PLANT-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Plant defense, herbivory, and the growth of *Cordia alliodora* trees and their symbiotic *Azteca* ant colonies

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Abstract The effects of herbivory on plant fitness are integrated over a plant's lifetime, mediated by ontogenetic changes in plant defense, tolerance, and herbivore pressure. In symbiotic ant–plant mutualisms, plants provide nesting space and food for ants, and ants defend plants against herbivores. The benefit to the plant of sustaining the growth of symbiotic ant colonies depends on whether defense by the growing ant colony outpaces the plant's growth in defendable area and associated herbivore pressure. These relationships were investigated in the symbiotic mutualism between *Cordia alliodora* trees and *Azteca pittieri* ants in a Mexican tropical dry forest. As ant colonies grew, worker production remained constant relative to ant-colony size. As trees grew, leaf production increased relative to tree size. Moreover, larger trees hosted lower densities of ants, suggesting that ant-colony growth did not keep pace with tree growth. On leaves with ants experimentally excluded, herbivory per unit leaf area increased exponentially with tree size, indicating that larger trees experienced higher herbivore pressure per leaf area than smaller trees. Even with ant defense, herbivory increased with tree size. Therefore, although larger trees had larger ant colonies, ant density was lower in larger trees, and the ant colonies did not provide sufficient defense to compensate for the higher herbivore pressure in larger trees. These results suggest that in this system the tree can decrease herbivory by promoting ant-colony growth, i.e., sustaining space and food investment in ants, as long as the tree continues to grow.

Keywords Allometry · Ant–plant mutualism · Chamela-Cuixmala Biosphere Reserve · Mexico · Ontogeny · Positive feedback

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Introduction

Anti-herbivory defense strategies and the impacts of herbivore damage can change considerably in the course of plant ontogeny. Greater investment in defense or tolerance may occur at life stages when a plant has more resources for defense allocation (Weiner 2004) or when the plant is most vulnerable to herbivores (Stowe et al. 2000). Synthesizing these theories, Boege and Marquis (2005) predicted nonlinear, substantial changes in defense in the course of a plant's life. However, empirical data for many plant species suggest that ontogenetic patterns in defense are idiosyncratic, depending on plant growth form, herbivore guild, and defensive trait strategy (Barton and Koricheva 2010). When plants employ biotic defenses, by providing rewards to defending mutualists, ontogenetic changes in plant defense depend on concurrent changes in the defensive efficacy of the mutualists. In symbiotic ant–plant protection mutualisms, plants (known as myrmecophytes) provide ants with nesting cavities known as domatia and, directly or indirectly, with food rewards. Ants, in turn, provide defense against herbivores and encroaching vegetation (Heil and McKey 2003), and, in some systems, supply the plant with additional nutrients (e.g., Defossez et al. 2011; Fischer et al. 2003; Solano and Dejean 2004).

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The effectiveness of ant defense over the host plant's life is related to the timing of ant colonization, the identity of the ant species, and the relative growth rates of the plant and its symbiotic ant colony. Myrmecophytes are colonized by ants some time after germination and production of the first domatia. Ant–plant systems differ in whether levels of direct (i.e., abiotic) defenses change after ant colonization (Del Val and Dirzo 2003; Llandres et al. 2010; Nomura et al. 2001; Trager and Bruna 2006). Once colonized, myrmecophytes spend most of their lives hosting ant mutualists, and the density of ant workers per unit leaf area, as opposed to colony size per se, determines how well plants are defended. The higher the density of ants in a plant, the more patrolling workers per unit leaf area, and thus the more likely ants are to encounter, attack, and chase away or consume herbivores (Duarte-Rocha and Godoy-Bergallo 1992; Pringle et al. 2011b). Young leaves are particularly valuable to plants (Harper 1989) and vulnerable to herbivores (Coley and Kursar 1996), so high densities of patrolling ants on young leaves in particular may confer important benefits to plants (Heil et al. 2004).

Both ant colonies and plants are modular organisms that grow by addition of sterile workers and shoots, respectively (Wulff 1985). The relative rates of worker and shoot production determine the density of ants per unit leaf area and therefore whether the ant colony continues to provide the same level of defense as the plant grows. The relative rates of worker and shoot production may also change as colonies and trees grow, depending on the allometric relationships between the production of workers or shoots and increasing ant-colony or tree size, respectively. In some ground-nesting ant species, as colonies grow, they may produce fewer new ant workers per unit size, exhibiting negative allometry (Smith and Tschinkel 2006; Tschinkel 1993, 1999); in other species, colonies produce the same number of new ant workers per unit size, exhibiting isometry (Gordon 1992). As juvenile trees grow into mature trees, they tend to produce more crown area per unit size, exhibiting positive allometry (Oliver and Larson 1990).

Few studies have assessed the effects of ant-colony growth over host-plant ontogeny, and the results to date have been variable. Fonseca (1993) found that the number of *Pseudomyrmex concolor* workers was highly correlated with total domatia space in *Tachigali* plants, and suggested that the size of the ant colony is limited by the number and size of domatia. If ant colonies grow faster than plants, colonies may grow so large that the marginal cost to the plant of providing food and space for each additional ant worker is greater than that worker's marginal contribution to herbivory reduction (Bronstein 1998; Fonseca 1993; Ness et al. 2006). In such cases, Fonseca (1993) argued, the plant should limit its investment in ants to keep colony size from surpassing the point where marginal costs exceed marginal benefits. In

contrast, Frederickson and Gordon (2009) measured plant growth and domatia occupation by ant colonies in two systems in the Peruvian Amazon, and found evidence for positive feedback between ant-colony and plant growth rates: when larger ant colonies do a better job of protecting plants, plants can grow larger because of this superior protection, and ant colonies grow as plants add domatia.

Herbivore pressure can change as trees grow, but few studies have measured herbivory continuously during plant development (but see Thomas et al. 2010). Larger trees attract higher abundances and richness of insect herbivores than smaller trees (Campos et al. 2006; Guedes et al. 2000). The greater relative architectural complexity of larger trees than of smaller trees may support more insects per unit of leaf area, or larger trees may support certain herbivore species that are rarely found on smaller trees (Lawton 1983). If larger plants experience higher herbivore pressure per unit of leaf area than smaller ones, and if ant defense scales linearly with colony growth, the relative growth of ant colonies must be greater than leaf production by the host plant to maintain a constant level of anti-herbivore defense. However, ant defense may not scale linearly with colony growth—for example, larger colonies may coordinate activities more effectively than smaller ones (e.g., Gordon 1987)—in which case even slow rates of ant-colony growth relative to tree leaf production may be sufficient to maintain low levels of herbivory.

To evaluate how ant-colony growth is related to plant growth, and whether plants benefit from sustaining continuous ant-colony growth over time, we measured herbivory, ant worker production, and tree shoot production in a range of ant-colony and juvenile-tree sizes in an ant–plant mutualism in a seasonally dry forest in Western Mexico. *Azteca pittieri* Forel (Formicidae: Dolichoderinae) ants form colonies in the domatia of *Cordia alliodora* (Ruiz and Pavón) Oken (Boraginaceae) trees, and defend their leaves from herbivores (Pringle et al. 2011b; Tillberg 2004). Using this system, we ask: (1) How does the rate of worker production change as *A. pittieri* ant colonies grow? (2) How do the rates of leaf and domatium production change as *C. alliodora* trees grow? (3) Do ant colonies produce new workers as quickly as trees produce new leaves that need to be defended? (4) Do bigger trees sustain more herbivore pressure per unit area than smaller trees? and (5) How do changes in both ant defense and herbivore pressure affect herbivory rates as trees grow?

Materials and methods

Study system

This study was conducted at the Chamela-Cuixmala Biosphere Reserve (19°30′N, 105°02′W) in Jalisco, Mexico in

July–August 2008 and July 2009. The site (hereafter "Chamela") is a seasonally dry tropical forest, with 85 % of the ~750 mm of yearly rain occurring from June to November (Bullock 1986).

Cordia alliodora is a common tree in the dry-season deciduous forest at the site (~80 individuals/ha; E.G. Pringle, unpublished data). Yearly rainfall, availability of light and soil nutrients, and herbivory all substantially affect the tree's growth (Haggar and Ewel 1997; Hazlett 1989; Menalled et al. 1998). Individuals at Chamela reach a maximum height of ~15 m (E.G. Pringle, unpublished data), and all life stages but seedlings are leafless for the duration of each year's dry season (Bullock and Solís-Magallanes 1990). The trees flush a completely new leaf set after the first rains of the wet season (which occurred during the study years on July 2 and June 19, respectively). All measurements reported here thus occurred within 1.5 months of new leaf production. It is unknown what the consequences of seasonality are for the evolution of this mutualism (see, e.g., Janzen 1973).

The tree produces domatia, in which symbiotic ants nest, at most stem nodes. The most common ant species to occupy *C. alliodora* domatia at the site is *A. pittieri*, which occupied ~97 % of all *C. alliodora* trees in surveys in 2007 ($n = 134$) and 2010 ($n = 117$) (E.G. Pringle, unpublished data). The number of *A. pittieri* ants is not strongly correlated with the amount of nesting space available in tree domatia (Pringle et al. 2011b), as it is in some other ant–plant symbioses (Fonseca 1993; Orivel et al. 2011). The tree does not produce food rewards for the ants, and the ants rely on honeydew-producing scale insects for much of their diet (Pringle et al. 2011b; Tillberg 2004; Wheeler 1942). The number of *A. pittieri* workers is tightly correlated with the number of scale insects; leaf herbivory is negatively related to ant density (Pringle et al. 2011b).

Allometries of tree and ant-colony growth

To determine the allometry of ant-colony growth, the number of ant brood were compared to the number of ant workers. Six juvenile *C. alliodora* trees occupied by *A. pittieri* colonies, from 1.6 to 4 m in height, were cut down in July 2008 on days when it was raining and ants were not active, or after spraying the tree heavily with water for 10 min. All the stems were collected in press-seal plastic bags containing alcohol-soaked paper towels. Stems were left in the bags for 12 h until ants had died. Domatia were then dissected individually, and all worker ants, larvae, and pupae were counted. Because $n = 6$ is a small sample size for determining an exponent of allometric growth, the relationship of brood to workers in Chamela was compared to the same relationship for *A. pittieri* ants in 12 more *C. alliodora* trees from other dry forests in Oaxaca,

Mexico, and Guanacaste, Costa Rica. The coefficient of the relationship was consistent across sites (Chamela only: $m = 1.08$; all sites: $m = 0.94$), and statistical significance increased as data from additional sites were added. For consistency, the analysis presented here includes only the six Chamela trees.

As *C. alliodora* trees grow, they add new shoots that support new leaves, and new domatia form at stem nodes. To determine the allometry of new leaf and domatia production as trees grow, the leaves and domatia of 42 juvenile *C. alliodora* trees of basal diameters 1–6 cm were counted in July 2008 and 2009. Number of leaves and number of domatia were regressed against the basal diameter of the tree to determine growth allometry. Although tree diameter can be affected by many factors other than age, Hummel (2000) documented a strong correlation between tree diameter and tree age in *C. alliodora*.

Ant density and tree size

To estimate whether ant density changed as trees grew, we calculated ant densities in a range of tree sizes. We used the counts of ant workers from the six juvenile trees dissected for the colony growth allometry estimation (above). We predicted total number of workers in an additional 14 trees from counts of ants in three domatia. The total number of ants counted in three domatia was multiplied by the total number of domatia and by the slope of the linear model relating actual counts to predicted counts for the six trees in which all ants were counted (for additional methods, see Pringle et al. 2011b). To estimate the error in predictions of total ant-colony size based on counts from a subset of three domatia, we randomly resampled counts from three domatia 100 times, with replacement, from all of the domatia of the six trees in which all ants were counted. We then calculated average percent difference between predicted and actual counts. Predicted ant-colony sizes overestimated actual counts by three percent on average (SE = 2 %; 95 % CI from −2 to 9 %).

Counts and estimates of numbers of ants in all 20 trees were converted to densities by dividing the number of ants by each tree's basal diameter. We used basal diameter in our estimate of ant density because it can be measured with low error on trees of all sizes, and it displays strong allometry with the area of the tree, including leaf area, that could be defended by ant mutualists (Fig. 1; Cole and Ewel 2006).

Herbivore pressure and tree size

To determine how herbivore pressure changed with tree size, we examined the relation of herbivory rate, in the absence of ants, with tree basal diameter. To measure leaf

Fig. 1 Ant colony and tree growth allometries (log-log relationships). **a** Relationship between the number of ant brood (both larvae and pupae) and number of adult worker ants ($R^2 = 0.65$, $P < 0.04$). *Filled circles* colonies from Chamela, for which the regression is calculated (see "Materials and methods"), *open circles* colonies from Guanacaste, Costa Rica, and *diamonds* colonies from Oaxaca, Mexico. **b** Allometric regression between the number of leaves and tree basal diameter ($R^2 = 0.75$, $P < 0.0001$). **c** Allometric regression between the number of domatia and tree basal diameter ($R^2 = 0.64$, $P < 0.0001$). **d** Allometric regression between the number of domatia and the number of leaves on a tree ($R^2 = 0.88$, $P < 0.0001$)
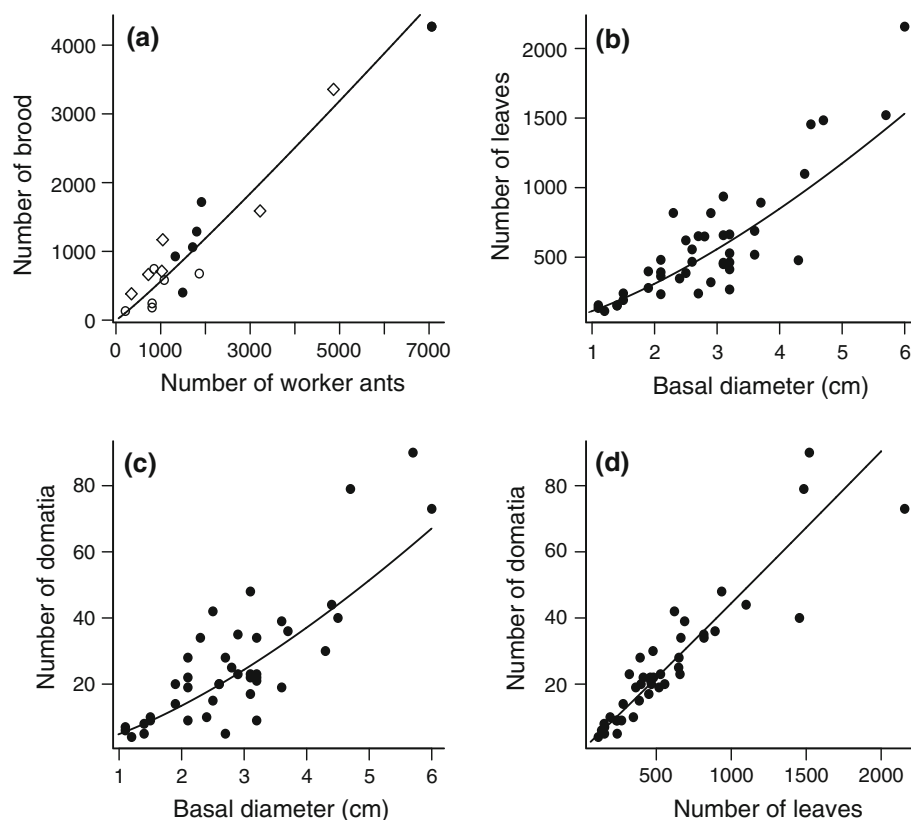
herbivory rates in the absence of ants, ants were excluded from leaves, and herbivory was measured over 3 weeks. Ants were excluded from two leaf whorls, with $\sim$7–10 leaves per whorl, on 39 trees of basal diameters 2–13 cm in the early rainy season, July–August 2008. A sticky barrier of Tanglefoot (Tanglefoot, Grand Rapids, MI, USA) was applied in 1.5-cm bands around the branch before the leaf whorl and distal to the nearest ant-occupied domatium. A single, newly expanded leaf from each whorl was marked with a colored wire for herbivory measurements. Herbivory was measured before and after the experiment by overlaying transparent sheets with $1/4$-cm^2 or 1-mm^2 grid cells on leaves and counting the number of cells for which leaf area was missing to herbivory. Total leaf area was estimated by multiplying measurements of length and width for each leaf by the coefficient of the relationship between length $\times$ width and total area. This coefficient was determined for 30 leaves before the start of the experiment. The percent leaf area eaten during the experiment, %LAE, was calculated as: (area eaten after the experiment $-$ area eaten before the experiment)/total leaf area. For each tree, we averaged %LAE for the two marked leaves.

Realized herbivory and tree size

To determine how herbivory in the presence of ant defense changed with tree size, we measured leaf herbivory in the

presence of ants in two ways. First, %LAE was measured over 3 weeks for a single leaf from each of two different leaf whorls to which ants had access (no Tanglefoot applied) on each of the same 39 trees over the same period used for herbivore pressure rates above. Second, to evaluate herbivory accumulated over the first $\sim$6 weeks of the early rainy season, standing levels of herbivory were determined for $\sim$100 individual leaves on each of the 39 trees at the end of the herbivory-rate experiments using the index of Dirzo and Domínguez (1995). Levels of standing herbivory were estimated by categorizing leaves, from at least three branches per tree, by percent leaf area eaten (%LAE) as follows: $0 = 0$ %, $1 = 1$–6 %, $2 = >6$–12 %, $3 = >12$–25 %, $4 = >25$–50 %, $5 = >50$–100 %. A single investigator (E.G.P.) estimated categories for all leaves on all trees, so that any deviations from these percentages were constant across individuals. An index of the average standing level of herbivory per leaf for each tree, from 0–5, was then estimated as the sum, for all categories, of the product of the number of leaves in each category and the category value, divided by the total number of leaves (Dirzo and Domínguez 1995).

Statistical analyses

Statistical analyses were conducted using JMP 8.0.2 (SAS Institute 2009) or R 2.8.1 (CRAN-r-project; R Development

Core Team 2008). For each regression relationship, a set of candidate models were tested that identified the relationship as linear, exponential, or power-law. The best model was determined for each relationship by ranking these models with the sample-size-corrected Akaike Information Criterion (AIC_c). Although in three cases there were no strong differences between log-linear and log-log models (Burnham and Anderson 2010), the model with the lowest AIC_c was used in each case. Results of regression model comparisons by AIC_c are shown in Table 1. All relationships were analyzed by Type-I regression, as is standard in allometric studies; only adjusted R^2 values are reported. Slopes of allometric growth curves for ant colonies and trees were compared to an isometric slope = 1.0 by t tests.

Results

Allometries of tree and ant-colony growth

Ant colonies and juvenile trees exhibited contrasting growth allometries. The rate of production of new ant workers remained constant relative to ant-colony size as colonies grew, whereas the rate of production of new leaves and domatia increased relative to tree size as juvenile trees grew. Numbers of ant brood increased isometrically (log-log plot,

$m = 1$) with the number of worker ants (Fig. 1a; $R^2 = 0.65$, $F_{1,4} = 10.19$, $P < 0.04$; Table 2). In contrast, the number of leaves and the number of domatia exhibited positive allometry (log-log plot, $m > 1$) with respect to tree basal diameter (Fig. 1b, c; leaves: $R^2 = 0.75$, $F_{1,40} = 125.10$, $P < 0.0001$; domatia: $R^2 = 0.64$, $F_{1,40} = 72.48$, $P < 0.0001$; Table 2). The number of new domatia increased at the same rate (log-log plot, $m = 1$) as the number of new leaves (Fig. 1d; $R^2 = 0.88$, $F_{1,40} = 311.46$, $P < 0.0001$; Table 2), which indicates that the relationship between plant investments in leaf growth and nest sites for ant partners was constant within the range of tree sizes included in this study. For allometric growth equations, results of t tests for isometry are shown in Table 2.

Ant density and tree size

The density of ant workers in a tree decreased with tree size in juvenile trees that had already been colonized by ants. There was a negative, marginally significant relationship between the density of worker ants and tree size (Fig. 2; $R^2 = 0.13$, $F_{1,18} = 3.76$, $P = 0.068$).

Herbivore pressure and tree size

In the absence of ant defense, rates of herbivory increased with tree size; larger trees suffer from higher herbivore

Table 1 AIC_c results for regression models

| Relationship	Model AIC_c		
	Linear (linear-linear)	Exponential (log-linear)	Power law (log-log)
No. ant brood versus no. worker ants	104.04	**24.38**	**23.31***
No. leaves versus tree basal diameter	578.47	39.15	**34.61**
No. domatia versus tree basal diameter	326.47	60.72	**57.80**
Density of ants versus tree basal diameter	297.38	**78.89**	**78.73***
Herbivory rate (no ants) versus tree basal diameter	285.35	**100.93***	102.51
Herbivory rate (with ants) versus tree basal diameter	274.12	**78.19**	82.58
Standing herbivory index versus tree basal diameter	57.89	**49.82**	54.18

The AIC_c evaluated whether the relationship was best described as linear, exponential, or power-law. The best model, with the lowest AIC_c, for each regression is highlighted in bold. For relationships with two equivalent models whose AIC_c difference was ≤ 2 (Burnham and Anderson 2010), both models are in bold, and the model with the lowest AIC_c that was used for the analysis is marked with an asterisk (*)

Table 2 Allometric growth of ant colonies and trees

Relationship	Slope (m)	Student's t	df	P
No. ant brood versus no. worker ants	1.08	0.23	4	0.8
No. leaves versus tree basal diameter	1.46	3.51	40	**0.002**
No. domatia versus tree basal diameter	1.46	2.69	40	**0.01**
No. domatia versus no. leaves	1.02	0.42	40	0.7

Significance of slope against a null hypothesis of isometry was tested by Student's t tests. For ant colonies, only the relationship for the six colonies from *Chamela* is reported

Significant differences from $m = 1.0$ are highlighted in bold
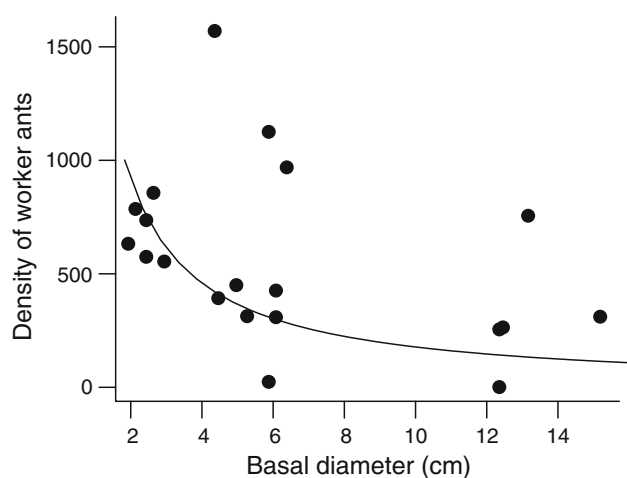
Fig. 2 Relationship between the density of worker ants per tree (the number of ants per unit basal diameter) and tree basal diameter (log-log relationship; $R^2 = 0.13$, $P < 0.07$). Note that the smallest tree diameter considered is 2 cm; ants colonize trees after domatia production, and the smallest trees may not be colonized or may have very small founding colonies

pressure. Per unit leaf area, herbivory rates in the absence of ant defense increased exponentially with tree basal diameter (Fig. 3; $R^2 = 0.18$, $F_{1,37} = 9.47$, $P < 0.004$; Table 1). This relationship was robust to exclusion of outliers.

Realized herbivory and tree size

In the presence of ant defense, herbivory rates and standing herbivory per unit leaf area increased exponentially with tree size within the size range included in this study. Thus, increases in the quality of ant defense did not match the
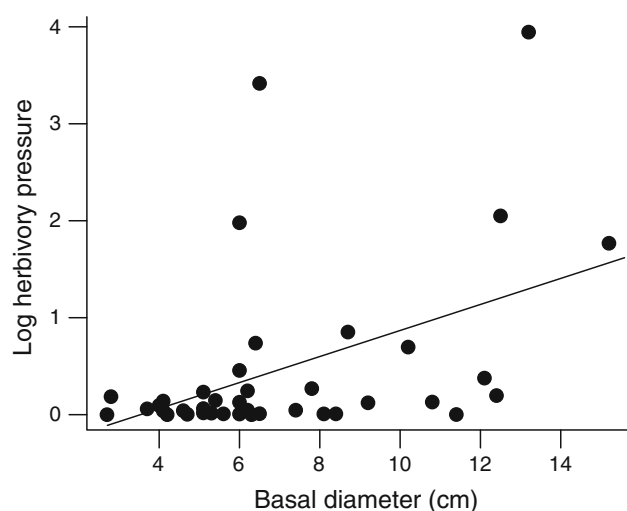
Fig. 3 Relationship between herbivore pressure and tree basal diameter ($R^2 = 0.18$, $P < 0.004$). Herbivore pressure is estimated as the rate of herbivory in the absence of ant defenders

plant's growth in defendable area and associated herbivore pressure as the mutualistic partners grew larger. Herbivory rates in the presence of ant defense were positively related to tree size (Fig. 4a; $R^2 = 0.30$, $F_{1,37} = 17.53$, $P < 0.0002$). It appears that ants reduced herbivory (Pringle et al. 2011b): the slope of the relationship between herbivory and tree size did not differ significantly when ants were present or absent ($t = 0.04$, $df = 74$, $P = 0.96$), but the intercept was lower when ants were present (ants excluded: -0.47 ± 0.34; ants present: -0.66 ± 0.25). This relationship was also robust to exclusion of outliers; we hypothesize that the large number of near-zero herbivory rates in both the absence and presence of ants reflects, at least in part, the stochastic aspect of whether experimental leaves were found by herbivores over the course of the 3-week experiment. Standing herbivory, accumulated in the presence of ants, also increased exponentially with tree size within the size range included in this study (Fig. 4b; $R^2 = 0.33$, $F_{1,37} = 20.00$, $P < 0.0001$).

Discussion

Ontogenetic changes in anti-herbivory defenses of myrmecophytic plants depend both on the relative growth rates of the plants and their symbiotic ant colonies, and on changes in herbivore pressure as plants grow larger. If ant colonies grow faster than plants produce new leaf area that needs to be defended, the marginal costs to the plant of supplying space and food rewards to each additional ant will increase faster than the herbivory reduction provided by that ant, resulting in a conflict of interest between plant and ant mutualists (Fonseca 1993). On the other hand, if there is positive feedback between plant and ant-colony growth, the interests of plant and ant mutualists are aligned (Frederickson and Gordon 2009), with potentially important consequences for the evolutionary stability of the mutualism (Weyl et al. 2010).

In this system, allometric growth patterns suggested that ant worker production per unit colony size remained constant as ant colonies grew, whereas leaf and domatia production per unit tree size increased as juvenile trees grew. In trees colonized by ants, the density of ants marginally decreased with increasing tree basal diameter, which suggests that production of new leaves significantly outpaced the production of new workers. Herbivore pressure increased with tree size. Thus, to maintain equivalent levels of anti-herbivory defense as the tree grows, the effectiveness of ant defense would need to improve faster than trees produce new leaves. However, herbivory per leaf area on ant-defended trees increased exponentially with tree size within the size range included in this study, suggesting that ant defense did not keep up with tree growth and increases

Fig. 4 Herbivory rates and cumulative herbivory in the presence of ant defenders. **a** Relationship between herbivory rates in the presence of ants and tree basal diameter ($R^2 = 0.30$, $P < 0.0002$). **b** Relationship between standing herbivory level per leaf per tree, an index (0–5) of percent leaf area eaten per leaf, and tree basal diameter ($R^2 = 0.33$, $P < 0.0001$)
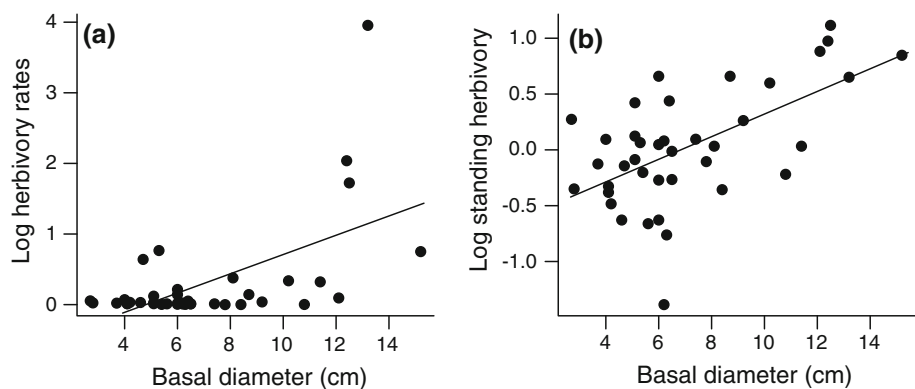
in herbivore pressure. For *C. alliodora* trees to maintain low levels of herbivory, they should sustain investment in ant-colony growth as long as they continue to grow. It is possible, however, that older trees are more tolerant of herbivory than younger trees (Warner and Cushman 2002).

Consistent with the hypothesis of positive feedback in ant–plant mutualisms (Frederickson and Gordon 2009; Heil et al. 2009; Pringle et al. 2011b), our results suggest that growing trees benefit from maximum herbivory reduction by sustaining ant-colony growth. Indeed, limiting ant colony growth may have other costs for trees, since trees would need to limit their investment either in domatia space or in the number of scale insects that the ants tend for honeydew. In this study, *C. alliodora* trees produced new domatia at the same rate as they produced new leaves relative to tree size; new domatia are produced at the nodes of most new shoots. To limit space for ant-colony growth, trees would have to limit their own growth, which could be disadvantageous in a competitive environment where size is linked to light access (Coomes and Allen 2007; Menalled et al. 1998; Potvin and Dutilleul 2009), or control whether individual nodes become hollow or solid, which *C. alliodora* apparently does not, as most nodes are hollow (Wheeler 1942; E.G. Pringle, personal observation). Although trees may be capable of changing phloem quality to control the number of scale insects (Berenbaum 1995), which in turn would limit ant colony size (Pringle et al. 2011b), the density of scale insects did not change with tree size in this study (data not shown).

The allometries of ant-colony and tree growth may differ because of the asymmetry in lifespan between the two partners. A long-lived tree will probably host several ant colonies during its lifespan (Frederickson and Gordon 2009; Palmer et al. 2010), and differences between colonies and trees in time to reproductive maturity may mean that ant colonies slow worker production to produce reproductive alates before trees slow vegetative growth to produce flowers and fruits (Orivel et al. 2011). Here, we have considered growth allometries of juvenile trees, but

tree growth allometries change as trees mature (Oliver and Larson 1990). Studies of *C. alliodora* in agroforestry plots have shown that juvenile trees make substantial investments in foliage, but that leaf production allometries level off and then become negative when trees reach their maximum size (Cole and Ewel 2006; Menalled et al. 1998). We note that the exponential increases in herbivore pressure and realized herbivory with tree size were detectable only in trees with basal diameters of up to 13 cm (Figs. 3, 4), whereas we measured positive allometries in tree growth up to diameters of 6 cm (Fig. 1). Although growth allometries may change in the range of 6–13 cm diameter (Cole and Ewel 2006), Olson et al. (2009) found that crown area in *C. alliodora* maintains positive allometry with stem diameter throughout this size range. Future studies are needed to assess whether isometric or negative allometric tree-growth allometries at maturity allow ant colonies developing in mature trees to attain densities per unit leaf area as high as those that they attain in small juvenile trees (Fig. 2).

Even if ants achieve similar densities in the largest trees as in the smallest trees, the quality of their defense would still need to be greater in larger trees to reduce herbivory equivalently because larger trees experienced higher herbivore pressure. In the dry tropical forest of Western Mexico, the leaves of *C. alliodora* exhibit very weak direct defenses relative to other species, with lower than average toughness, trichome density, latex, leaf mass per area, and aqueous chemical defenses, and slightly higher than average nitrogen content (Pringle et al. 2011a). The removal of trees' indirect defenses by means of ant exclusion thus represents an important reduction in total defenses. The increases in herbivory with tree size that we observed in the absence of ants suggest that herbivore pressure is positively related to tree size per se, and probably not to concomitant changes in defenses as trees grow. Our experiment measured herbivore pressure when ants were excluded from individual branches; true herbivore pressure may be even higher when entire trees are undefended by ants (E.G.

Pringle, unpublished data). Further work measuring changes in herbivory pressure with tree size when ants are excluded from entire trees is warranted.

An important caveat to our conclusions is that, by using replicate trees with instantaneous measurements of ant colonies and trees, we assume that plants of similar sizes grow and interact with ants and herbivores similarly in different locations and at different times. These assumptions allow us to evaluate a greater range of life stages in long-lived trees (Landis and Peart 2005), and to make accurate estimates of ant-colony size in a species that is not limited by available space by using destructive sampling (Pringle et al. 2011b). However, future studies that avoid these assumptions by following individuals through time will be informative.

Here, we have considered what density of ant workers and associated colony size is optimal for trees. There may also exist an optimal mature colony size for the ants (Bronstein 1998). Colony sizes can vary substantially across locations (Kaspari 2005; Pringle et al. 2011b) and seasons (Tschinkel 1993), and the effects of such variation on reproductive output and fitness are not well understood (but see Frederickson and Gordon 2009; Smith and Tschinkel 2006; Wagner and Gordon 1999). Future studies are needed to determine whether increased rewards from growing host plants positively affect ant colony fitness. It will be particularly interesting to compare the relative growth rates of plant and ant mutualists in systems where tree domatia space apparently limits colony growth (e.g., Fonseca 1993; Orivel et al. 2011) and systems, such as this one (Pringle et al. 2011b), in which it does not. Such comparisons in combination with studies of changes in plant defense before and after ant occupation (Del Val and Dirzo 2003; Llandres et al. 2010; Nomura et al. 2001; Trager and Bruna 2006) will elucidate the ontogeny of plant defense in ant–plant mutualisms.

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