which are not usually considered an obligate mutualist with *Cecropia* in Costa Rica, although they, along with other genera (Yu and Davidson 1997), are found on some *Cecropia* elsewhere such as central Amazonia (Bonato et al. 2003) and the island of Hawaii (Wetterer 1997). We found that the *Azteca* ants were no more effective against preventing herbivory than the *Crematogaster* ants were.

Our qualitative assessment of available light—sunny, semi-shady, and shady—might not have been accurate enough to show an effect of available light on ant activity in the *Cecropias*, which might explain why our results were non-significant. Most of the *C. obtusifolia* (N = 22) were in shady or semi-shady lighting, while only twelve were in sunny areas. Increased light has been shown to increase the production of Müllerian

bodies and thus should make the tree more attractive to ants (Folgarait and Davidson 1994, Kersch and Fonseca 2005), but we did not find such a trend.

Tree height did not have an effect on percent herbivory. While it has been shown that trees are colonized by ants at a young age, and, therefore, a shorter height (Cindy Sagers, pers. comm.), the leaves we were testing were young—the second and third newest—and thus the herbivory they had experienced had to have been relatively recent compared to that of the older leaves. Taller trees should not have experienced more herbivory by default, since their ants had already been established and should have been protecting the tree since before leaves two and three appeared.

The presence of ants had no effect on the type of herbivory that the tree had undergone, although only one ant-inhabited tree out of thirteen had experienced *Atta* herbivory. This suggests that with a larger sample size, results may have shown that the trees occupied by ants had experienced significantly less *Atta* herbivory. However, we found that when ants are present on *C. obtusifolia*, they are effective against encroaching vines and mosses. When vines are present on trees, they increase the tree's chances of being pulled down along with other falling trees; mosses provide a stepping-stone on which vines and other epiphytes can grow (Van der Heijden et al. 2008).

While we did not find enough *C. insignis* to have a viable sample size, we found that the one individual with an *Azteca* colony had less herbivory than the mean of the other six individuals.

Experiments have shown that when *A. constructor* ants are removed from juvenile *C. obtusifolia*, their growth rates decrease and they experience significantly more herbivory and epiphytic growths than trees with active colonies (Schupp 1986). Shorter, vine-covered trees that have had their photosynthetic capabilities reduced would be more likely to have lower reproductive success; ant occupation may indeed influence survival until maturity (Schupp 1986). In this study the presence of ants significantly reduced herbivory levels and epiphytic growths, suggesting that the ants increase the fitness of the trees and are therefore beneficial members of a mutualism.

Our results suggest that the classic understanding of the *Cecropia-Azteca* mutualism still holds true, at least at the La Selva Biological Station in Costa Rica. When ants are present on *Cecropia*, the trees experience much lower levels of herbivory and

have less of a chance of being damaged by epiphytes—two factors that are debilitating to tree fitness and can decide whether or not the tree survives long enough to reproduce.

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Table 1: The species of ant present on *C. obtusifolia* did not cause a significant difference in percent herbivory on the trees. *Azteca* ants are the genus typically associated with *Cecropia*, especially in Costa Rica, though *Crematogaster* is known to inhabit the trees as well.

Species	Number of Trees	Mean Percent Herbivory	Std Dev
<i>Crematogaster</i> spp.	4	0.0331	0.0206
<i>A. Alfari</i>	4	0.0631	0.1158
<i>A. Constructor</i>	1	0.0355	0.0517
<i>A. Xanthochroa</i>	4	0.0168	0.031

Table 2: When ants were present on *C. obtusifolia*, a significantly lower number of vines and mosses were found growing on the trees. This suggests that the ants increase the overall fitness of the trees, as vines and mosses can physically damage the trees' structure.

	Vines/mosses Absent	Vines Present	Mosses Present
Ants Absent	10	4	7
Ants Present	11	0	2

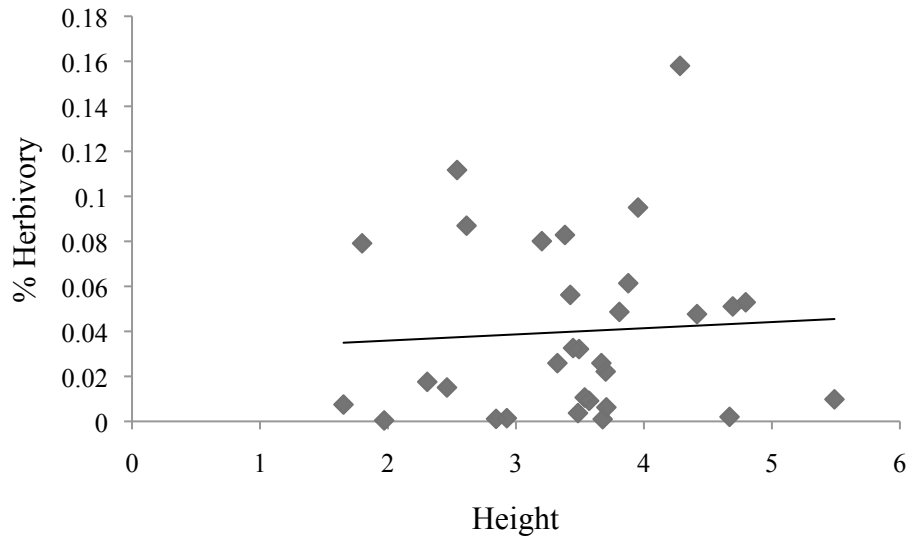


Figure 1: Percent herbivory on *C. obtusifolia* leaves as a function of juvenile tree height at La Selva Biological Station, Costa Rica. Height had no significant effect on herbivory levels. N = 31.

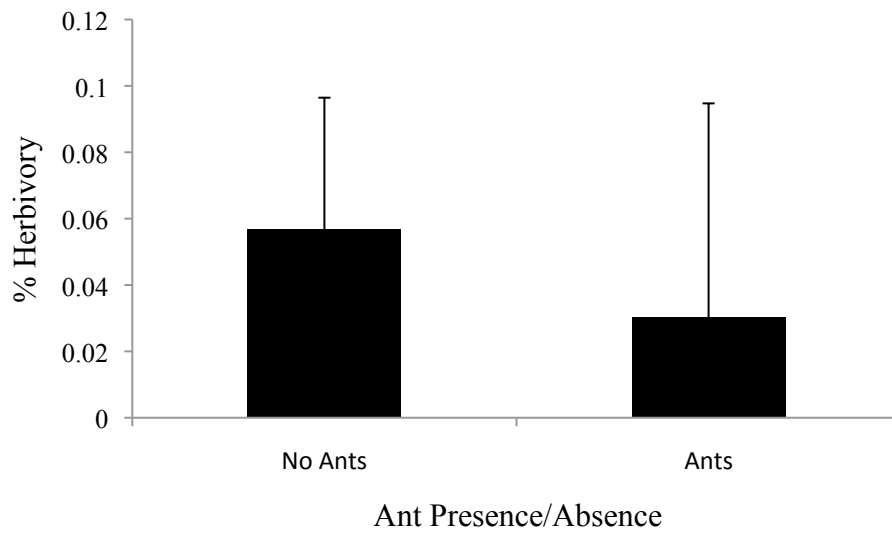


Figure 2: Comparison of herbivory levels in *C. obtusifolia* with and without active ant colonies. Error bars represent \pm SE.