BIOTROPICA 43(3): 343–350 2011 10.1111/j.1744-7429.2010.00700.x

Weighing Defensive and Nutritive Roles of Ant Mutualists Across a Tropical Altitudinal Gradient

Genoveva Rodríguez-Castañeda[1,7], **Rebecca E. Forkner**[2], **Eric J. Tepe**[3,4], **Grant L. Gentry**[5], and **Lee A. Dyer**[6]

[1] Department of Ecology and Environmental Science, Umeå Universitet, Sweden

[2] Department of Environmental Science and Policy, George Mason University, Fairfax, Virginia 22030, U.S.A.

[3] Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221, U.S.A.

[4] Department of Biology, 257 South 1400 East University of Utah, Salt Lake City, Utah 84112, U.S.A.

[5] Samford University, Howard College of Arts and Sciences, Samford University, 800 Lakeshore Drive, Birmingham, Alabama 35229, U.S.A.

[6] Department of Biology, University of Nevada, Reno, Nevada 89557, U.S.A.

ABSTRACT

The diversity of mutualistic interactions influences many ecological components of community structure, including biodiversity and ecosystem stability. However, mutualistic interactions are not well resolved because of a historical bias toward examining antagonistic interactions. Here we examine both antagonistic and facilitative interactions between tropical plants and arthropods by characterizing the biotic interactions between a common myrmecophytic shrub, *Piper immutatum* Trel. (Piperaceae), the ants hosted by this plant, *Pheidole* sp. (Formicidae: Myrmicinae), and their associated communities of herbivorous and predatory arthropods. To determine if ant mutualists affect the altitudinal distribution of Neotropical myrmecophytes, *P. immutatum* interactions with arthropods were quantified across a tropical elevational gradient. *Piper immutatum* was most abundant in lower montane forests (1000–1600 m asl) and disappeared above 1600 m asl, and colonies of *Pheidole* sp. inhabited 90 percent of the sampled plants. The myrmecophyte was then transplanted within and beyond its altitudinal range, excluding ants from half of the transplanted plants. Plant survival was affected primarily by elevation, with only 20 percent surviving above 1600 m asl. Ant exclusion did not significantly affect plant mortality. Nevertheless, ant colony size did affect both herbivory and nutrient availability for surviving *P. immutatum*, with nutrient availability having a stronger effect than antiherbivore defense on growth and biomass. This approach of studying the contributions of ant mutualisms across the myrmecohpyte's habitat range yields an improved picture of the role of mutualistic interactions in determining community structure.

Abstract in Spanish is available at http://www.blackwell-synergy.com/loi/btp.

Key words: elevation; mutualistic ants; myrmecophytes; nutrient procurement; plant defense.

ANT-PLANT INTERACTIONS ARE GEOGRAPHICALLY WIDESPREAD, common in many ecological communities and important in plant defense against herbivores. As such, spatial variation in plant–ant interactions can depend on the environmental and ecological context in which they occur (Holland *et al.* 2005). Obligate ant–plant mutualisms, which are uniquely tropical, comprise an important part of the diversity of resource–consumer interactions, especially in lowland wet forests. Facultative ant–plant mutualisms are even more widespread and also contribute significantly to interaction diversity. More than 40 angiosperm orders contain species producing ant attractants (Rico-Gray & Oliveira 2007). In a meta-analysis of terrestrial trophic cascades, ants were the main predator in 62 percent of studies, and exclusion of these predators significantly increased plant damage (Schmitz *et al.* 2000). Mutualisms are as important to community stability and diversity as any other multispecies interactions (Christian 2001, Hay *et al.* 2004, Savage & Peterson 2007, Bulleri *et al.* 2008), but greater attention to the mechanisms by which these mutualisms evolve and are maintained is necessary to understand the community role of mutualistic interaction diversity. For ant–plant mutualisms in particular, it is important to understand how specific interactions are linked to other species, such as herbivores or potential competitors (Holland *et al.* 2005). One way by which mutualistic interactions can affect populations and communities is via altering the acceptable habitat range of the mutualistic populations. Here we consider how ant mutualists might affect the altitudinal range of their host plants and examine the mechanisms by which ants enhance conditions for these plants.

Myrmecophytes are broadly defined as plants that host ant colonies in modified plant tissues that provide domatia or nesting sites (*e.g.*, Janzen 1966; Letourneau 1983, 1998; Folgarait & Davidson 1994, 1995; Fiala *et al.* 1999). In most well-characterized plant–ant mutualistic interactions, ants act as predators of insect herbivores in exchange for extrafloral nectar, food bodies, and shelter (Janzen 1966; Risch & Rickson 1981; Letourneau 1983, 1998; Fischer *et al.* 2002; Rico-Gray & Oliveira 2007). Myrmecotrophy, the potential ability of plants to absorb nutrients from debris piles of ant nests, is another benefit of housing ants (Benzing 1991, Sagers *et al.* 2000, Fischer *et al.* 2003, Rico-Gray & Oliveira 2007). Despite much variation in the nature of myrmecotrophic mutualisms, they usually occur under suboptimal survival conditions such as sites that are poor in nutrients (Rico-Gray & Oliveira 2007). Myrmecophytes that are also myrmecotrophs can receive

Received 28 March 2010; revision accepted 30 May 2010.

[7] Corresponding author; e-mail: genoveva.rodriguez@emg.umu.se

both protective and nutritive benefits from their resident ants. As part of understanding the roles of mutualisms in affecting communities, we also explore the relative roles of these benefits.

The strength of plant–ant interactions is expected to depend on changes in the ant species, herbivores, environmental conditions, and array of antiherbivore defenses used by the host plant (Agren & Schemske 1993, Gaume *et al.* 2005, Kersch & Fonseca 2005). For example, ant colony size, a measure of effectiveness of defense, and colony relocation are correlated with soil nutrient availability (Heil *et al.* 2001, 2002; McGlynn *et al.* 2003). As a result, plants in nutrient-rich soils, such as those of tropical montane soils, could harbor larger ant colonies that are more effective at protection than plants on poor soils, such as tropical lowland ultisols. On the other hand, the lower temperatures in the montane forests can weaken plant–ant interactions (*e.g.*, Rico-Gray *et al.* 1998, Rosumek *et al.* 2009). Thus, the outcome of ant–plant–herbivore interactions can vary among communities, as well as across species' geographical ranges (Thompson 1997, Abdala-Roberts & Marquis 2007). To better understand the plasticity of myrmecophyte–ant interactions, it is helpful to study associations across geographical gradients because different combinations of ants, plant defenses, herbivores, and environmental conditions will determine how effective and costly a plant's defenses are and how much damage plants can sustain without a reduction in fitness (Guimarães *et al.* 2008).

Pheidole–Piper interactions, in which the *Piper* plant provides shelter in the form of domatia and food in the form of food bodies, have been documented for five *Piper* species in Costa Rica (Tepe *et al.* 2007) and two in the northeastern Andes of Ecuador (Tepe *et al.* 2009). Several studies in lowland forests have demonstrated that *Pheidole* ants have an indirect positive effect on *Piper cenocladum* biomass and fitness through decreasing herbivory by *Eois* (Geometridae) caterpillars and other herbivores (Letourneau 1983, 1998). The relationship between *Pheidole* ants and the myrmecophyte *Piper immutatum* is not as well established (Tepe *et al.* 2009). We utilized observational and experimental approaches to examine how the ant mutualists of *P. immutatum* might affect its altitudinal range and examined the mechanism by which ants enhance conditions for these plants, addressing these specific questions: (1) What are the indirect effects of elevation on interactions between *P. immutatum*, *Pheidole*, and associated herbivores? (2) Are ants benefitting *P. immutatum* by providing nutrients or by reducing herbivory (Fig. 1)? (3) Which environmental conditions, within and outside the plants' elevational range, favor nutrient procurement vs. antiherbivore defense?

METHODS

STUDY SYSTEM.—Study sites were located in the northeastern Andes of Ecuador in the provinces of Napo and Sucumbios along an elevational gradient from the Amazonian lowland rain forest to the Andean paramo (200–3200 m asl). Temperature along this gradient drops linearly with elevation at a rate of approximately −0.5°C/200 m in elevation. Precipitation ranges from 3900 to 4500 mm/yr. The sampled area includes three mountain ranges: the Sumaco, Huacamayos, and Reventador regions, as well as continuous lowland and mountainous rain forests, which are part of the Cayambe-Coca and Antisana Gran Sumaco natural reserves.

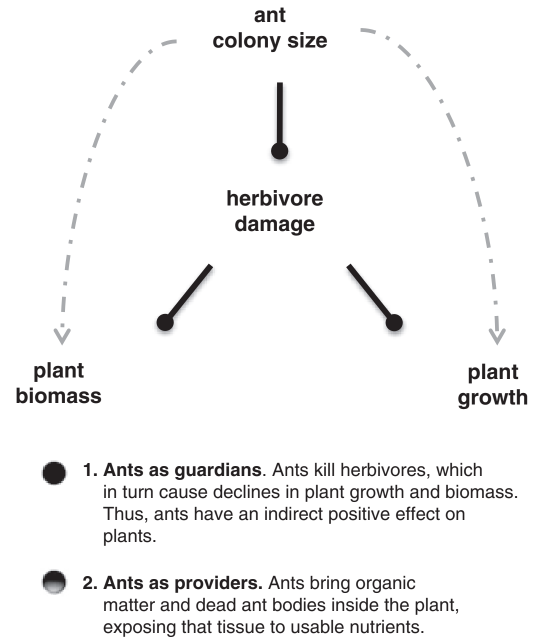

FIGURE 1. Hypothesized causal relationships between *Piper immutatum* biomass, *Pheidole* ant colony size, herbivory, and elevation. Solid lines indicate direct effects and dashed lines denote indirect effects; arrow heads indicate positive effects and bulleted heads denote negative effects; the width of the line is proportional to the hypothesized magnitude of the interaction.

Piper immutatum is an understory shrub that ranges in height from 50 cm to 2 m and occurs in the eastern Andes from Bolivia to Colombia. Its altitudinal distribution in these mountains ranges from 325 to 1500 m asl (Tropicos, MOBOT, 2008). This tropical shrub hosts colonies of a single species of ant throughout its range: *Pheidole* sp. nov. (J. Longino, reference number JTL 174, 2008). This study exclusively includes observations and experiments involving this species of ant from *P. immutatum*. The stems are hollowed out by the ants, and the plants produce food bodies early in plant development (Tepe *et al.* 2009). This adaptation is known from other myrmecophytic *Piper* species that produce food bodies when ant colonies are present (Risch *et al.* 1977, Risch & Rickson 1981). *Pheidole* workers have been observed patrolling new leaves of *P. immutatum* and removing small eggs and early instar *Eois* herbivores from leaves. Based on observations of other *Pheidole–Piper* mutualism, we assumed that the number of queens inside the stem is correlated with ant colony size (Risch *et al.* 1977).

OBSERVATIONAL METHODS.—We pooled data from surveys of *P. immutatum* in 10 m diam vegetation plots (98 surveys in total). We sampled 7 ± 0.81 plots per 200-m interval along a gradient that ranged from 280 to 3200 m. The plots included three main altitudinal transects within the Cayambe-Coca and Antisana natural reserves: the Sumaco region (0°40′ S, 77°44′ W; 1400–2000 m asl), the Huacamayos ridge (0°37′ S, 77°50′ W; 400–2600 m asl) and areas surrounding Volcán Reventador and Cayambe-Coca (0°06′ S, 77°34′ W; 1000–3200 m asl). Inside each plot we tagged the base of all *Piper* plants present, and then harvested all of the leaves and stems of each individual plant leaving the root systems intact. We excavated the roots of all *Piper* plants and kept each individual in a separate bag, identified by plot number and plant number, and transported them to laboratories and rearing centers at Yanayacu Biological Station (2200 m asl) and Jatun Sacha (400 m asl). At the laboratory, each *Piper* plant was weighed and identified to morphospecies. We counted all leaves, measured the leaf area of three leaves per plant, estimated percent herbivory per leaf and carefully surveyed plants for interactions (*i.e.*, herbivorous and predatory arthropods). When the plants were occupied by ants, we placed the ants into labeled vials with 75 percent ethanol. Each caterpillar found was assigned a unique number and reared to adult. Plant specimens were pressed, dried, and sent to the Herbario Nacional del Ecuador, where E. J. Tepe identified the *Piper* spp.; J. Miller at the American Museum of Natural History identified the adult moths; and the ants were sent to J. Longino for identification.

To estimate leaf quality, we collected three fully expanded young leaves of each plant inside each 10 m diam plot for a total of 12 plots for every 200 m in elevation within the Tena-Huacamayos mountain range. Leaves were oven-dried and ground, and nitrogen:carbon ratios were analyzed using combustion analysis at the Tulane University Coordinated Instrumentation Facility.

TRANSPLANT EXPERIMENT.—In order to understand the effect that foraging ants had on *P. immutatum* and to evaluate how elevation and ants influence plant growth, we performed a transplant experiment. We collected 120 *P. immutatum* cuttings (> 50 cm tall, 5 ± 0.11 leaves, each) from a border population located near 1400 m asl (0°40′96″ S, 77°48′27″ W) and transplanted in pots ten cuttings each to locations at 200 m elevational intervals from lowland Amazon forest (400 m asl) to high montane forest (2600 m asl, Table S1). Transplants at 1400 m asl, where all cuttings were obtained, were treated as control plants for elevation and transplant effects. In order to test for the direct effect of ants on *P. immutatum* at the different elevations, we excluded ants from half of the transplanted plants by injecting 1 cm^3 of dieldrin (10%) inside the plant stem once before the plants were transplanted. Dieldrin is a chlorinated hydrocarbon that kills ants or modifies their behavior by blocking inhibitory neurotransmitters in the central nervous system, thus it can have both population-level and behavioral effects on ants. Dieldrin prevents ants from recolonizing plants for at least 1 mo (Campbell 1966, Haynes 1988). Additionally, we blocked ant access to the plants by adding 'Tanglefoot' (The Tanglefoot Company, Grand Rapids, Michigan, U.S.A.) to the edge of the plant pot and clipping any neighboring plants. Tanglefoot application and clipping of neighbor plants were repeated every 3 mo for the first 9 mo of the experiment. All plants were planted in the understory with 95 percent canopy cover. Every 3 mo, we collected data for each individual plant on the number of new leaves, total leaf area, specialist and generalist herbivory and observed species of herbivore and predatory insects. Specialist and generalist herbivory were differentiated via the characteristic damage caused by specialist caterpillars and curculionid or chrysomelid beetles on the leaves (Dyer *et al.* 2010). We measured leaf area of the complete leaf even when it had parts lost due to herbivores. In order to correct for transplant mortality, we replaced any dead plants in all treatments after the first 3 mo. After 18 mo we harvested the plants, estimated ant colony size per plant, weighed the plants, counted and measured the number of new leaves and estimated mean herbivory rates on the new leaves per plant.

STATISTICAL ANALYSIS.—We calculated plant abundance, herbivory, and ant colony size across the elevational distribution of *P. immutatum* to determine the natural distribution of the ant–plant association. Mean plant abundance across the elevational gradient was calculated for each 200 m interval of elevational rise (corresponding to a −0.5°C temperature change) as well as the correlation between elevation and the proportion of plants with ant colonies at 200 m intervals across the altitudinal gradient. We also utilized a logit model with plant mortality as a response variable and elevation and treatment as predictor variables. The gradient was divided into three strata: lowland rain forests (0–1000 m asl), low montane forest (1000–1600 m asl), and a third stratum where *P. immutatum* was transplanted outside its natural range (1600–2600 m; Table 1). Using these three elevation zones, we determined whether treatment or elevation predicted plant survival by the logit model, followed by two-dimensional contingency tables.

To evaluate the effects ants had on the surviving transplants; we grouped plants into three different categories depending on final ant colony size: No ants, < 50 ants, and > 50 ants. We did so to evaluate ant effects on plants because ant exclusions were followed by some ant colonization and some plants without exclusion treatment had no ant colonies. We used MANOVA analysis to evaluate the effects elevation and ant exclusion on net weight as well as the net production of new leaves and percent herbivory by specialists and generalists. The net biomass and new leaf production were measured as the difference between initial and final measurements (*i.e.*, after 18 mo of being transplanted). We then used path analysis to test causal hypotheses about the effects of ants on: (1) final plant biomass (measured as wet plant weight); (2) plant growth (measured from new leaf production); and (3) herbivory (measured as the sum of generalist and specialist herbivory). All statistical analyses were conducted using SAS 9.1.

TABLE 1. *Elevational strata with the elevations and number of transplanted plants included at each stratum of the experiment.*

Elevation zones (m asl)	Specific elevations included (m asl)	Transplanted plants (*n*)	Transplanted plants surviving after 1.5 yr (final *n*)
400–1000	400, 600, 800, 1000	40	25
1100–1600	1200, 1400, 1600	30	15
1700–2600	1800, 2000, 2100, 2200, 2400, 2600	60	18

RESULTS

We surveyed *Piper* species in 98 plots and found that the genus *Piper* occurs up to 3200 m asl along our gradients, but *P. immutatum* is restricted to 400–1600 m asl, with the highest mean abundance at 1400 m asl. This species was absent from elevations above 1600 m asl (Fig. 2). We found a total of 300 individual *P. immutatum* plants. It was the most abundant *Piper* species in plots at riparian sites in lower montane forests (1000–1400 m). Ant colonies in this myrmecophytic plant ranged from 1–15 queens per plant, and *Pheidole* sp. nov. ants occurred on 90 ± 4 percent of the *P. immutatum* individuals collected. *Piper immutatum* plants from lowland forests had 47 percent more queens than the plants found in montane forests (*t*-test = 2.53, $P < 0.01$). In addition to *Pheidole*, we found ants in the subfamilies Ponerinae and Myrmecinae foraging on 5 percent of the lowland plants. Other natural enemies of *P. immutatum* herbivores were spiders (present on 17% of the sampled plants) and predatory beetles.

Mean leaf damage on *P. immutatum* across all elevations was consistent at 13.1 ± 0.6 percent, and there was no significant difference between the leaf damage inflicted by specialist (*Eois* spp.: Geometridae and beetles) vs. generalist herbivores (mainly Phasmatidae, and leafcutter ants 5.4 ± 1.2 and 6.9 ± 1.1, respectively; Wilks' $\lambda = 0.98$, $F = 0.05$, $P > 0.95$). Fifty-seven percent of the myrmecophytes sampled hosted internally feeding herbivores (*i.e.*, leaf miners and gall midges), while exposed feeders (arctiid, geometrid, and pyralid caterpillars, spittlebugs, tettigoniids, gryllids, and phasmatids) were found only on 14 percent of the sampled plants. The remaining plants had no herbivores or herbivore damage. We found three species of specialist caterpillars in the genus *Eois* (Larentiinae: Geometridae) feeding on *P. immutatum*: *Eois nigricosta* Prout., *Eois fusicosta* Dognin, and *Eois* sp. nov. 12.

Path analysis of observational data across the altitudinal range supported the causal hypothesis that increases in elevation cause decreases in: the abundance of *P. immutatum* plants, the mean leaf area per plant, and the colony size of resident ants; leaf nitrogen content increased with elevation (Fig. 3; model fit to the data: $\chi^2 = 0.073$, df = 2, $P = 0.96$). In addition, ant colony size had a positive effect on leaf nitrogen, while leaf nitrogen had a negative effect on herbivory; thus, ant colony size had an indirect negative effect on herbivory, through increasing leaf nitrogen (Fig. 3).

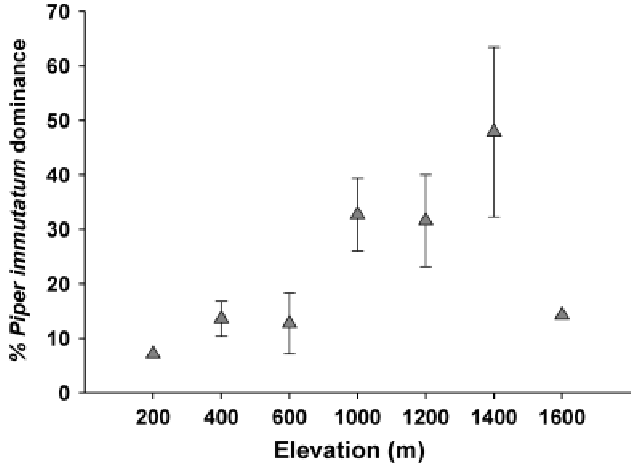

FIGURE 2. *Piper immutatum* dominance (Mean ± SE). Percent of all *Piper* species that were *P. immutatum* in 10 m diam plots along the plant's altitudinal range of distribution.

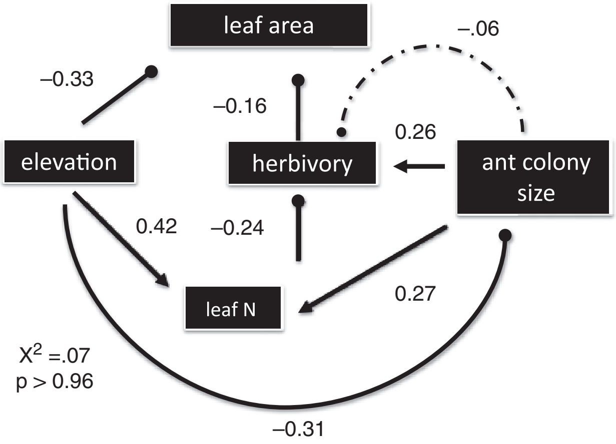

FIGURE 3. Path analysis examining relationships between variation of *Piper immutatum* biomass, herbivory, and ant colony size across a continuous elevational gradient. Solid lines indicate direct effects and dashed lines denote indirect effects. Arrow heads indicate positive effects and bulleted heads denote negative effects; the width of the line is proportional to the magnitude of the path coefficient. Only path coefficients greater or equal to 0.1 are included. $P < 0.05$ for all path coefficients.

Although half of the transplants died, the logit model showed no effect of treatment on plant mortality ($\chi^2 = 0.01$, df = 2, $P > 0.93$). However, the transplanted plants had higher chances of dying at elevations outside of their natural range (greater than 1600 m; $\chi^2 = 6.49$, df = 2, $P < 0.01$; Fig. 4). Survival at elevations ≥ 2200 m was 20 ± 14 percent. After 18 mo, the surviving plants that hosted large colonies of ants had higher leaf production and

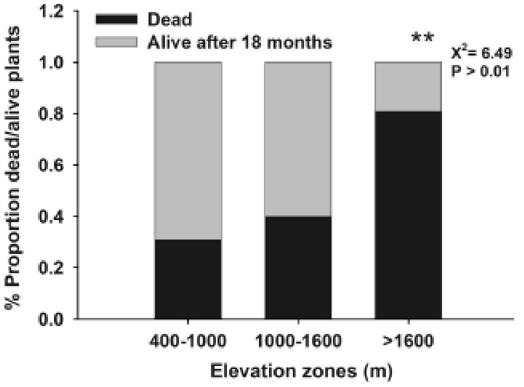

FIGURE 4. Proportion of *Piper immutatum* transplants that died and survived at three elevation zones. **The elevations at which death/survival probabilities were significantly different based on a χ^2 contingency table ($P < 0.01$).

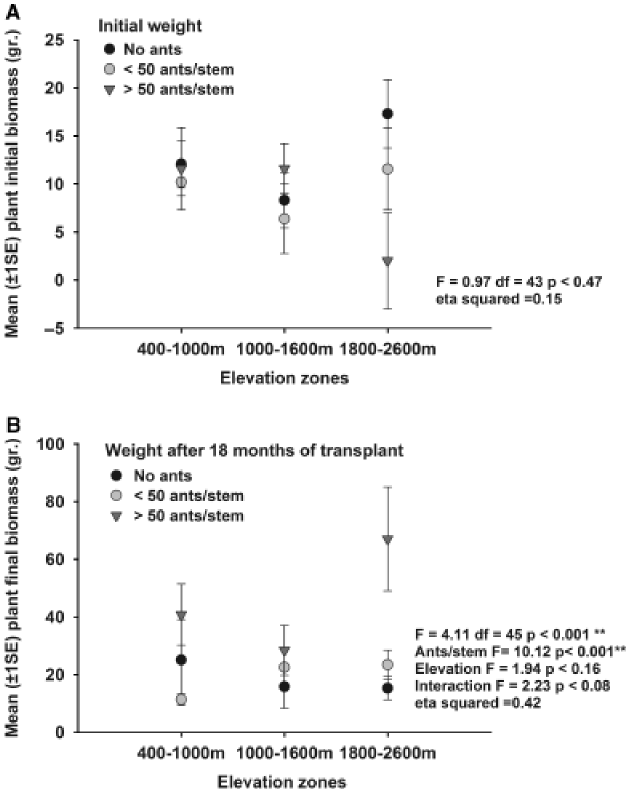

FIGURE 5. Mean (± 1 SE) plant biomass of surviving *Piper immutatum* transplants in response to elevation zones and ant colony size. *F* (Wilks' λ) and *P* values are from MANOVA, with the net growth and biomass as response variables. (A) Initial biomass of cuttings; (B) Biomass after 18 mo.

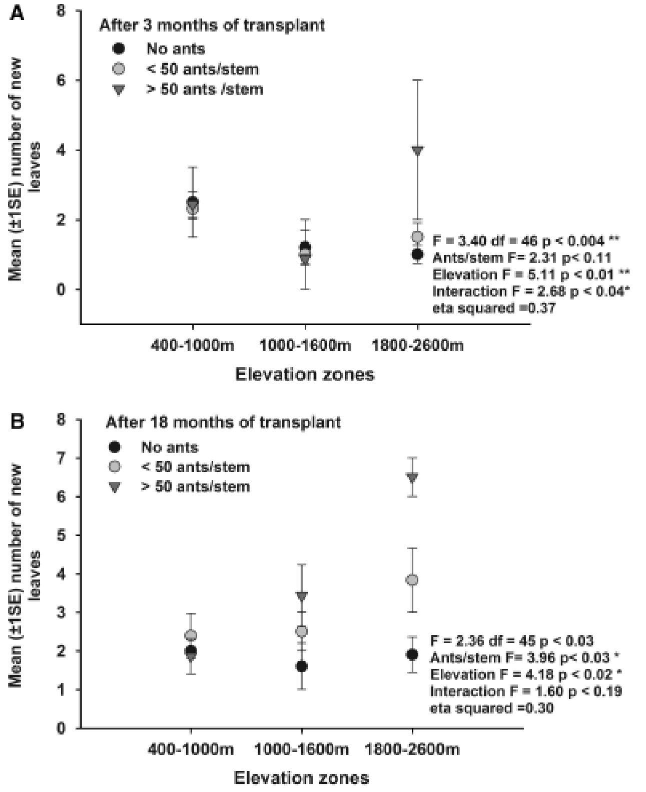

FIGURE 6. Mean (± 1 SE) initial new leaf production of surviving *Piper immutatum* transplants in response to elevation zones and ant colony size. *F* (Wilks' λ) and *P* values are from MANOVA, with net growth and biomass as response variables. (A) After the first 3 mo of transplant; (B) After 18 mo.

biomass. The initial biomass of surviving transplants did not differ among location or ant exclusion treatment ($F = 0.97$, df = 43, $P > 0.47$; Fig. 5A); after 18 mo, we found a significant effect of resident ants on plant biomass, especially at montane elevations outside the species' natural range ($F = 4.11$, df = 45, $P < 0.001$; Fig. 5B).

After the first 3 mo, elevation had a significant effect on plant growth (measured as number of new leaves per plant) and there was a significant interaction with ant colony size ($F = 3.40$, df = 46, $P < 0.004$; Fig. 6A). The effect of ant colony size on plant growth, especially at elevations outside of the species' natural range, was stronger after 18 mo ($F = 2.36$, df = 45, $P < 0.03$; Fig. 6B). We did not find effects of ant colony size or elevation on specialist herbivory after 3 and 18 mo ($F = 0.50$, df = 41, $P > 0.85$, and $F = 0.52$, df = 46, $P > 0.84$, respectively). In addition we did not find effects of ant colony size on generalist herbivory after 3 and 18 mo ($F = 0.43$, df = 39, $P > 0.9$, and $F = 1.30$, df = 46, $P > 0.26$, respectively).

For the surviving plants, after 18 mo the path model of ants protecting and facilitating nutrients to plants across the elevational gradient was a good fit to the data ($\chi^2 = 0.25$, $P = 0.96$; Fig. 7). Based on this model and path coefficients, elevation did not have a significant effect on ant colony size (path coefficient = − 0.02), plant biomass (− 0.03), plant growth (0.004), or herbivory (0.08). We found evidence indicating that ants' facilitation of nutrients explained a large proportion of the variation in plant biomass (0.53) and growth (0.4). Notably, we found the ants also protect the plant from herbivores (0.19) and herbivores had a negative effect on plant biomass (0.11). However it does not cascade to an increase in plant biomass or growth (0.02).

DISCUSSION

Our study documented a robust ant–plant interaction, with ant colonies living inside 90 percent of *P. immutatum* individuals collected throughout the plant's altitudinal range. Furthermore, our transplant experiment supports the hypothesis that the primary benefit of resident ants to *P. immutatum* was the nutrients that the ants provided to the host plant. This effect was more evident at sites outside the plants' range where overall transplant survival rates were the lowest. Plant defense was a secondary benefit, mainly due to low herbivory rates suffered by plants with or without ants. It is clear that such mutualistic interactions are an important component of determining the range of multiple interacting species, because these myrmecophytes also supported specialist herbivores.

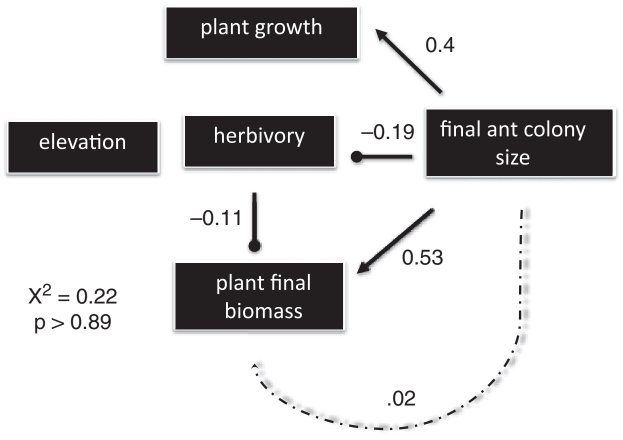

FIGURE 7. Path analysis integrating nutrient procurement and top–down cascade effects of ants on plant biomass and plant growth across an altitudinal gradient. Arrow heads indicate positive effects and bulleted heads denote negative effects; the width of the line is proportional to the magnitude of the path coefficient. Only path coefficients greater or equal to 0.1 are included. $P < 0.05$ for all path coefficients.

In this study, ant colony size positively affected leaf nitrogen content of *P. immutatum* (Fig. 3), and the surviving transplants with large ant colonies had greater biomass and produced a greater number of new leaves (Figs. 5A–B). Thus, our results support the hypotheses that ants are nourishing *P. immutatum* and that the effects of facilitating nutrients to the plants are stronger at elevations outside the plants' natural range. Other *Piper* species for which nutrients are provided by *Pheidole* ants include *Pheidole fimbriulatum*, *Pheidole obliquum*, and *Pheidole sagittifolium* (Letourneau 1998, Fischer *et al.* 2003). *Pheidole bicornis* provide nutrients to these plants via piling organic matter and dead ant bodies inside plant cavities (Letourneau 1998), and *Piper* species can incorporate up to 25 percent of the ants' nitrogen into leaf and stem tissue (Fischer *et al.* 2003). Plant–ant interactions where plants absorb the nitrogen provided by ants (myrmecotrophs) have been documented for several tropical plant genera. Ants in the genus *Philidris* provide carbon through ant respiration and the nitrogen from ant middens to the climber plant *Dischidia major* (Asclepiadaceae) (Treseder *et al.* 1995). The amount of nitrogen provided by the ants can be quite high as in, for example, the lowland Amazon shrub in the genus *Maieta* (Mellastomataceae) which incorporates up to 80 percent of its nitrogen from *Pheidole* ants living in petiolar domatia (Solano & DeJean 2004). Because of the paucity of similar myrmecotrophic studies, especially for nonarboreal plants, the diversity or abundance of this type of myrmecophytic interaction is completely unknown. However, increased nitrogen availability in plants can also increase plant defenses (Coley *et al.* 1985), which is the case for several defensive imides and amides in *Piper* (Dyer *et al.* 2004); thus ants may also have an indirect negative effect on herbivores through increasing plant defenses.

Path analysis of the observed *P. immutatum–Pheidole* interaction in the northeasterm Andes suggests that ants may only have a weak positive indirect effect on plant biomass via reduction in herbivory (Fig. 3). When we eliminated the effect of elevation on ant colony size by transplanting cuttings with and without ants across the gradient, we found evidence that ants protect plants from herbivores. However, the strength of this interaction was weak because leaf herbivory was low for all the *P. immutatum* plants we transplanted. *Pheidole* ants are probably too small to be effective against large generalist predators but can be very effective at cleaning lepidopteran eggs and early instar caterpillars from leaf surfaces (Letourneau 2004). In the lowland forests in Costa Rica, this passive defense can result in trophic cascades when herbivory is high (*e.g.*, Dyer & Letourneau 2003), but a meta analysis on trophic cascades revealed that biotic interactions that cause lower plant productivity are milder in cloud forests (Rodríguez-Castañeda 2009).

Several *Piper* species occur above 3200 m asl, yet the range of *P. immutatum* is limited to below 1600 m asl. In fact, both ants and plants disappeared at higher elevations, which are associated with sharp decreases in temperature that are known to reduce ant abundance (Novonty *et al.* 1999, O'Donnell & Kumar 2006, Sanders *et al.* 2007). Other studies have demonstrated that lower temperatures at high elevations are associated with smaller colony size in social insects (Purcell & Aviles 2007) and lower ant activity (Kaspari *et al.* 2000, O'Donnell *et al.* 2007). Such effects on ant populations are likely to be associated with lower interaction diversity, given the importance of ants in a variety of antagonistic and mutualistic interactions. Plant–ant interactions disappear from habitats with low temperatures and high precipitation (*e.g.*, Koptur 1985, Rico-Gray *et al.* 1998). Nevertheless, the transplanted *P. immutatum* in our experiments showed increased performance at high elevations when ant colonies are present. We suggest the contribution of nutrients by *Pheidole* sp. nov. to *P. immutatum* have important effects on the plant's survival and this effect may become more important when the plant is in a marginal habitat or acclimating to a new habitat where additional nutrient availability in the form of ant middens may be crucial. In combination with other studies demonstrating important roles of antagonistic interactions in determining plant distribution (*e.g.*, Fine *et al.* 2006), our results indicate that characterizing and quantifying antagonistic and beneficial interactions will provide insight into community assemblages.

ACKNOWLEDGMENTS

Funding came from the US National Science Foundation, BSI DEB 0346736 and 0849361. We thank N. J. Sanders and R. Dunn for their comments on an earlier version of this manuscript. Field assistants included: Toni Walters, Andrew Townsend, Wilmer Simbaña, José Simbaña, Lauren Loe, Joseph Sexton, and Kimberly Sheldon. Special thanks to H. Greeney for his support on our creative studies and to J. Longino for identifying the *Pheidole* ants. Finally, we thank the Yanayacu Biological Station and Center for Creative Studies and the Nucanchi Causai community for providing lodging and logistical support.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

TABLE S1. *Latitude, longitude and elevation of sampling. Plots were situated within the elevation range denoted for each site.*

LITERATURE CITED

Abdala-Roberts, L., and R. J. Marquis. 2007. Test of local adaptation to biotic interactions and soil abiotic conditions in the ant-tended *Chamaecrista fasciculata* (Fabaceae). Oecologia 154: 315–326.

Agren, J., and D. W. Schemske. 1993. The cost of defense against herbivores, An experimental study of trichome production in *Brassica rappa*. Am. Nat. 141: 338–350.

Benzing, D. H. 1991. Myrmecotrophy: Origins, operation and importance. *In* C. R. Huxley and D. F. Cutler (Eds.). Ant-plant interactions, pp. 353–373. Oxford University press, Oxford, UK.

Bulleri, F., J. F. Bruno, and L. Benedetti-Cecchi. 2008. Beyond competition: Incorporating positive interactions between species to predict ecosystem invasibility. PLoS Biol. 6: 1136–1140.

Campbell, M. H. 1966. Theft by harvesting ants of pasture seed broadcast on unploughed land. Aust. J. Exp. Agr. Anim. Husb. 6: 334–338.

Christian, C. E. 2001. Consequences of a biological invasion reveal the importance of mutualism for plant communities. Nature 413: 635–639.

Coley, P., J. P. Bryant, and F. S. Chapin III. 1985. Resource availability and plant antiherbivore defense. Science 230: 895–899.

Dyer, L. A., C. D. Dodson., D. K. Letourneau, M. A. Tobler, A. Hsu, and J. O. III Stireman. 2004. Ecological causes and consequences of variation in defensive chemistry of a neotropical shrub. Ecology 85: 2795–2803.

Dyer, L. A., and D. Letourneau. 2003. Top-down and bottom-up diversity cascades in detrital vs. living food webs. Ecol. Lett. 6: 60–68.

Dyer, L. A., D. Letourneau, G. Vega Chavarria, and D. Salazar Amoretti. 2010. Herbivores on a dominant understory shrub increase local plant diversity in rain forest communities. Ecology. in press.

Fiala, B., A. Jakob, and U. Maschwitz. 1999. Diversity, evolutionary specialization and geographic distribution of a mutualistic ant-plant complex: *Macaranga* and *Crematogaster* in South East Asia. Biol. J. Linn. Soc. 66: 305–331.

Fine, P. V. A., Z. J. Miller, I. Mesones, S. Irazuzta, H. M. Appel, M. H. H. Stevens, I. Saaksjarvi, L. C. Schultz, and P. D. Coley. 2006. The growth-defense trade-off and habitat specialization by plants in Amazonian forests. Ecology 87: S150–S162.

Fischer, R. C., A. Richter, W. Wanek, and V. Mayer. 2002. Plants feed ants: Food bodies of myrmecophytic *Piper* and their significance for the interaction with *Pheidole bicornis* ants. Oecologia 133: 186–192.

Fischer, R. C., W. Wanek, A. Richter, and V. Mayer. 2003. Do ants feed plants? A N-15 labeling study of nitrogen fluxes from ants to plants in the mutualism of *Pheidole* and *Piper*. J. Ecol. 91: 126–134.

Folgarait, P. J., and D. W. Davidson. 1994. Antiherbivore defenses of myrmecophytic *Cecropia* under different light regimes. Oikos 71: 305–320.

Folgarait, P. J., and D. W. Davidson. 1995. Myrmecophytic *Cecropia* antiherbivore defenses under different nutrient treatments. Oecologia 104: 189–206.

Gaume, L., M. Zacharias, V. Grosbois, and R. M. Borges. 2005. The fitness consequences of bearing domatia and having the right ant partner: Experiments with protective and non-protective ants in a semi-myrmecophyte. Oecologia 145: 76–86.

Guimarães, P. R. Jr, V. Rico-Gray, P. S. Oliveira, T. Izzo, S. F. Reis, and J. N. Thompson. 2007. Interaction intimacy affects structure and coevolutionary dynamics in mutualistic networks. Curr. Biol. 17: 1797–1803.

Hay, M. E., J. D. Parker, D. E. Burkepile, C. C. Caudill, A. E. Wilson, Z. P. Hallinan, and A. D. Chequer. 2004. Mutualisms and aquatic community structure: The enemy of my enemy is my friend. Annu. Rev. Ecol. Evol. Syst. 35: 175–197.

Haynes, K. F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Annu. Rev. Entomol. 33: 149–168.

Heil, M., B. Fiala, U. Maschwitz, and K. E. Linsenmair. 2001. On benefits of indirect defense: Short- and long-term studies of antiherbivore protection via mutualistic ants. Oecologia 126: 395–403.

Heil, M., A. Hilpert, B. Fiala, R. Bin Hashim, E. Strohm, G. Zotz, and K. E. Linsenmair. 2002. Nutrient allocation of *Macaranga triloba* ant plants to growth, photosynthesis and indirect defense. Funct. Ecol. 16: 475–483.

Holland, J. N., D. L. Deangelis, and J. L. Bronstein. 2002. Population dynamics and mutualism: functional responses of benefits and costs. American Naturalist 159: 231–244.

Janzen, D. H. 1966. Coevolution of mutualism between ants and acacias in Central America. Evolution 20: 249–275.

Kaspari, M., S. O'donnell, and J. R. Kercher. 2000. Energy, density, and constraints to species richness: Ant assemblages along a productivity gradient. Am. Nat. 155: 280–293.

Kersch, M. F., and C. R. Fonseca. 2005. Abiotic factors and the conditional outcome of an ant-plant mutualism. Ecology 86: 2117–2126.

Koptur, S. 1985. Alternative defenses against herbivores in *Inga* (Fabaceae, Momosoidea) over an elevational gradient. Ecology 66: 1639–1650.

Letourneau, D. K. 1983. Passive aggression—An alternative hypothesis for the *Piper-Pheidole* association. Oecologia 60: 122–126.

Letourneau, D. K. 1998. Ants, stem-borers, and fungal pathogens: Experimental tests of a fitness advantage in *Piper* ant-plants. Ecology 79: 593–603.

Letourneau, D. K. 2004. Mutualism, antiherbivore defense, and trophic cascades: *Piper* ant-plants as a mesocosm for experimentation. *In* L. A. Dyer and A. Palmer (Eds.). Piper: A model genus for studies of phytochemistry, ecology, and evolution, pp. 5–32. Kluwer Academic/Plenum Publishers, New York, New York.

McGlynn, T. P., M. D. Shotell, and M. S. Kelly. 2003. Responding to a variable environment: home range, foraging behavior, and nest relocation in the costa rican rainforest ant *Aphaenogaster araneoides*. Journal of Insect Behavior 16: 687–701.

Novonty, V., Y. Basset, J. Auga, W. Boen, C. Dal, P. Drozd, M. Kasbal, B. Isua, R. Kutil, M. Manumbor, and . K. Molem. 1999. Predation risk for herbivorous insects on tropical vegetation: A search for enemy-free space and time. Aust. J. Ecol. 24: 477–483.

O'Donnell, S., and A. Kumar. 2006. Microclimatic factors associated with elevational changes in army ant density in tropical montane forest. Ecol. Entomol. 31: 491–498.

O'Donnell, S., J. Lattke, S. Powell, and M. Kaspari. 2007. Army ants in four forests: Geographic variation in raid rates and species composition. J. Anim. Ecol. 76: 580–589.

Purcell, J., and L. Aviles. 2007. Smaller colonies and more solitary living mark higher elevation populations of a social spider. J. Anim. Ecol. 76: 590–597.

Rico-Gray, V., J. G. Garcia-Franco, M. Palacios-Rios, C. Diaz-Castelazo, V. Parra-Tabla, and J. A. Navarro. 1998. Geographical and seasonal variation in the richness of ant-plant interactions in Mexico. Biotropica 30: 190–200.

Rico-Gray, V., and P. S. Oliveira. 2007. The ecology and evolution of ant–plant interactions. University of Chicago press, Chicago, Illinois, 332pp.

Risch, S. J., and F. R. Rickson. 1981. Mutualism in which ants must be present before plants produce food bodies. Nature 291: 149–150.

Risch, S., M. McClure, J. Vandermeer, and S. Waltz. 1977. Mutualism between 3 species of tropical *Piper* (Piperaceae) and their ant inhabitants. Am. Midl. Nat. 98: 433–444.

Rodríguez-Castañeda, G. Tropics are not flat: Tri-trophic interactions and herbivore diversity across an altitudinal gradient in the north-eastern Andes of Ecuador. Ecology and Evolutionary Biology, Tulane University, Doctoral dissertation, 2009.

Rosumek, F. B., F. A. O. Silveira, F. D. Neves, N. P. D. Barbosa, L. Diniz, Y. Oki, F. Pezzini, G. W. Fernandes, and T. Cornelissen. 2009. Ants on plants: A meta-analysis of the role of ants as plant biotic defenses. Oecologia 160: 537–549.

Sagers, C. L., S. M. Ginger, and R. D. Evans. 2000. Carbon and nitrogen isotopes trace nutrient exchange in an ant-plant mutualism. Oecologia 123: 582–586.

Sanders, N. J., J. P. Lessard, M. C. Fitzpatrick, and R. R. Dunn . 2007. Temperature, but not productivity or geometry, predicts elevational diversity gradients in ants across spatial grains. Global Ecol. Biogeogr. 16: 640–649.

Savage, A. M., and M. A. Peterson. 2007. Mutualism in a community context: The positive feedback between an ant-aphid mutualism and a gall-making midge. Oecologia 151: 280–291.

Schmitz, O. J., P. A. Hamback, and A. P. Beckerman. 2000. Trophic cascades in terrestrial systems: A review of the effects of carnivore removals on plants. Am. Nat. 155: 141–153.

Solano, P. J., and A. Dejean. 2004. Ant-fed plants: comparison between three geophytic myrmecophytes. Biol. J. Linn. Soc. 83: 433–439.

Tepe, E. J., W. A. Kelley, G. Rodríguez-Castañeda, and L. A. Dyer. 2009. Characterizing the cauline domatia of two newly discovered Ecuadorian ant plants in *Piper*: An example of convergent evolution. J. Insect Sci. 27: 1–9.

Tepe, E. J., M. A. Vincent, and L. E. Watson. 2007. Stem diversity, cauline domatia, and the evolution of ant-plant associations in *Piper* sect. *Macrostachys* (Piperaceae). Am. J. Bot. 94: 1–11.

Thompson, J. N. 1997. Evaluating the dynamics of coevolution among geographically structured populations. Ecology 78: 1619–1623.

Treseder, K. K., D. W. Davidson, and J. R. Ehleringer. 1995. Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. Nature 375: 137–139.

Tropicos, Missouri Botanical Garden (MOBOT). 2008. http://www.tropicos.org (accessed 11 August 2008).