Ant-tended Hemiptera in Amazonian myrmecophytes: Patterns of abundance and implications for mutualism function (Hymenoptera : Formicidae)

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Ant-Tended Hemiptera in Amazonian Myrmecophytes: Patterns of Abundance and Implications for Mutualism Function (Hymenoptera: Formicidae)

by

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ABSTRACT

We assessed how the abundance of ant-tended Hemiptera associated with two Amazonian myrmecophytes, *Tococa bullifera* and *Maieta guianensis*, varied as a function of resident ant species. We collected five species or morpho-species of adult hemiptera in the domatia of *M. guianensis*, with four of these species also found in *Tococa bullifera*. *Maieta guianensis* plants inhabited by *Crematogaster laevis* had over four-fold more hemiptera in them than plants inhabited by *Pheidole minutula*. In contrast, the density of hemiptera in *Tococa bullifera* domatia was independent of the species of ant resident. For each of the two ant species inhabiting *Maieta guianensis*, there was a positive and significant relationship between the abundance of hemiptera and workers inhabiting a plant. This relationship was also significant and positive for the *Tococa bullifera* plants inhabited by *C. laevis*. However, there was no relationship between *Azteca* worker and hemipteran density, although there was a trend towards a positive relationship. Our results indicate that hemipteran abundance can vary significantly between different myrmecophyte species, but that the nature of this relationship is mediated by the identity of the ant associate. Because hemipterans are herbivores, the costs and benefits of different ant partners to the host plant may vary in ways that are often overlooked.

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INTRODUCTION

Mutualistic associations between plants and ants have long been recognized as one of the defining features of tropical forests (Beattie 1985, Huxley & Cutler 1991). In some cases the mutualisms are facultative – ants may simply forage on leaf surfaces or feed from extrafloral nectaries, with varying degrees of specialization. However, many ant species obligately establish colonies in hollow thorns, swollen petioles, leaf pouches, or other specialized plant structures known as domatia. Because ant residents may confer benefits such as defense against herbivores, they have been the subject of considerable observational and experimental research (reviewed in Beattie 1985, Huxley & Cutler 1991, Bronstein 1998, Heil & McKey 2003).

Obligate ant-plant mutualisms almost always involve a third partner: scale insects and mealybugs (coccids and pseudococcids, respectively, both Hemiptera). These herbivores are often tended by ants, which use the "honeydew" they excrete as a food resource (Way 1963, Carroll & Janzen 1973). Their presence putatively has both costs and benefits to the host plant – they feed on sap and are potential vectors for plant diseases (McPheron & Chung-Kim 1993), but they can also influence the number of ants patrolling leaves via their influence on ant behavior and colony structure (Buckley 1987). They can therefore alter the outcome of plant-ant interactions along the continuum from mutualism to parasitism (Gaume *et al.* 1998).

There is an extensive body of literature on ant-plant-hemipteran interactions (reviewed in Way 1963, Buckley 1987), motivated in part by their importance as agricultural pests. However, few of these studies experimentally investigate the ecology of hemiptera associated with obligate domatia-dwelling ants (but see Gaume *et al.* 1998, Heckroth *et al.* 1998, Gaume *et al.* 2000, Itino *et al.* 2001, Gaume & McKey 2002). This is due primarily to the difficulty of conducting manipulations with domatia residents and the need for destructive sampling of ant colonies. An additional complicating factor is that we have only a limited understanding of the taxonomy, systematics, distribution, and abundance of most hemiptera found in ant-plants. Such data are an important prerequisite for investigating how hemiptera may influence the dynamics of ant-plant mutualisms.

In this study we investigated the relationship between the ants and hemiptera associated with two Amazonian myrmecophytes – *Maieta* *guianensis* and *Tococa bullifera* (both Melastomataceae). As with most ant-plants, these species are associated with a suite of ant species (Fowler 1993, Fonseca & Ganade 1996) though each individual plant is colonized by a single species at a time (Vasconcelos and Davidson 2000). Our previous work in these systems has demonstrated important interspecific variation in several aspects of plant-ant interactions (Lapola *et al.* 2003, Bruna *et al.* 2004), and we expect the same is probably true of the relationship between ants, plants, and hemiptera as well. With this in mind, we addressed the following three questions: First, what hemiptera species are associated with *T. bullifera* and *M. guianensis*? Second, does hemipteran abundance vary with respect to the identity of host-plants and their ant residents? Third, does the abundance of hemiptera vary with respect to the ant-worker abundance?

MATERIALS AND METHODS

Study site and system

Fieldwork was conducted from January to February 2004 in Reserve 1501 of the Biological Dynamics of Forest Fragments Project (BDFFP), located c. 70 km north of Manaus (2° 25'S, 59" 48'W). This reserve of approximately 800 ha is embedded in more than 10,000 ha of continuous *terra firme* rain forest. Annual precipitation ranges from 1900-3500 mm, with a distinct dry season from May-December. For a complete description of the site see Bierregaard *et al.* (2002).

Maieta guianensis and *Tococa bullifera* are two of the sixteen species of myrmecophytes that have been identified in Reserve 1501 (Fonseca & Ganade 1996, Bruna *et al.* 2005). Both are understory shrubs, reaching heights of 2 and 3 m, respectively, with swollen pouches at the bases of leaves in which ant queens establish colonies. In our study sites, approximately 30% of the *T. bullifera* individuals and 14% of the *M. guianensis* individuals are occupied by *Crematogaster laevis*. The remaining 86% of *M. guianensis* and 70% of *Tococa bullifera* are occupied by *Pheidole minutula* and an unidentified species of *Azteca*, respectively (Vasconcelos & Davidson 2000).

Data collection and statistical analysis

To elucidate the relationship between plants, ants, and hemiptera, we collected five randomly selected domatia from 10 *T. bullifera* and 10 *M. guianensis* inhabited by each ant species (total N = 40 plants). For each plant we counted (a) the total number of ant workers in the five domatia and (b) the total number of number of adult hemiptera. We identified hemiptera to species or morpho-species; voucher specimens are deposited in the entomological collection of Brazil's Instituto Nacional de Pesquisas da Amazônia (INPA).

To compare the abundance of (a) hemiptera in plants inhabited by each ant species and (b) ant workers of different species inhabiting the same plant species, we summed the number of hemiptera and ant workers in the five domatia collected from each plant and compared the total abundance using t-tests. We log-transformed all totals prior to analysis to meet the assumptions of parametric statistics, but throughout the manuscript we present back-transformed values. Each plant species was compared separately. To test for a relationship between hemipteran abundance and worker abundance (both log-transformed) we used simple linear regression. Each plant *versus* ant species combination was compared separately.

RESULTS

We collected five species or morpho-species of adult hemiptera in the domatia of *M. guianensis*: *Nipaecoccus* morpho-species 1, *Nipaecoccus* morpho-species 2, *Dysmicoccus* morpho-species 1 (all Pseudococcidae), *Coccus viridis*, and *Coccus hesperidum* (both Coccidae). Although the identification of immature individuals is difficult, we also collected immature individuals that we are provisionally assigning to the genus *Alichtensia* and the family Margarodidae. All of these species were also found in the domatia of *T. bullifera*, with the exception of *Nipaecoccus* morpho-species 1.

Maieta guianensis plants inhabited by *Crematogaster laevis* had over four-fold more hemiptera in them than plants inhabited by *Pheidole minutula* (231.6 ± 88.07 SE vs. 49.1 ± 6.63 SE, respectively, Fig. 1A), a highly significant difference (t=3.301, df = 18, P = 0.004). In contrast, the density of hemiptera in *Tococa bullifera* domatia was independent of the species of ant resident (t=0.498, df = 18, $P = 0.62$). The average abundance of hemipterans in plants inhabited by *C. laevis* was 82.1 ± 22.22 SE, while in plants inhabited by *Azteca* sp. it was 94.5 ± 26.78 SE (Fig. 1A).

The summed abundance of *C. laevis* workers collected from the domatia of *T. bullifera* plants was over 1.5 times that of *Azteca* sp. workers (295.8 ± 41.48 SE vs. 186.6 ± 52.43 SE, respectively, Fig. 1B). A similar pattern was seen in *Maieta guianensis* – plants with *C. laevis* colonies in them had twice the number of workers in domatia as those inhabited by *P. minutula* (208.5 ± 28.33 SE vs. 130.8 ± 16.28 SE, respectively). However, the difference in worker abundance was only significant for *T. bullifera* (*T: bullifera*: t = 2.27, df = 18, $P = 0.04$; *M. guianensis*: t = 0.63, df = 18, $P = 0.09$, Fig. 1B).

For each of the two ant species inhabiting *Maieta guianensis*, there was a positive and significant relationship between the abundance of hemiptera and workers inhabiting a plant (*C. laevis*: MS = 0.849, $F_{1,8}$=

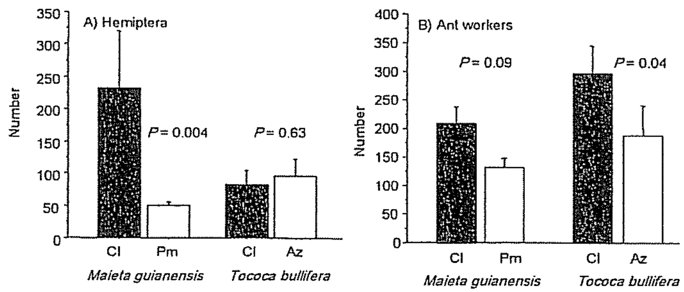

Fig. 1. Summed abundance of (A) hemiptera and (B) ant workers in five domatia inhabited by different ant species colonizing the Amazonian myrmecophytes *Maieta guianensis* and *Tococa bullifera*. Comparisons for each plant species were conducted separately (see text for details).

6.07, $P = 0.04$, $R^2 = 0.43$; *P. minutula*: MS=0.849, $F_{1,8}= 6.07$, $P = 0.04$, $R^2 = 0.43$, Fig. 2A). This relationships was also significant and positive for the *Tococa bullifera* plants inhabited by *C. laevis* (MS=1.88, $F_{1,8}$=6.69, P= 0.04, R^2 = 0.46, Fig. 2B). However, there was no relationship between *Azteca* worker and hemipteran density, although there was a trend towards a positive relationship (MS=1.56, $F_{1,8}$=2.40, P= 0.16, R^2 = 0.23).

DISCUSSION

Domatia-dwelling hemiptera are a common partner in obligate ant-plant mutualisms. The ecology of these interactions continues to be understudied, however, particularly in the Neotropics. While studies of *Macaranga* trees in southeast Asia have suggested that some hemipteran species may have obligate associations with single plant species (Heckroth *et al.* 1998), two of the species we collected – *Coccus hesperidum* (i.e., the soft brown scale) and *Coccus viridis* (i.e., the green soft scale) – have wide host ranges and cosmopolitan geographic distributions (Dekle & Fasulo 2001). Much less is known regarding the host-plant preference and geographic range of the other genera we collected (e.g., *Nipaecoccus*, *Dysmicoccus*), despite their importance as agricultural pests (reviewed in Ben-Dov & Hodgson 1997). Comprehensive surveys of the hemiptera associated with Neotropical ant-plants, such as those Heckroth *et al.* (1998) have conducted with *Macaranga*, are clearly needed.

Plant identity, ant identity, and the abundance of hemiptera

Our results indicate that hemipteran abundance can vary significantly between different myrmecophyte species, but that the nature of

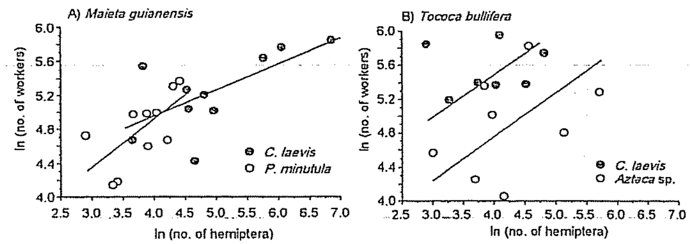

Fig. 2. Relationship between hemipteran and ant worker abundance in (A) *Maieta guianensis* and (B) *Tococa bullifera* inhabited by different ant species. For *M. guianensis* the linear regression equations describing the relationships are: ln(no. of *P. minutula* workers) = 2.62 + 0.57 * ln(no. of hemiptera), R^2 = 0.44; ln(no. of *C. laevis* workers) = 3.72 + 0.31 * ln(no. of hemiptera), R^2 = 0.43 For *T. bullifera* the equations are: ln(no. of *Azteca* sp. workers) = 2.67 + 0.52 * ln(no. of hemiptera), R^2 = 0.23; ln(no. of *C. laevis* workers) = 3.49 + 0.50 * ln(no. of hemiptera), R^2 = 0.46.

this relationship is mediated by the identity of the ant associate. *Maieta guianensis* plants inhabited by *C. laevis* colonies had four times more hemiptera in them than those inhabited by *P. minutula*. This suggests *C. laevis* is more heavily dependent on hemipteran honeydew as a food resource than *P. minutula*, a conclusion that is the consistent with the results of previous work conducted in this system. Vasconcelos and Davidson (2000) found that the proportion of nitrogen in *C. laevis* workers was lower than in workers of *P. minutula*, indicating animal protein made up a lower proportion of their diet. Because as much as 25% of a *C. laevis* colony may be in satellite nests in twigs at the base of the plant (Vasconcelos, unpubl. data) some of the honeydew collected in domatia may also be going to workers without access to hemipterans.

Why then is the number of hemiptera tended by *C. laevis* so much lower when this species inhabits *T. bullifera*? At least three non-mutually exclusive mechanisms could explain these results. First, insect prey may be more abundant on the larger *T. bullifera* plants than on *M. guianensis* (e.g., *T. bullifera* may be more "apparent", sensu Feeny 1976) thereby reducing the dependence of *C. laevis* on hemipteran honeydew. Alternatively, the glandular trichomes on the interior surface of *T. bullifera*'s domatia may serve as an alternative source of carbohydrates (Roth 1976), thereby reducing the need to tend large numbers of hemiptera. Finally, we cannot discount the possibility that our results reflect the relatively limited number of colonies we sampled (N = 10 for each species x host plant combination). It may be that hemipteran abundance varies as a function of colony characteristics we did not control (e.g., colony age, microhabitat), and that increased sampling would reduce the disparity between *C. laevis* colonies in different host-plant species. Regardless of the mechanism, our results suggest the relationship between domatia-inhabiting ants and hemiptera may be context-dependent in ways that have previously remained unexplored. While earlier comprehensive studies (e.g., Fonseca 1993, Gaume *et al.* 1998, Gaume *et al.* 2000, Gaume & McKey 2002) have investigated the causes and consequences of variation in hemipteran abundance within a single plant species, most plant-ants are actually associated with a suite of geographically co-occurring taxa (e.g., Heckroth *et al.* 1998). Our results clearly indicate that conclusions from studies conducted with one plant species may not readily be generalized to others, even when these species are closely related.

Is ant colony size limited by hemipteran abundance?

The size of ant colonies in myrmecophytic plants are thought to be regulated by two principal factors: nesting space (i.e., domatia volume) or the availability of food (Hölldobler & Wilson 1990, Gaume *et al.* 1998). Previous studies investigating how honeydew, food bodies, or other food resources influence the size of ant colonies associated with myrmecophytes have drawn contradictory conclusions. While Fonseca (1993) found the volume of domatia most strongly limited colony size, other have found hemiptera appear to exert a strong influence on colony size and behavior (Gaume *et al.* 1998, Gaume & McKey 2002). These patterns may in part be influenced by the source of nutritional resources – it has been argued that when the plant indirectly provides nutritional resources (i.e., via hemiptera), colonies are more likely to be limited by space rather than food (Gaume *et al.* 1998). The results of our analyses, however, are not consistent with this hypothesis. In three of four ant species x plant species combinations, there was a positive and significant relationship between ant worker abundance and hemipteran abundance. This suggests that in contrast to other myrmecophytic systems in our study sites (Fonseca 1993), the abundance of hemiptera and other food sources could potentially limit colony size. However, other factors are clearly important, since hemipteran abundance explained only 43-46% of the variance in worker number. We suggest future studies investigating the factors limiting plant-ant colony size move beyond the correlative framework used in this and other studies, and instead conduct experimental manipulations of food abundance and colony size.

Finally, interspecific variability in hemipteran tending has importance consequences for plants as well. Because mealybugs and scales are herbivores, the maintenance of high densities by ant colonies could

conceivably have costs for plant growth and reproduction. Our results suggest these costs would be similar for *T. bullifera* plants irrespective of their ant inhabitant, since both *C. laevis* and *Azecta* sp. tended similarly low numbers of hemiptera. However, the disparity in hemipteran abundance observed in *M. guianensis* suggests they may incur much higher costs when hosting *C. laevis* colonies than when inhabited by *P. minutula*. These costs include direct costs from hemipteran herbivory, as well as indirect costs resulting from reduced patrolling of leaves by ants (sensu Gaume *et al.* 1998). Such variable costs are frequently overlooked in studies evaluating the benefits to plants of their ant associates, and could conceivably diminish the purported benefits of ant colonies substantially.

CONCLUSION

Our study is one of a limited number to have compared the abundance of hemiptera found in the domatia of different myremcophytic plant species (see also Heckroth *et al.* 1998, Itino *et al.* 2001). We found that the density of hemiptera varies within and between plant species, and that this can be a function of the identity of ant colonists. As such, the costs and benefits of different ant partners to the host plant may vary in ways that are often overlooked. This may help explain the observed disparities in colonization frequencies by the different ant species associated with ant-plants (Longino 1989, Fonseca & Ganade 1996, Bruna *et al.* 2005). Previous work has focused almost exclusively on ant defensive behavior, habitat specificity, and tradeoffs between competitive ability and queen dispersal as the principal drivers of distribution patterns (e.g., Yu & Davidson 1997, Stanton *et al.* 2002, Bruna *et al.* 2004). We propose the ubiquitous intermediate trophic level could be playing a critical role as well. We also suggest studying the ecology of coccids and pseudococcids may yield insights into the post-colonization dynamics of ant-plant mutualisms. The chemical composition of the honeydew ants consume can vary as a function of the location on the plant where insects are feeding, plant age, plant species, and season (reviewed in Way 1963, Molyneux *et al.* 1990). If variation in honeydew composition influences the intensity of ant-tending, it may indirectly influence ant patrolling behavior and hence levels of herbivory. Finally, we end by encouraging other researchers working on tropical ant-plant mutualisms to at the very least collect voucher specimens of the coccids and pseudococcids inhabiting ant-plants. Information on the geographic distribution of these cryptic insects and their host-plant associations is a necessary first step in elucidating their impacts on the dynamics of ant-plant mutualisms.

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