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Reviewed work(s):

Source: The American Naturalist, Vol. 179, No. 6 (June 2012), pp. 768-778

Published by: The University of Chicago Press for The American Society of Naturalists

Stable URL: http://www.jstor.org/stable/10.1086/665654

Accessed: 12/06/2012 11:49
The Direct and Ecological Costs of an Ant-Plant Symbiosis

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Submitted July 12, 2011; Accepted February 3, 2012; Electronically published April 25, 2012

Abstract: How strong is selection for cheating in mutualisms? The answer depends on the type and magnitude of the costs of the mutualism. Here we investigated the direct and ecological costs of plant defense by ants in the association between Cordia nodosa, a myrmecophytic plant, and Allomerus octoarticulatus, a phytoecious ant. Cordia nodosa trees produce food and housing to reward ants that protect them against herbivores. For nearly 1 year, we manipulated the presence of A. octoarticulatus ants and most insect herbivores on C. nodosa in a full-factorial experiment. Ants increased plant growth when herbivores were present but decreased plant growth when herbivores were absent, indicating that hosting ants can be costly to plants. However, we did not detect a cost to ant colonies of defending host plants against herbivores. Although this asymmetry in costs suggests that the plants may be under stronger selection than the ants to cheat by withholding investment in their partner, the costs to C. nodosa are probably at least partly ecological, arising because ants tend scale insects on their host plants. We argue that ecological costs should favor resistance or traits other than cheating and thus that neither partner may face much temptation to cheat.

Keywords: Ant-plant interactions, cheating, costs of resistance, evolution of mutualism, indirect plant defense.

Introduction

Mechanisms that maintain cooperation and prevent cheating have been heavily emphasized in the mutualism literature (e.g., Pierce 1987; Sachs et al. 2004; Foster and Kokko 2006; Douglas 2008; Weyl et al. 2010). Although individuals may reap net benefits through cooperation, they may do even better by cheating and taking the benefits of cooperation without paying the costs of reciprocation. However, for natural selection to favor cheating over cooperation, it is necessary (although not sufficient) for cooperation to be costly. There is simply nothing to be gained from cheating in cost-free interactions, which are often termed “by-product mutualisms” (Connor 1995; Sachs et al. 2004; Douglas 2008). Therefore, understanding the costs of cooperation is central to predicting when cheating might evolve.

Ant-plant interactions are classic examples of mutualism (e.g., Janzen 1966) and have figured prominently in the literature on the evolution of mutualism (Bronstein 1998; Yu 2001; Heil and McKey 2003; Sachs et al. 2004; Frederickson 2009; Heil et al. 2009; Weyl et al. 2010). Numerous plant species make food or housing to attract ants, which defend the plants against herbivores or other enemies (reviewed by Heil and McKey 2003). These ants collect extraloral nectar or food bodies from plants, and symbiotic species nest inside live plant cavities called domatia. These ant-plant relationships range from facultative interactions, in which plants typically associate with a diversity of free-living partners, to obligate interactions, which tend to be more species specific (Chamberlain and Holland 2009; Trager et al. 2010). In obligate associations, the plants and ants rely upon each other during much of their life cycles and are rarely found without each other. Here we refer to plant species that make domatia as myrmecophytic and ant species that nest in domatia as phytoecious.

Research on the costs of plant resistance to herbivores provides a useful framework for considering the costs of ant-plant interactions (e.g., Heil 2002; Strauss et al. 2002). The costs of ants to plants are direct costs if they arise from a trade-off between allocating resources to ants versus allocating resources to other functions like growth or reproduction. However, ants can also impose ecological costs on plants if they negatively affect the interactions between their host plants and other organisms in the environment. For example, ants can deter pollinators (Ness 2006) or increase herbivore loads on their host plants (Frederickson and Gordon 2007; Palmer et al. 2008), such as by tending scale insects or aphids (Styrsky and Eubanks 2007). Direct and ecological costs are tightly linked (e.g., investing in
ants instead of growth may reduce a plant’s competitive ability; see also Heil 2002), but nonetheless we think the distinction is useful because of the different implications of direct and ecological costs for the evolution of cheating. When mutualisms involve direct costs, cheaters can achieve higher fitness by investing more in growth or reproduction at the expense of the mutualism. However, it is less clear how individuals might minimize ecological costs by cheating (a term that we use throughout this article to mean withholding the benefits of mutualism). Instead, ecological costs may result in selection for resistance or other traits that do not amount to cheating. Returning to the above example, to keep ants off of flowers, where they can deter pollinators, some ant-acacias have evolved to defend their flowers with ant repellents (Willemer and Stone 1997; Ghazoul 2001; Raine et al. 2002; Nicklen and Wagner 2006) while still provisioning ants with domatia, food bodies, and/or extrafloral nectar.

Mutualisms can also involve other costs, but again they are unlikely to impose selection for cheating. Interacting with a mutualist can result in physical damage, such as the destruction of host reproductive structures by phytoecious ants (Yu and Pierce 1998; Stanton et al. 1999; Izzo and Vasconcelos 2002; Frederickson 2009). This cost of mutualism is also thought to result in selection for defense (e.g., tolerance; Edwards and Yu 2008) more often than in selection for cheating (but see Izzo and Vasconcelos 2002). The evolution of specialization can be considered to be another cost of mutualism (e.g., Bronstein 2001) because the participants lose evolutionary flexibility, such as the ability to live independently or associate with a more beneficial partner. However, costs resulting from specialization cannot be overcome by cheating and should instead result in selection for facultative associations or reversions to autonomy (Sachs and Simms 2006). Furthermore, specialization may also constrain the ability of mutualists to cheat (Moran and Werngreen 2000). Hence, of the many varied costs of mutualism, only direct costs can unambiguously result in selection for cheating.

What do we already know about the direct costs inherent in ant-plant mutualisms? On the plant side, several studies have compared investment in ant rewards with investment in other functions. O’Dowd (1980) estimated that Ochroma pyramidale saplings invest about 1% as much in extrafloral nectar and food bodies as they do in leaves, and Heil et al. (1997) found that Macaranga triloba allocates about 5% of its aboveground biomass to food bodies. Four experimental studies have investigated the costs to plants of maintaining their associated ants. Three found equivocal evidence of costs: Rutter and Rausher (2004) found no negative genetic correlation between fitness and extrafloral nectar production in ant-excluded Chamaecrista fasciculata, and Rudgers (2004) and Rudgers and Strauss (2004) found no fitness differences between ant-excluded Gossypium thurberi producing normal and experimentally reduced amounts of extrafloral nectar. These results are surprising, given the apparent investment by the plant in providing food for ants, although the absence of evidence for costs in these studies does not necessarily amount to evidence of the absence of costs. In addition, C. fasciculata and G. thurberi form facultative associations with ants, and we might expect greater costs in obligate ant-plant symbioses. Indeed, the fourth study concluded that hosting ants is costly to the obligate ant-plant Acacia drepanolobium, on the basis of the results of a multiyear experiment in which exterminating ant colonies actually increased growth and/or reproduction of their host trees (Stanton and Palmer 2011).

On the ant side, few if any attempts have been made to measure the costs to ants of defending plants, even though this behavior is often assumed to be costly (e.g., Yu 2001; Edwards et al. 2006). Phytoecious ants that attack herbivores may or may not also eat them. If they do eat most of the herbivores they attack, then plant defense is likely to be directly beneficial to ants and herbivory reduction should be considered a cost-free or by-product benefit of ants foraging for insect prey on plant surfaces. Alternatively, if a phytoecious ant colony depends entirely on extrafloral nectar or food bodies for food (e.g., Heil et al. 2004) and workers mostly just chase away herbivores, then any resources the colony invests in patrolling or defending a plant may represent a direct cost to the ant colony. For example, workers might expend energy or suffer increased mortality while protecting their host plant.

Here we investigated the direct and ecological costs of defense by the phytoecious ant Allomerus octoarticulatus (Formicidae: Myrmicinae) to the myrmecophytic plant Cordia nodosa (Boraginaceae). We manipulated the presence of ants and most insect herbivores on C. nodosa saplings for almost 1 year in a full-factorial experiment. We predicted that if maintaining A. octoarticulatus colonies is costly to C. nodosa, then in the absence of herbivores, having ants should reduce plant performance. Similarly, if defending C. nodosa against herbivores is costly to A. octoarticulatus, then colonies should perform better in the absence than in the presence of herbivores.

Material and Methods

Study Site and System

This study was conducted at the Los Amigos Research Center (12°34’S, 70°05’W; elevation, ~270 m), in the Department of Madre de Dios, Peru. Surrounding the research center is the Los Amigos conservation concession, which comprises 146,000 ha of primary tropical rain forest.
on a mixture of upland terraces and floodplains. Annual rainfall at Los Amigos is between 2,700 and 3,000 mm, with more than 80% of the precipitation falling during the October–April wet season (Pitman 2008). Mean monthly temperatures range from 21° to 26°C (Pitman 2008).

At Los Amigos and elsewhere in the western Amazon Basin, *Cordia nodosa* associates with several species of ants. The most common is *Allomerus octoarticulatus* (Myrmicinae), which occupies 40%–80% of *C. nodosa* trees in this region (Yu and Pierce 1998; Frederickson 2009). Other *C. nodosa* are occupied by *Azteca* spp. (Dolichoderinae, 10%–35% of trees), *Myrmelachista schumanni* (Formicinae, <2% of trees), or other twig-nesting ant species (very rarely), or else they are not occupied by ants at all (10%–20% of primarily very young trees; Yu and Pierce 1998; Frederickson 2009). *Cordia nodosa* produces domatia—hollow stem swellings on otherwise slender branches (fig. 1)—regardless of whether ants are present. Each time a *C. nodosa* tree grows a new shoot, it produces one domatium together with a whorl of new leaves. If the tree has ants, the colony quickly fills this new domatium with brood and workers. *Cordia nodosa* trees produce food bodies on the surfaces of young leaves and shoots as a reward for ants (Solano et al. 2005). *Allomerus octoarticulatus* colonies also obtain additional food from the honeydew-producing scale insects (Hemiptera: Sternorrhyncha: Coccoidea) they tend inside the domatia (fig. 1). In southeastern Peru, *A. octoarticulatus* appears to be an obligate symbiont of *C. nodosa* (Yu and Pierce 1998), but this species specificity breaks down at larger geographic scales. In Brazil, ants identified as *A. octoarticulatus* have been collected from *Duroia sacifera*, *Hirtella myrmecophila*, *Hirtella physophora*, *Remejia physophora*, and *Tococa* spp. (Wheeler 1942; Fonseca 1999; Izzo and Vasconcelos 2002; Fernández 2007). Although further work is needed to determine whether these collections represent a single species or a complex of closely related species (Fernández 2007), genetic data indicate that throughout the Peruvian Amazon the *Allomerus* ants found in *C. nodosa* are all from one species (Debout et al. 2009).

Previous research on this system has shown that *A. octoarticulatus* defends *C. nodosa* against folivores: the presence of *A. octoarticulatus* ants significantly reduces folivory in ant-exclusion experiments (Yu and Pierce 1998; Fred-

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**Figure 1**: Interaction web showing the principal cost and benefit pathways between *Allomerus octoarticulatus* ant colonies and *Cordia nodosa* plants, as well as the participants manipulated and the variables measured in this experiment. *Cordia nodosa* houses ants and scale insects in domatia (black swellings next to asterisks). Ants reduce folivory but facilitate scale insects. Direct costs to plants of hosting ants come from the production of ant rewards (i.e., domatia, food bodies), whereas ecological costs include the loss of resources to ant-tended scale insects. Solid lines are direct interactions and dashed lines are indirect interactions; some direct and many indirect pathways are omitted for clarity. Lifetime fitness measurements for plants and ants (e.g., seed or alate production) were not possible over the timescale of our experiment, but plant performance is a good surrogate. See text for details.
In 2008, we collected more than 100 fruits from 20 mature
C. nodosa plants at Los Amigos, removed the pulp, and
abraded the seeds and sowed them in seedling trays. We put
plants in grow bags inside two large Lumite screen outdoor
cages (BioQuip Products) to keep them free of ants and
erbivores, and then we left the plants to grow under
ambient light and rainfall conditions for 1 year.

We added queenright A. octoarticulatus colonies to C.
nodosa saplings in the A+ treatment. We collected the
colonies from small, naturally occurring C. nodosa trees
and counted the workers. We then transferred each colony
to a small plastic bag and hung the bags on the recipient
plants; over the next 1–5 days, the ant colonies moved
from the bags into the domatia of the recipient plants. All
but two colonies survived the transfer to new host plants;
the two colonies that died were replaced with new ant
colonies, using the same protocol.

Larger plants received larger ant colonies, but otherwise,
ant colonies were assigned to recipient plants at random.
Because of a scarcity of small C. nodosa trees at the study
site and to allow for some worker mortality during the
transfer, we collected ant colonies from plants that were
somewhat larger than the experimental plants onto which
the colonies were moved. Recipient plants with one do-
matium received ant colonies from plants with one to four
domatia; recipient plants with two domatia received ant
colonies from plants with four to seven domatia; recipient
plants with three domatia received ant colonies from plants
with seven or eight domatia; and recipient plants with four
to eight domatia received ant colonies from plants with
nine domatia. As a result, there was a significant relation-
ship between plant size (i.e., number of domatia, square-
root transformed) and ant colony size (i.e., number of
workers, square-root transformed) at the beginning of the
experiment (\( n = 26, R^2 = 0.21, P = .017 \)). A similar cor-
relation is observed in naturally occurring A. octoarticu-
latus colonies nesting in C. nodosa trees (L. M. Arcila Her-
nández, unpublished data).

To prevent ants from colonizing plants assigned to the
A− treatment, we injected with a syringe 0.2 mL of a
dilute, nonsystemic, pyrethroid insecticide (Cypermethrin,
0.2 mg/mL) into each domatium at the beginning of the
experiment (August 2009) and again in November 2009
and February 2010. All A− plants received insecticide.
When applying the insecticide, we took care to avoid con-
tact between the insecticide and any plant parts other than
the inside of domatia. Leaf-chewing insects fed on the
leaves associated with insecticide-treated domatia; damage
by folivores to A−H+ plants was high (see “Results”). We
did not monitor visits or damage by sap-sucking insects
or other herbivores, and so we do not know whether the
insecticide reduced other forms on herbivory on the A−
plants. However, in a pilot study, no significant differences
were observed in plant growth over 3 months between
ant-free C. nodosa plants treated with Cypermethrin and
ant-free controls (\( n = 12 \); results not shown).

To maintain similar light environments among treat-
ments, all plants were planted inside mesh nets. The nets
covering plants in the H− treatment were staked securely
to the ground. The nets covering plants in the H+ treat-
ment were rolled up so their bottoms hung ~30 cm off
the ground, which is a height that allowed access by most
insect herbivores but kept leaves similarly shaded under-
neath the nets as in the H− treatment. We used a Li-Cor
LI-250A light meter equipped with a LI-190SA quantum
sensor (Forestry Suppliers) to measure photosynthetically
active radiation (PAR) inside and outside the nets at the
beginning (August 2009), middle (February 2010), and

Experimental Addition or Exclusion of
Ants and Herbivores

In 2008, we collected more than 100 fruits from 20 mature
C. nodosa trees at Los Amigos, removed the pulp, and
abraded the seeds and sowed them in seedling trays. When
a seed had germinated and produced two fully expanded
cotyledons, we transplanted the seedling to its own grow
bag. We put plants in grow bags inside two large Lumite
cages to keep them free of ants and herbivores, and then we
left the plants to grow under ambient light and rainfall
conditions for >1 year.

In July 2009, 52 C. nodosa saplings with at least one
domatium were ready for transfer from their outdoor
cages. For each of these saplings, we measured the height
of the sapling, counted the number of leaves and domatia,
and then marked each domatium individually with a thin,
plastic-coated wire. We divided the plants into 13 blocks
of four plants on the basis of their sizes, and within each
block we assigned each plant at random to one of four
treatments. The treatments were as follows: (1) ants ex-
cluded, herbivores present (A−H+); (2) ants added, her-
bizores present (A+H+); (3) ants excluded, herbivores
excluded (A−H−); and (4) ants added, herbivores ex-
cluded (A+H−). We planted the saplings in the rain forest
understory such that each sapling in a block formed the
corner of a 2 m × 2 m square; blocks were separated by
3–30 m along a trail. We hung a mosquito net over each
C. nodosa sapling as we planted the trees. We then built
1-m-tall wire fences around each group of four plants to
prevent mid- to large-sized mammals from making holes in
the mesh nets.

We added queenright A. octoarticulatus colonies to C.
nodosa saplings in the A+ treatment. We collected the
colonies from small, naturally occurring C. nodosa trees
and counted the workers. We then transferred each colony
to a small plastic bag and hung the bags on the recipient
plants; over the next 1–5 days, the ant colonies moved
from the bags into the domatia of the recipient plants. All
but two colonies survived the transfer to new host plants;

Some important folivores of C. nodosa are
adult and larval leaf beetles (i.e., Chrysomelidae) and
orthopterans. It has also been shown that A. octoarticulatus
sterilizes C. nodosa inflorescences, reducing C. nodosa fruit
production by up to 100%, which benefits the ants because
sterilized plants produce more domatia and thus house
larger, more fecund ant colonies (Yu and Pierce 1998;
Dejean et al. 2004; Frederickson 2005, 2009; Szilágyi et al.
2009). In this study, C. nodosa plants were harvested before
they reached reproductive maturity, so the sterilization be-
havior of A. octoarticulatus was not included as a factor
in our experimental design.

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end (June–July 2010) of the experiment. The light environment did not differ among treatments, nor did it differ significantly between the outside and the inside of the nets; see the appendix, available online, for the complete PAR results and analysis. Between August 2009 and May 2010, the plants were monitored monthly. We checked the integrity of the mesh nets and repaired holes as necessary. In February 2010, all 52 mesh nets were replaced with new, larger mosquito nets.

Plants spent a total of 314–329 days in the experiment. In late June and early July 2010, we harvested the plants, collecting one block of four plants each day. We first measured the height of each plant and then cut off, counted, and labeled all of the leaves, aspirating any ants on the leaves into a plastic vial. We also cut off domatia and placed them into individually labeled envelopes that, along with the plant stems, were sealed into Ziploc bags to prevent ants from escaping. *Allomerus octoarticulatus* workers do not venture off their host plants, even when disturbed, so we are confident that we collected almost all of the ants in each colony. To kill the ants and other insects, bags of domatia and stems as well as vials of aspirated ants were frozen for 24–72 h. We cut open each domatium and counted all of the worker ants and scale insects (i.e., mealy bugs and soft scales) inside, and then we preserved a subset of the scale insects (i.e., mealy bugs and soft scales) inside, and then we preserved the entire ant colony (workers, brood, and queen) in 95% ethanol. We also preserved a subset of the scale insects that we found in the domatia; taxonomic identification revealed that the scale insects included the mealy bugs *Dysmicoccus texensis*, *Dysmicoccus brevipes*, *Nipaecoccus cf. nipae*, and *Leptococcus neotropicus* and the soft scales *Coccus hesperidum* and *Pulvinaria floccifera*. Because we did not collect all of the scale insects from each plant, other species may also have been present.

In the field, one of us (G.B.) visually estimated percent folivory for each leaf (*n* = 4,196), using a score of 0–20. We also digitally photographed each leaf against a white background. For a random subsample of 25 leaves per treatment, we measured percent folivory in the digital images, using ImageJ. We analyzed the relationship between the visually estimated scores and the ImageJ results (fig. A1, available online; *R*² = 0.83; *P* < .001) and used the least squares regression equation to convert the visual scores to percent folivory values. We calculated percent folivory for each plant as the mean value for all of its leaves.

### Statistical Analysis

Four of the 13 blocks of plants did not finish the experiment with all of the treatments intact, so these blocks were excluded from the data analysis. In three blocks, one or both of the ant colonies died before the end of the experiment (two colonies in the H+ treatment and two in the H− treatment). In the fourth block, one of the plants lost all of its leaves, apparently because of a fungal pathogen. To improve normality, we square-root transformed all count data (i.e., numbers of ants, scale insects, domatia, and leaves), as well as folivory data, then log-transformed the height data. When we measured several correlated variables, we used principal components (PC) analysis to reduce the data. We used mixed-model ANOVAs or ANCOVAs to analyze the effects of the experimental treatments on folivory, plant size, ant colony size, and number of scale insects, all measured at the end of the experiment. For the folivory data, the ANOVA model included ant treatment (*A*+ or *A*−), herbivore treatment (*H*+ or *H*−), and their interaction effect as fixed factors and block and block × treatment interaction effects as random factors. The ANCOVA model for final plant size had all of these same factors as well as initial plant size as a covariate. For ant colony size and number of scale insects, we modeled data only from plants with ants (i.e., *A*+ plants). The ANCOVA model for final colony size included the final number of domatia and initial colony size as covariates and herbivore treatment (*H*+ or *H*−) as the categorical variable. Similarly, the model for the number of scale insects included final colony size as a covariate and herbivore treatment as a fixed factor. All statistical analyses were performed in JMP 9.0.0. Data are deposited in the Dryad repository (Dryad Data: http://dx.doi.org/10.5061/dryad.144v45c6).

### Results

#### Folivory

Ants significantly reduced folivory on plants, but they were not as effective in reducing folivory as the herbivore-exclusion treatment (fig. 2; ant treatment effect: *F*₁,₈ = 14.93, *P* = .005; herbivore treatment effect: *F*₁,₈ = 22.31, *P* = .002; ant × herbivore interaction effect: *F*₁,₈ = 14.04, *P* = .006). Folivory was very localized, as indicated by the significant block × herbivore treatment interaction effect (*F*₁,₈ = 10.02, *P* = .002). However, the block effect (*F*₁,₈ = 0.851, *P* = .589) and the block × ant treatment interaction effect (*F*₁,₈ = 0.868, *P* = .577) were not significant.

#### Plant Growth

The effect of ants on plant growth depended on whether herbivores were present (fig. 3). We combined our three highly correlated measures of plant size—namely, height, number of domatia, and number of leaves—using PC analysis (see the appendix for the complete univariate re-
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Figure 2: Standing percent folivory on Cordia nodosa plants at the end of the experiment. Shown are back-transformed least squares means; error bars indicate 95% CIs. Most insect herbivores had access to plants symbolized by filled circles (H\(^+/\)H\(^{-}\)) but were excluded from plants symbolized by open circles (H\(^{-}/\)H\(^{-}\)). Plants with and without ants are indicated by A\(^+/\)H\(^{-}\) and A\(^{-}/\)H\(^{-}\), respectively.

Ant Colony Growth
At the end of the experiment, ant colonies were larger in the presence than in the absence of herbivores (least squares means [95% CI]: for H\(^+\), 817 [511–1,195] workers; for H\(^-\), 495 [265–797] workers), but the difference was not statistically significant (herbivore treatment effect: \(F_{1,84} = 1.96, P = .184\)). There was a significant effect of final plant size (i.e., number of domatia: \(F_{1,84} = 4.65, P = .049\)) but not initial colony size (\(F_{1,84} = 0.01, P = .944\)) in the ANCOVA model. We did not find winged males or females in the Allomerus octoarticulatus colonies we collected, which suggests that the colonies were not yet reproductively active.

Scale Insects
Compared with plants in the ant-addition treatments, plants in the ant-exclusion treatments had few scale insects (back-transformed means [95% CI]: for A\(^+\), 60 [37–90] scale insects; for A\(^-\), 1.2 [0–4.5] scale insects). This difference is unlikely to be an artifact of applying insecticide to A\(^-\) domatia. Similarly small numbers of scale insects were found in ant-free Cordia nodosa domatia that had not been treated with insecticide; the four A\(^+\) plants with ant colonies that died during the experiment harbored totals of zero, one, two, and four scale insects at the end of the experiment. Thus, in the absence of ants, few scale insects recruit to or survive in C. nodosa domatia.

Figure 3: Size of Cordia nodosa plants at the end of the experiment, measured as the first principal component (PC1) of height, number of domatia, and number of leaves. Shown are least squares means; error bars indicate 95% CIs. Most insect herbivores had access to plants symbolized by filled circles (H\(^+\)) but were excluded from plants symbolized by open circles (H\(^-\)). Plants with and without ants are indicated by A\(^+\) and A\(^-\), respectively.
Figure 4: Relationship between the number of scale insects and the number of *Allomerus octoarticulatus* workers in a *Cordia nodosa* plant. The line represents the least squares regression. Most insect herbivores had access to plants symbolized by filled circles (H+/H11001) but were excluded from plants symbolized by open circles (H-/H11002).

In the ant-addition treatments, the total number of scale insects was strongly and positively correlated with ant colony size at the end of the experiment, and this relationship did not differ between herbivore treatments (fig. 4; herbivore treatment effect: $F_{1,15} = 1.51, P = .239$; ant colony size effect: $F_{3,15} = 32.83, P < .001$). Therefore, the mesh nets that excluded other herbivores from *C. nodosa* had little impact on scale insect populations, either because the crawlers were small enough to pass through the mesh or because some scale insects colonized plants when the *A. octoarticulatus* colonies were moved at the beginning of the experiment.

**Discussion**

Costs of Ant Defense to *Cordia nodosa*

We found that hosting an *Allomerus octoarticulatus* colony is costly to *C. nodosa*. In the absence of herbivores, plants with ants were 18% shorter and had 40% fewer domatia and 36% fewer leaves than plants without ants at the end of the experiment. The only other experimental study to find evidence for costs in an ant-plant system likewise found that hosting ants is apparently costly for the plant, although the design of that study did not allow the costs to be measured separately from the benefits of using ants for defense (Stanton and Palmer 2011). Numerous studies have found that ant rewards are produced sparingly, which certainly suggests that they are costly to make. For example, Heil et al. (2001) and Palmer et al. (2008) showed that both domatia and extrafloral nectar production can be induced by herbivory, and plants often produce extrafloral nectar and food bodies on only young, expanding leaves, which are the most vulnerable to folivory (Coley and Barone 1996). However, other previous tests of the costs of ants to plants have been inconclusive (i.e., Rudgers 2004; Rudgers and Strauss 2004; Rutter and Rausher 2004).

If larger *C. nodosa* plants have higher fitness, our results show that there is a selective disadvantage to hosting ants when they are not needed to attack or deter herbivores. Previous work on this system suggests that plant size is a key component of fitness for *C. nodosa*. In a multiyear study of *C. nodosa* demography, Frederickson and Gordon (2009) found that smaller plants are more likely to die than larger plants and *C. nodosa* trees live much longer than their ant colonies: an estimated 77 years for a *C. nodosa* tree, compared with only 7.8 years for an *A. octoarticulatus* colony. The same study showed that after an ant colony dies, a plant will get smaller until it also dies, unless a new queen founds a colony in its domatia and reverses the growth trend; larger plants are therefore more likely to survive periods between ant occupations and reach sexual maturity. An unresolved issue is what effect sterilization of *C. nodosa* floral buds by *A. octoarticulatus* has on plant fitness (Frederickson 2009). We know that sterilized plants grow faster (Frederickson 2009); larger plants, in turn, are more likely to survive until they are colonized by other, nonsterilizing ant species (Frederickson and Gordon 2009), and once they are occupied by nonsterilizing ants, larger *C. nodosa* trees produce more fruit (Yu and Pierce 1998). Therefore, although sterilization substantially reduces current reproduction (Frederickson 2009; Szilágyi et al. 2009), it may enhance future reproduction and ultimately have little or even a positive effect on lifetime fitness (as suggested for *Acacia drepanolobium* in Palmer et al. 2010). Cumulatively, the evidence indicates that the smaller size of plants with ants than without ants that we observed in the absence of herbivores likely has fitness implications for *C. nodosa*, but the benefits of housing ant colonies outweigh the costs when herbivores are present.

The costs to *C. nodosa* of hosting *A. octoarticulatus* could be direct costs if they result from the allocation of resources to feeding or housing ants. However, even though making domatia may be costly, this is unlikely to explain the results of our experiment because *C. nodosa* produces domatia regardless of whether ants are present (fig. A2b, available online), and the presence of ants does not increase domatia size (M. E. Frederickson, A. Ravenscraft, L. M. Arcila Hernández, and G. A Miller, unpublished data). Unlike some
Costs of Plant Defense to A. octoarticulatus

We could not detect a cost to A. octoarticulatus of defending C. nodosa against herbivores. Much like larger plants, larger ant colonies are likely to have higher fitness, because the production of reproductive by A. octoarticulatus colonies is highly correlated with C. nodosa size (Yu and Pierce 1998; Frederickson 2006; Frederickson and Gordon 2009), which is also correlated with ant colony size (i.e., number of workers; this study). Although ant colonies were almost twice as large on average in the H+ treatment than in the H− treatment at the end of the experiment, this difference was not statistically significant because of the large variance in ant colony size and the small number of replicates in each
treatment (nine); further study will be necessary to investigate this possible relationship.

Another explanation for our results is that the costs to A. octoarticulatus of patrolling and defending C. nodosa are fixed and did not vary significantly between treatments. During the experiment, we observed large numbers of workers on the young leaves of both the H+ and the H– plants. A previous study of this system assumed that patrolling young leaves imposes a direct cost to A. octoarticulatus colonies, because the resources tied up in patrollers could otherwise have been used to make reproductive (Edwards et al. 2006). However, workers not only patrol plants, they simultaneously engage in actively gathering food for their colonies. Allomerus octoarticulatus workers that visit young leaves both collect food bodies and attack the insect herbivores that are attracted to these tender, nutritious leaves. Attacking insect herbivores may represent defense to C. nodosa, but it represents foraging for food to A. octoarticulatus and may directly benefit A. octoarticulatus colonies.

A similar argument has been made about pollinators going back at least as far as Darwin (1876, p. 419), who said,

That insects should visit the flowers of the same species for as long as they can, is of great importance to the plant, as it favours the cross-fertilization of distinct individuals of the same species; but no one will suppose that insects act in this manner for the good of the plant.

Unlike in plant-pollinator interactions, however, in symbiotic ant-plant associations an ant colony can derive both a direct benefit from foraging for insects as food and an indirect benefit from the increased growth of their host plant when the plant is released from herbivory. Some phytoecious ants may consume nothing but extraloral nectar and/or food bodies (e.g., Pheidole bicornis [Fischer et al. 2002] and Pseudomyrmex on Acacia [Heil et al. 2004]), even though they have been shown to significantly reduce herbivory on their hosts (Janzen 1967; Letourneau et al. 1998), in which case they may reap only the indirect benefits of plant defense. But many, such as A. octoarticulatus, eat at least some of the insects that visit their host plants (e.g., Yu and Pierce 1998; Tillberg 2004). These ants are probably not under strong selection to cheat by not protecting their host plants against herbivores, and they might be expected to be more effective against palatable herbivores than unpalatable ones.

Cheating and the Evolution of Mutualism

How strong is the temptation to cheat in the ant-plant symbiosis between A. octoarticulatus and C. nodosa? Our results show that hosting A. octoarticulatus is costly to C. nodosa, so one might therefore expect that plant investment in ant rewards should be subject to stabilizing selection, with the optimal investment determined by the balance between the benefits of having ants to reduce herbivory and the costs of feeding or housing them. However, although C. nodosa could reduce its direct costs by skimping on the production of food bodies or other ant rewards, it is less clear how C. nodosa might cheat to reduce its ecological costs, such as the loss of resources to scale insects. We speculate that this cost may instead result in selection for resistance traits, such as tough inner walls in domatia or the production of secondary chemicals in plant sap. Similarly, another cost of hosting A. octoarticulatus that becomes apparent only when C. nodosa begins to flower is host-plant sterilization by this ant species (Yu and Pierce 1998; Frederickson 2009), and again C. nodosa may have evolved a defensive mechanism to minimize this cost: Edwards and Yu (2008, p. 95) proposed that C. nodosa “tolerates castration by directing floral growth to new shoots,” where ants are less likely to attack flowers. Therefore, even in situations where mutualism is maintained at a cost, selection may not favor cheating if the cost is not a direct one.

Our results suggest that plant defense may be a byproduct benefit of A. octoarticulatus workers foraging for insect prey on their host plants. In general, we expect cheating by not defending a host plant to evolve more readily in systems where ants rely exclusively on their host plants for food than in those where resident ants eat herbivorous insects. Other ant behaviors, such as pruning vegetation or removing epiphytes from host plants, may be more prone to invasion by cheaters, although even these behaviors have been suggested to be directly beneficial to the ants in some ant-plant systems (Davidson et al. 1988).

Most plant-animal mutualisms, not just ant-plant systems, result from plants benefiting from animal foraging. For example, most animals that transfer pollen between flowers or disperse seeds do so as they forage for nectar, pollen, or fruit. In general, vectoring pollen or seeds is probably no more costly to these animals than protecting C. nodosa by foraging for insect prey is to A. octoarticulatus. Therefore, selection for cheating (by failing to move pollen or seeds while still consuming nectar, pollen, or fruit) within pollinating or seed-dispersing animal lineages should be relatively rare and confined to those interactions in which reciprocating the mutualism is costly. This may explain why many “cheaters” in plant-animal mutualisms are not derived evolutionarily from mutualistic lineages (i.e., they are “cheating from without,” sensu Bronstein et al. 2006). Conversely, rewarding animals is often costly to plants (e.g., Pyke 1991), but whether these costs are largely direct or ecological is an open question. Thus, the asymmetry in costs that we observed between C. nodosa and
A. octoarticulatus may be the norm in plant-animal mutualisms. Measurement of direct and ecological costs in other systems may provide further insight into the evolution of cheating and the persistence of mutualisms.

Acknowledgments

We thank G. Watson of the California Department of Food and Agriculture and D. Kondo of the Corporación Colombiana de Investigación Agropecuaria (CORPOICA) for identifying scale insects; I. Aggarwal for analyzing leaf images; A. Coral, L. Flores Quijpe, and J. Sanders for field assistance; the staff at the Los Amigos Research Center for logistics; and the Peruvian Ministry of Agriculture for issuing permits (394-2009-AG-DGFFS-DGEEFS and 79-2008-INRENA-IFFS-DCB). We also thank members of the Thomson and Frederickson laboratories at the University of Toronto for comments and discussion and M. Heil, M. Stanton, and three anonymous reviewers for their insightful comments on earlier drafts. This research was funded by a Natural Sciences and Engineering Research Council Discovery Grant (to M.E.F.) and a National Science Foundation grant (SES-0750480; to N.E.P.). G.A.M. was supported by a Foundational Questions in Evolutionary Biology Postdoctoral Fellowship from Harvard University (funded by the Templeton Foundation), A.R. was supported by a Benjamin A. Trustman Fellowship from Harvard University, and G.B. was funded by the Independen Experiential Study Program of the Faculty of Arts and Science at the University of Toronto.

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