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Protection Mutualisms and the Community: Geographic Variation in an Ant-Plant Symbiosis and the Consequences for Herbivores

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Abstract

Protection mutualisms mediate trophic interactions in many systems, but their effects on the surrounding community are rarely studied. Ant-plant symbioses are classic examples of protection mutualisms: myrmecophytic plants provide nesting space and food for symbiotic ants in exchange for ant defense. Ant defense should thus reduce the abundance of herbivores, but studies of ant-plant symbioses usually measure damage to the plant without quantifying the herbivores themselves. In this study, we investigated whether geographic variation in the quality of ant defense in a symbiotic mutualism between *Cordia alliodora* trees and *Azteca* ants was associated with the abundance and species richness of plant herbivore communities. In three tropical-dry-forest sites in Middle America, we found that the density of *Azteca* ants within trees was negatively associated with the levels of leaf herbivory. At sites where ants were effective tree defenders, tree herbivores were less abundant and herbivore assemblages on trees exhibited lower species richness than at a site where ants were poor defenders. In addition, in a site where ants reduced herbivory, herbivore communities were less abundant and diverse in the presence of ants than in their absence, where as in a site where ants did not reduce herbivory, there were no differences in herbivore abundance or richness between trees with or without ants. We conclude that geographic variation in the quality of ant defense drives variation in myrmecophytic-plant herbivore communities. Moreover, ant-plant protection mutualisms should have important but rarely considered effects on herbivore population dynamics and food-plant specialization.

Introduction

Mutualistic interactions between species can strongly affect the structure of ecological communities (Stachowicz, 2001; Bruno et al. 2003; Hay et al., 2004). For example, animal seed dispersal can determine the composition of tropical-tree communities (Terborgh et al., 2008), and pollinator preferences can favor the spread of certain plant species (Chittka and Schürkens, 2001). Protection mutualisms, in which a mutualist defends its partner against natural enemies, are frequently shown to affect the growth and fitness of individual organisms, but very little is known about how these interactions structure the communities in which they are embedded. The paucity of such studies may give the false impression that the community effects of protection mutualisms are rare

or unimportant, when in fact the studies so far suggest that protection tends to have strong effects on local diversity and abundance. Interestingly, it has been suggested that the overall effect of protection is to reduce local species diversity (Rudgers and Clay, 2008; Rudgers et al., 2010; but see Jani et al., 2010), which contrasts with other well-known mutualisms that clearly enhance local diversity (e.g., fig trees, corals) (Terborgh, 1986; Stachowicz, 2001; Hay et al., 2004). Such community consequences have been so little studied, however, that we do not know how general such local effects may be, or how these mutualisms affect systems on larger spatial and temporal scales.

In terrestrial communities, ant protection frequently determines the strength of trophic cascades (*sensu* Hunter and Price, 1992). Ants both decrease herbivory as mutualists



of plants by predated and harassing herbivores (Hölldobler and Wilson, 1990) and increase herbivory as mutualists of honeydew-producing Hemiptera by protecting them from other predators (Way, 1963; Del-Claro and Oliveira, 2000). In both mutualistic roles, ant protection can reduce the diversity and abundance of other arthropods in a system, but the strength of these effects varies among taxa and depends on environmental context (Fowler and Macgarvin, 1985; Wimp and Whitham, 2001; Kaplan and Eubanks, 2005; Oliveira and Del-Claro, 2005; Mooney, 2007; Rudgers et al., 2010). Indeed, although ant-plant interactions tend to have positive effects on individual plants (Styrsky and Eubanks, 2007; Chamberlain and Holland, 2009), which suggests that ants should reduce the abundance, if not also the diversity, of untended herbivores on those plants, these effects are often non-significant at both plant- and experimental-plot-scales (Kaplan and Eubanks, 2005; Mooney, 2007; Rudgers et al., 2010). This is inconsistent with the evidence that ants reduce herbivory levels (Chamberlain and Holland, 2009; Styrsky, 2007) unless most herbivores collectively consume very little and ants defend only against the few herbivores that inflict the most damage. Whether or not such conditions are generally true, they raise interesting and mostly unanswered questions about the relative abundances of herbivore species in these systems, and about the relative specialization of those species on ant-defended food plants.

The strength of any given ant-plant interaction is geographically variable. The identities and abundances of ants, herbivores, and even other predators vary over space and affect how much ants benefit plants (Bronstein, 1994; Rudgers and Strauss, 2004; Mooney, 2007; Rosumek et al., 2009). Geographic variation in the effectiveness of ant defense will also affect food availability for herbivores of a given plant, with potential cascading effects on herbivore population sizes and even food-plant specialization (i.e., in a geographic mosaic of coevolution, sensu Thompson, 2005).

In one of the few studies to examine the community effects of a geographically variable ant-plant interaction, Rudgers et al., (2010) reported that, in three Arizona populations of wild cotton, there was a positive relationship between how much ant presence within a site increased plant fitness and influenced arthropod community composition. Geographic variation in the effects of ants in that system resulted from differences in total ant abundance and the relative abundance of a particularly aggressive ant species (Rudgers and Strauss, 2004). Surprisingly, however, even at the site where ants produced the greatest benefits for plants, by decreasing the abundance of a specialist caterpillar (Rudgers and Strauss, 2004), ants did not reduce the per-plant abundances of total arthropods or generalist herbivores (Rudgers et al., 2010).

Ant-plant symbioses are specialized mutualisms: myrmecophytic plants supply ants with nesting space and food, and ants defend plants against herbivores (Davidson and McKey, 1993; Heil and McKey, 2003). Such highly specialized ant-plant interactions should consistently reduce the

abundances of the herbivores that eat the plant. Yet although ants' capacity to reduce herbivory and increase plant fitness in such systems is well documented (Chamberlain and Holland, 2009; Rosumek et al., 2009), and some studies have contrasted the effects on plants of different herbivore species (e.g., Frederickson 2005; Palmer and Brody, 2013), very few studies have investigated the community of herbivores associated with a given myrmecophyte (but see, e.g., Janzen, 1967; Gaume et al., 1997; Itino and Itioka, 2001). This lacuna probably results in part from the difficulty of identifying insect larval forms, which often inflict most of the leaf damage in the diverse tropical forests where myrmecophytes are common (Davidson and McKey, 1993). Surmounting this difficulty will be essential for understanding the community role played by these classic study systems.

The myrmecophytic tree *Cordia alliodora* (Ruiz & Pavón) Oken (Boraginaceae) hosts colonies of *Azteca* ants (Hymenoptera: Formicidae: Dolichoderinae) in Neotropical forests. Ants nest in hollow swellings, known as domatia, at stem nodes, and tend honeydew-producing scale insects (Hemiptera: Coccidae and Pseudococcidae) that feed on plant phloem from inside the domatia. *Azteca* ants can reduce folivory on *C. alliodora* (Tillberg, 2004; Trager and Bruna, 2006; Pringle et al., 2011), but the magnitude of these effects depends on the size of the ant colony, the size of the tree, and geographic location (Pringle et al., 2011; Pringle et al., 2012a). There have been few studies of *C. alliodora* herbivores, but many of the lepidopteran larvae that eat the tree's leaves in Middle America can be identified by comparison with a database of caterpillars and their food plants from northwestern Costa Rica (Janzen and Hallwachs, 2009).

In this study, we investigated geographic variation in *C. alliodora* herbivore communities. We investigated variation in the density of ants within and among *C. alliodora* trees and in the effectiveness of ant defense in three sites in Middle America. By conducting surveys of the herbivore assemblages on individual trees, we asked whether the abundance and species richness of known *C. alliodora* herbivores were negatively associated with the density of ants and the effectiveness of ant defense at a site. We then asked whether trees occupied by *Azteca* ants supported lower abundance and richness of known herbivores than unoccupied trees within each of two sites that differed in the typical effectiveness of ant defense. Finally, we consider the implications of our results for herbivore population dynamics and food-plant specialization.

Material and Methods

Study sites and system

The study was conducted in three tropical dry forests located on the Pacific Coast of Middle America: the Chamaela-Cuixmala Biosphere Reserve, Jalisco, Mexico (19°30' N, 105°02' W), Huatulco National Park, Oaxaca, Mexico (15°42' N, 96°10' W), and the Area de Conservación Guana-

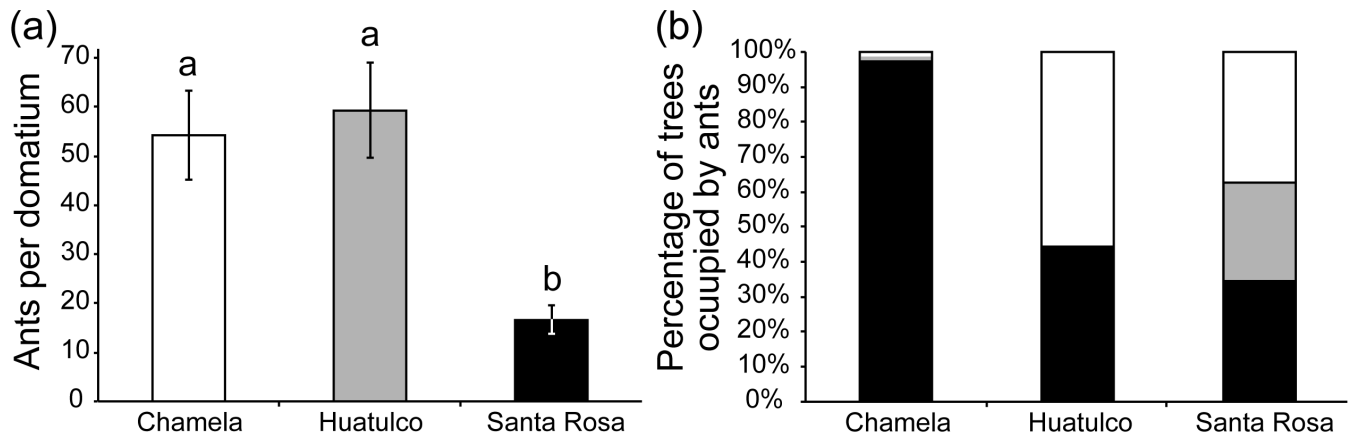


Figure 1. (a) Number of worker ants per tree domatium (mean \pm SE) averaged for all domatia within trees at the three sites (Chamela $N = 19$, Huatulco $N = 6$, Santa Rosa $N = 20$), listed from north to south. Different letters indicate significant differences by ANOVA and Tukey-Kramer HSD ($P < 0.008$). (b) Percentage of *C. alliodora* trees occupied by *Azteca* ants in surveys of trees at the three sites (Chamela $N = 225$, Huatulco $N = 91$, Santa Rosa $N = 226$). Black portions of the bar indicate trees occupied by *Azteca* spp.; gray portions indicate trees occupied by other ant species (*C. setulifer*, *Crematogaster* spp., *P. viduus*); white portions indicate trees unoccupied by ants.

caste, Sector Santa Rosa, Guanacaste, Costa Rica (10°50' N, 85°36' W). These three sites (henceforth Chamela, Huatulco, and Santa Rosa) are all characterized by strong rainfall seasonality: the annual 4–6 mo rainy season is followed by an intense dry season (Bullock, et al., 1995). Average annual precipitation between 1979 and 2009 was 778.5 mm in Chamela, 1033.5 mm in Huatulco, and 1686.4 mm in Santa Rosa, according to records from meteorological stations associated with the protected areas in Chamela and Santa Rosa and with nearby towns in Huatulco.

Cordia alliodora is common throughout the Neotropics, from Mexico to Argentina, including the Caribbean, and populations have recently been introduced to East Africa and the South Pacific (Dawson et al., 2008). The most common ant symbiont throughout Middle America is the *C. alliodora* specialist *Azteca pittieri* Forel, but other ant species do colonize the tree, especially at lower latitudes (Longino, 1996; Pringle et al., 2012b). Notable among these other species are *Azteca beltii* Emery, which is a generalist live-stem nester and the second-most-common *Azteca* species found in *C. alliodora* after *A. pittieri*, and *Cephalotes setulifer* Emery (Hymenoptera: Formicidae: Myrmicinae), which is the second-most-common *C. alliodora* specialist after *A. pittieri*, and whose known range extends from El Salvador and Honduras to Panama (Longino, 1996). *Cephalotes setulifer* provides *C. alliodora* with little or no defense against herbivores (Tillberg, 2004). Because it is difficult to distinguish *A. pittieri* workers from those of other *Azteca* species in the field (Longino, 2007), we will refer to *Azteca* ants throughout the rest of the manuscript by the genus name only. However, genetic data gathered from random samples of colonies in the field indicate that these ants are usually *A. pittieri* at all three sites (Pringle et al., 2012b).

Azteca density estimates

To estimate the density of *Azteca* ants in trees, a variable that is positively related to the effectiveness of ant defense against herbivores (Pringle et al., 2011), we divided the number of ants in a tree by the number of domatia counted or estimated for that tree. We counted the number of ants and domatia for six entire trees (of heights 1.6–4.5 m) at each of the three sites by cutting trees down, collecting domatia in press-seal bags, and opening all domatia to count ants. We estimated the number of ants and domatia in 14 additional trees (of heights 2–8 m) in Chamela and Santa Rosa. Estimates were made from counts of the ants in three domatia and diameter measurements of the trees (for more details, see Pringle et al., 2011). We tested whether ant density varied among sites with an ANOVA that treated site as a fixed factor and post-hoc Tukey-Kramer HSD.

Survey of tree ant occupants

We conducted a survey of tree ant occupants to determine the proportion of trees at each site occupied by *Azteca* spp. In Chamela and Santa Rosa, three 0.5-ha tree plots were established in July–August, 2007; all *C. alliodora* individuals were located within these plots, and the ant occupant was identified when possible (Santa Rosa $N = 170$ of 302 total trees, Chamela $N = 117$ of 166 total trees). When trees were too tall to examine domatia directly, we shook the tree vigorously and examined the branches for ants with binoculars. Trees in which no ants were visible were recorded as unknowns and were not included in the analysis. At all three sites, we also surveyed trees along the trail system, looking for *C. alliodora* individuals whose branches were low enough to examine domatia for the ant occupant(s) (Chamela $N = 108$,

Huatulco $N = 91$, Santa Rosa $N = 56$). Trees were recorded as unoccupied by ants only when an extensive examination of domatia revealed no ant entrance holes.

Herbivory estimates

To compare the leaf herbivory experienced by trees in the presence of *Azteca* spp. between sites, we measured standing levels of herbivory. Levels of herbivory were estimated on $N = 40$ trees in Chamela in August 2008, $N = 27$ trees in Huatulco in July 2009, and $N = 41$ trees in Santa Rosa in June 2008. Herbivory was estimated by E.G. Pringle on ~100 leaves from ≥ 3 branches per tree of 2-8 m height using a standardized index (Dirzo and Domínguez, 1995). Leaves were categorized by eye according to the following levels of leaf area eaten: 0 = 0%, 1 = 1-6%, 2 = 6-12%, 3 = 12-25%, 4 = 25-50%, 5 = 50-100%. The number of leaves in each category was multiplied by its category value, and the sum of these products was divided by the total number of leaves to generate the index. Because herbivory is positively related to tree size in this system (Pringle et al., 2012a), differences among sites were analyzed with an ANOVA, in which site was treated as a fixed factor and tree basal diameter as a covariate, followed by a post-hoc Tukey HSD test.

Herbivore surveys

To investigate differences in the abundance and species richness of *C. alliodora* herbivore communities at the three sites, we conducted surveys in the early rainy season in June-August 2009 and 2010. Because herbivory in tropical dry forests is highest early in the rainy season (Janzen, 1988; Dirzo and Domínguez, 1995), herbivore surveys were conducted within the first third of the rainy season at each site in both years. In both years, we surveyed ~40 trees of 3 m average height (height ranges: Chamela 1.3-7.5 m; Huatulco 1.2-12.0 m; Santa Rosa 0.9-8.5 m) haphazardly chosen along the trail system at each site (Chamela: 2009 $N = 43$, 2010 $N = 40$; Huatulco: 2009 $N = 53$, 2010 $N = 39$; Santa Rosa: 2009 $N = 38$, 2010 $N = 39$).

Surveys were performed by visually scanning the tree, and particularly the undersides of the leaves, for 5 min. We searched the leaves from the ground, from a 2-m ladder, and with binoculars if necessary. Tree size (basal diameter) was used as a covariate in all analyses. Surveys were conducted simultaneously by three investigators, except in Santa Rosa in 2009 and Chamela in 2010, when, for logistical reasons, they were conducted by two investigators.

Herbivores were identified to order and morphospecies were assigned to individuals on each tree. Lepidopteran larvae were identified to genus, and to species when ~3rd instar or larger, based on the database of Janzen and Hallwachs (2009). In addition, larvae of *Coptocyclus leprosa* Boheman (Coleoptera: Chrysomelidae) beetles are *C. alliodora* foli-

vores (Trager and Bruna, 2006) and are common and easily identified at all sites.

All arthropods seen during the survey were identified as non-herbivorous (predators, scavengers) or potentially herbivorous (*i.e.*, omnivorous insects were classified as potential herbivores); potentially herbivorous morphospecies were then hierarchically classified as: (1) herbivorous arthropods and (2) known *C. alliodora* herbivores. The second category was based on food-plant records of Janzen and Hallwachs (2009), which come in part from the Santa Rosa site, and on personal observations by E.G. Pringle.

The ant occupant of each tree used for an herbivore survey was identified by examining domatia. In Santa Rosa, trees that were occupied by both *Azteca* spp. and *C. setulifer* were treated in analyses as trees occupied by *Azteca*. In Chamela, all survey trees were occupied by *Azteca* spp.; in Huatulco and Santa Rosa, some survey trees were not occupied by *Azteca* spp. (Huatulco: 2009 $N = 18$, 2010 $N = 9$; Santa Rosa: 2010 $N = 19$). Because we were interested in how geographic variation in *Azteca* defense affects herbivore communities, only trees occupied by *Azteca* spp. were included in the analysis of differences in herbivore communities between sites. Analysis was conducted using a nested ANOVA, in which site and year nested within site were treated as fixed factors and tree size was included as a covariate, followed by post-hoc Tukey HSD.

To test whether differences in herbivore communities between sites were caused in part by differences in ant defense between sites, and not just by other environmental variables, we also compared herbivore communities between *Azteca*-occupied trees and unoccupied trees within Huatulco and within Santa Rosa.

Stepwise regression of ant occupant and survey year with the Huatulco data indicated that survey year was non-significant in all cases (herbivorous arthropod abundance $P = 0.3$, *C. alliodora* herbivore abundance $P = 0.6$, *C. alliodora* herbivore richness $P = 0.08$), so Huatulco data from 2009 and 2010 were pooled for this analysis. The analysis of Santa Rosa data included only the 2010 survey, because we did not survey unoccupied trees in 2009. Differences between *Azteca*-occupied trees and unoccupied trees were assessed with an ANOVA, in which ant occupant was the factor and tree size was included as a covariate.

Statistical software

All analyses were conducted with JMP® Pro 10.0 (SAS Institute Inc. 2010).

Results

The density of *Azteca* ants within trees and of *Azteca* colonies among trees varied significantly between sites. Ant workers nested at higher densities within trees in Chamela and Huatulco than in Santa Rosa (Fig 1a; ANOVA $F_{2,42} = 10.19$,

$P < 0.0002$; Tukey-Kramer HSD $P < 0.008$). A higher proportion of trees contained colonies of *Azteca* spp. in Chamela (97.3%) than in Huatulco (44.0%) or Santa Rosa (34.5%) (Fig 1b). Chamela contained the fewest trees completely unoccupied by ants (1.3%), and there were fewer completely unoccupied trees in Santa Rosa (37.6%) than in Huatulco (56.0%) (Fig 1b). In Chamela, 1.3% of trees were occupied by *Crematogaster crinosa* Mayr; in Santa Rosa, 25.7% of trees were occupied by *C. setulifer* alone (an additional 4.0% of trees contained both *C. setulifer* and *Azteca* spp., although this is probably an underestimate because not all of the domatia were examined in any given tree (Longino, 1996)), 1.3% of trees were occupied by *Crematogaster curvispinosa* Mayr, and 0.9% of trees were occupied by *Pseudomyrmex viduus* Smith.

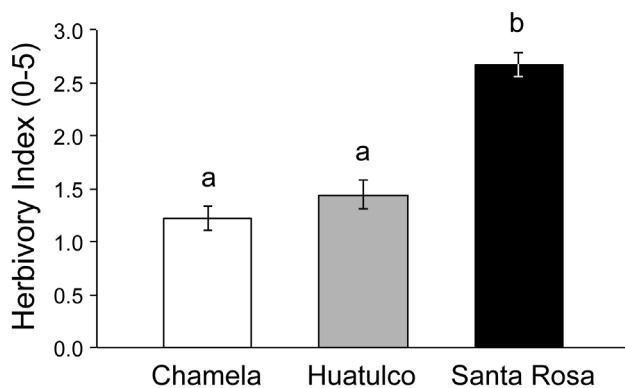


Figure 2. Standing herbivory level based on an index of percent leaf area eaten (0-5; mean \pm SE) on trees occupied by *Azteca* ants at the three sites (Chamela $N = 40$, Huatulco $N = 27$, Santa Rosa $N = 41$). Different letters indicate significant differences by a mixed-effect ANOVA and Tukey HSD ($P < 0.05$).

Azteca-occupied trees experienced less herbivory in sites where ants occupied trees at higher densities. Standing levels of herbivory in *Azteca*-occupied trees were significantly lower in Chamela and Huatulco than in Santa Rosa (Fig 2; ANOVA: $F_{3,104} = 38.07$, $P < 0.0001$, site: $F = 41.53$, $P < 0.0001$, tree basal diameter: $F = 7.05$, $P < 0.01$).

The abundance and richness of herbivores found on *C. alliodora* trees was significantly higher in Santa Rosa, the site where ants occupied the tree at the lowest densities and appeared to be the least effective plant defenders. Herbivores belonged to the orders Coleoptera, Hemiptera, Lepidoptera, Orthoptera, and Phasmida. The composition of herbivorous arthropod orders varied between sites (Table 1); the proportion of individuals from Coleoptera and Lepidoptera was highest in Santa Rosa.

The abundance of herbivorous arthropods was greater in Santa Rosa than in the other two sites (Fig 3a; ANOVA $F_{6,198} = 9.25$, $P < 0.0001$, site: $F = 13.51$, $P < 0.0001$, year[site]: $F = 9.32$, $P < 0.0001$, tree basal diameter: $F = 2.73$, $P = 0.1$). In addition, both the abundance and the species richness of known *C. alliodora* herbivores per tree was higher in Santa Rosa than

in either Chamela and Huatulco (Fig 3b-c; abundance: ANOVA $F_{6,198} = 13.35$, $P < 0.0001$, site $F = 20.48$, $P < 0.0001$, year[site] $F = 14.17$, $P < 0.0001$, tree basal diameter $F = 3.00$, $P = 0.08$; richness: ANOVA $F_{6,198} = 22.67$, $P < 0.0001$, site $F = 31.93$, $P < 0.0001$, year[site] $F = 27.27$, $P < 0.0001$, tree basal diameter $F = 3.89$, $P < 0.05$).

In all cases, the significant effect of survey year was driven by Santa Rosa, not by the other two sites. Santa Rosa

Table 1. Percentage of the total herbivorous arthropods found at each site on *Azteca*-occupied *C. alliodora* trees belonging to each of five insect orders.

Site	N	Orders of herbivorous arthropods				
		Coleoptera	Hemiptera	Lepidoptera	Orthoptera	Phasmida
Chamela	67	34%	21%	19%	24%	1%
Huatulco	146	21%	42%	29%	8%	0%
Santa Rosa	239	42%	1%	54%	3%	0%

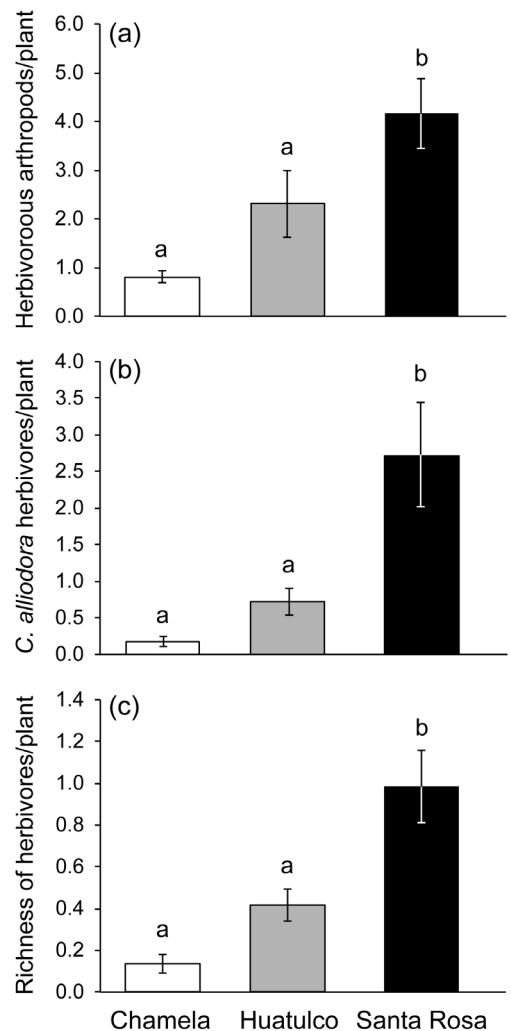


Figure 3. Herbivore communities harbored by *C. alliodora* trees occupied by *Azteca* ants at the three study sites (Chamela, Huatulco, and Santa Rosa). (a) Abundance of all herbivorous arthropods. (b) Abundance of herbivores known to eat *C. alliodora* per plant. (c) Species richness of herbivores known to eat *C. alliodora* per plant. Bars indicate mean \pm SE. Different letters indicate significant differences by ANOVA and Tukey HSD ($P < 0.05$).

surveys in 2010 showed higher abundances of herbivorous arthropods (ANOVA $F_{1,55} = 19.78$, $P < 0.001$), abundances of *C. alliodora* herbivores ($F_{1,55} = 14.07$, $P < 0.0004$), and species richness of *C. alliodora* herbivores ($F_{1,55} = 33.86$, $P < 0.0001$) than in 2009.

Trees occupied by *Azteca* spp. harbored significantly fewer herbivores and lower herbivore species richness than unoccupied trees in Huatulco, where ants nested at high densities and appeared to reduce leaf herbivory, but not in Santa Rosa, where they did not (Fig 4). In Huatulco, the abundance and richness of *C. alliodora* herbivores was significantly lower in the presence of an *Azteca* colony (Fig 4a-b; abundance: ANOVA $F_{2,89} = 8.89$, $P < 0.003$, presence of *Azteca* $F = 16.83$, $P < 0.0001$, tree basal diameter $F = 0.26$, $P = 0.6$; richness: ANOVA $F = 9.71$, $P < 0.0002$, presence of *Azteca* $F = 16.78$, $P < 0.0001$, tree basal diameter $F = 0.01$, $P = 0.9$); the abundance of all herbivorous arthropods was lower on *Azteca*-occupied trees, but not significantly so (ANOVA $F_{2,89} = 2.24$, $P = 0.1$). In Santa Rosa, *Azteca* presence did not affect the abundance of herbivorous arthropods ($F_{2,36} = 1.42$, $P = 0.3$), the abundance of *C. alliodora* herbivores ($F_{2,36} = 1.28$, $P = 0.3$), or *C. alliodora* herbivore species richness ($F_{2,36} = 0.56$, $P = 0.6$) (Fig 4c-d).

Discussion

Despite the prominence of ant-plant mutualisms as study systems for evolutionary ecology (Bronstein 1998; Heil and McKey 2003), very few studies have quantified the effects of ant defense on herbivore communities. Here we show that *Azteca* symbiotic plant-ants can significantly reduce both the abundance and diversity of herbivores on their *C. alliodora* host plants, but that these reductions occur only at geographic locations where ants are effective plant defenders.

Janzen (1966) reported the first experimental evidence that symbiotic ants can reduce herbivory on myrmecophytic acacia plants, and he went on to identify the common acacia herbivores and characterize their interactions with defensive ants (Janzen, 1967). Since that time, much more attention has been paid to how ants affect plant damage than to how they affect the plant's herbivores (but see, e.g., Gaume et al., 1997; Itino and Itioka, 2001). The few studies so far of the community-level effects of ant protection have focused on free-living (*i.e.* non-symbiotic) mutualisms, and have shown that the effects of ants on plant arthropod communities are context-dependent (Fowler and Macgarvin, 1985; Wimp and Whitham, 2001; Kaplan and Eubanks, 2005; Mooney, 2007; Rudgers et al., 2010). Although herbivory reduction by ants tends to be greater in symbiotic mutualisms than in free-living ones (Chamberlain and Holland, 2009; Rosumek et al., 2009), our results demonstrate that the effects of symbiotic ants on plant herbivore communities are also context-dependent, depending strongly on geographic variation in the

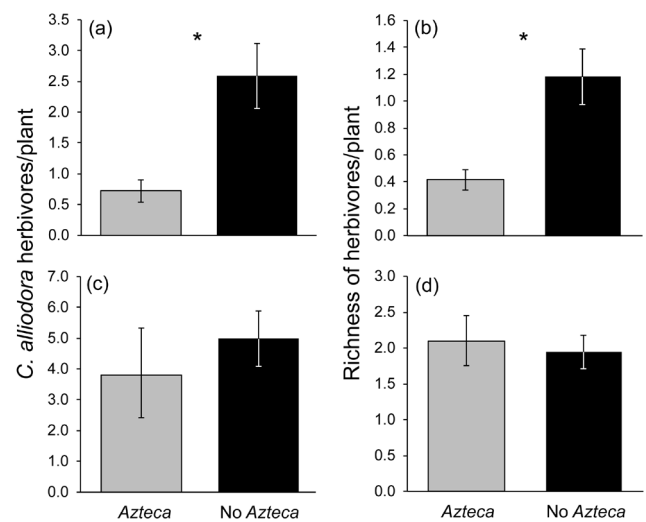


Figure 4. Abundance and richness of *C. alliodora* herbivores on trees occupied by *Azteca* ants or not in Huatulco (a,b) and Santa Rosa (c,d). (a,c) Abundance of known *C. alliodora* herbivores per plant. (b,d) Species richness of known *C. alliodora* herbivores per plant. Bars indicate mean \pm SE. Asterisks (*) indicate significant differences by ANOVA ($P < 0.0001$).

effectiveness of ant defense.

Understanding the evolutionary ecology of species interactions requires studies at large spatial scales (Thompson 2005), but interpreting such studies is challenging because there are many potential explanatory variables. In this study, the site with the least effective ant defense exhibited the highest per-plant abundance and diversity of herbivores, but ant defense is one of many factors that vary among the sites. For example, there is also considerable variation in annual rainfall among these sites, which can directly affect insect communities (Janzen and Schoener 1968). In support of an important role for ant defense among these variables, however, we also found that the presence of *Azteca* ants was associated with lower abundance and richness of *C. alliodora* herbivores within a site where ants appeared to be effective plant defenders (Huatulco), but not within a site where they did not (Santa Rosa). This latter result is consistent with the lack of effect of experimental ant exclusion on herbivory rates in Santa Rosa (Pringle et al., 2011). Overall, our results concord with those of Rudgers et al. (2010), who reported stronger effects of free-living ants on cotton-plant arthropod community composition at geographic locations where ants more strongly increased plant fitness. Geographic comparisons of species interactions are necessary to decipher the selection pressures on species across their ranges, and such within-site controls render these comparisons more interpretable.

We found that the effectiveness of ant defense was negatively associated with herbivore species richness across sites. Species richness is an important metric for community comparisons, but its meaning can be confounded by differences in abundance (Gotelli and Colwell, 2001). This problem is particularly acute in diverse tropical forests where commu-

nities are usually undersampled, such that increased sample size usually leads to increased richness estimates. Although there may be true differences in herbivore alpha diversity among the sites studied here, the negative association we found across sites between ant defense and herbivore richness results at least partly from the differences across sites in herbivore abundance (Table 1). Many of the lepidopteran herbivore species that we find regularly in Santa Rosa are also found in Chamela, but less frequently (E.G. Pringle, personal observation). When ant defense is effective, therefore, the primary effect seems to be to reduce overall herbivore abundance. The species that can reach the highest densities typically persist on *Azteca*-defended trees, whereas other species persist only rarely or on unoccupied trees. Interestingly, the rank order of species abundances for *C. alliodora* herbivores was similar in our three sites. For example, in our surveys, *Cropia connecta* (Lepidoptera: Noctuidae) caterpillars comprised 13.3% of known *C. alliodora* herbivores in Chamela, 12.8% in Huatulco, and 13.0% in Santa Rosa. Future work is necessary to identify species and evaluate variation among sites within non-lepidopteran orders.

This and previous studies represent only the first step towards a comprehensive understanding of the effects of ants on herbivore communities. In particular, our results raise two important questions for future research about the community effects of protection mutualisms. First, are ants driving differences in the population sizes of herbivore species among sites? And second, what is the relationship between ant defense and herbivore food-plant specialization?

Ant defense of plants affects realized food availability for herbivores, which should affect herbivore population sizes. Many ant-plant mutualisms conflate the traditional definition of trophic levels (sensu Hairston et al., 1960), and the resulting distinction between "bottom-up" and "top-down" control of herbivore populations, because plants attract ants that evolved from predatory ancestors but that may not actually eat their herbivores (Janzen, 1966). Higher ant density in trees leads to a higher probability that ants will attack and chase away herbivores (Pringle et al., 2011; Palmer and Brody, 2013), which should reduce the survivorship of vulnerable arthropod larvae (Vencl and Srygley, 2013). Higher ant densities may also affect food availability by reducing female oviposition (Sendoya et al., 2009). Within a site, food availability will be affected not just by ant density within plants, but also by ant and tree population sizes, and the resulting proportion of trees that are occupied by defensive ants. We found a positive association between ant density within trees and colony density among trees at two of our sites (Chamela and Santa Rosa), but not at our third site (Huatulco), where individual ant colonies grew large within trees, but more than half of the surveyed trees did not contain ants (Fig 1). This suggests that *C. alliodora* food availability is intermediate in Huatulco, which may lead to intermediate herbivore population sizes at this site.

Ant defense is of course not the only potential driver of herbivore population sizes: site climate also has important direct and indirect effects. Seasonality in tropical dry forests leads to particularly dramatic indirect effects on herbivores through changes in food availability. The near-synchronous flushing of new foliage in the early rainy season strongly affects the first generation of herbivores (Janzen, 1988; Dirzo and Domínguez, 1995). Variation in the timing and amount of rainfall early in the rainy season can also dramatically affect the survivorship and fitness of dry-forest herbivores (Agosta, 2008), and these effects may have driven the significant temporal variation in Santa Rosa herbivore communities between our surveys in 2009 and 2010.

Abundance of herbivorous arthropods was much lower in our 2009 surveys than in 2010 in Santa Rosa (2.2 ± 0.5 versus 7.8 ± 1.5 herbivorous arthropods per plant, respectively), and these differences may have resulted from less overall rain in 2009 or from the "false start" to the 2009 rainy season, which began in April but did not continue until mid-May. In addition to its effects on leaf flush, precipitation may also drive patterns of local adaptation in *Azteca* (Pringle et al., 2012b), and these effects may extend to variation in ant defensive behaviors. Indirect effects of climate on herbivore population sizes are rarely documented (Boggs and Inouye, 2012), but such effects can be important, even for arthropods that are considered food-plant generalists (Belovsky and Slade, 1995). A next important step will be determining the relative importance of these different direct and indirect effects on herbivore population sizes over time.

Although any herbivore that eats *C. alliodora* could be affected by geographic variation in ant defense, the effects on specialist herbivores may be particularly important and complex. Of the three most abundant lepidopteran larvae in our surveys, *C. connecta* is a specialist on Boraginaceae (and also eats a few species in the Malvaceae (sensu Stevens, 2001)), *Paridnea holophaealis* (Lepidoptera: Pyralidae) has only been found to eat *Cordia* spp. and *Varronia* spp. (Boraginaceae), and *Stauropides persimilis* (Lepidoptera: Noctuidae) has only been found to eat *Cordia* spp. (Janzen and Hallwachs 2009). We consider food-plant specialism to be a continuum, and thus these three most abundant lepidopteran herbivores are specialists, considering the many thousands of plant species found in northwestern Costa Rica that they do not eat. Moreover, ongoing DNA barcoding of these Lepidoptera continues to reveal cryptic species, and often further specialism, within the present taxonomic nomenclature (Hajibabaei et al., 2006).

It is unclear what the outcome of geographic variation in ant defense should be for population sizes of specialist herbivores. On the one hand, specialist herbivores of ant-defended plants should have strategies that allow them to continue feeding in the presence of ants (Heads and Lawton, 1985), which could buffer the effects of geographic variation in ant defense on food availability. On the other hand, spe-

cialist herbivores have fewer alternatives if *C. alliodora* is a primary food plant and ant density within and among trees is high.

Caterpillar defenses against ants in this system, which include thrashing, biting, regurgitating, dropping, and shelter-building, should also be effective against other predators and parasitoids (Gentry and Dyer, 2002), and it remains unclear whether any of these defenses have evolved in response to *Azteca* specifically (see, e.g., Vencel and Srygley, 2013). Nevertheless, the evolution of defenses against natural enemies may be one reason that tropical Lepidoptera are generally more specialized than temperate Lepidoptera (Dyer et al., 2007). Interestingly, Gange (2002) suggested that plant-mycorrhizal mutualisms may also increase herbivore food-plant specialization by enhancing plant chemical defenses, which suggests that although plant protection mutualisms seem to decrease diversity on a per-plant scale (Rudgers et al., 2010), they may actually increase diversity at a community scale.

Nearly 50 years of research on ant protection has revealed that ants usually benefit plants by reducing herbivory (Styrsky and Eubanks, 2007; Chamberlain and Holland 2009; Rosumek et al. 2009; Trager et al., 2010), but the effects of ants on herbivore communities remain unexplored. Detailed studies are necessary to determine how important protection mutualisms are to their communities, an important step towards the greater inclusion of mutualisms in ecological theory (Stachowicz, 2001; Bruno et al., 2003; Hay et al., 2004). Moreover, such research will address questions that have rarely been considered in the tropical environments where ant-plant interactions are most abundant. In particular, it seems likely that these interactions drive arthropod population dynamics, which are important to understand in the face of rapid environmental change. In addition, such research would shed light on the role of mutualisms in such key processes as food-plant specialization and plant-herbivore coevolution.

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