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No Defensive Role of Ants throughout a Broad Latitudinal and Elevational Range of a Cactus

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ABSTRACT

Defensive mutualisms mediated by extrafloral nectaries are particularly variable; their net results may change with seasons, communities and environmental contexts. Particularly, an environmental factor that can promote changes in outcomes of ant-plant interactions is elevation in mountainous regions. We tested whether (1) the interaction between the cactus *Opuntia sulphurea* and ant visitors of extrafloral nectaries is a defensive mutualism; and (2) ant-plant interaction outcomes vary with elevation as a result of changes in herbivory rate and ant activity. To evaluate if the outcome of interactions was consistent at two extremes of the range distribution of *O. sulphurea*, we performed an ant-exclusion experiment with plants at two growth conditions (natural or potted) in two sites with contrasting elevation (1235–1787 m asl) in a temperate region (Villavicencio Nature Reserve, Mendoza, Argentina), and in a tropical region (Huajchilla, La Paz, Bolivia). Although herbivory rate and ant visitation frequency increased with elevation, herbivore damage, plant reproductive success, and cladode growth rate were similar between plants excluded and non-excluded from ants among sites, geographic regions and growth conditions. These results do not support the hypotheses that the interaction between *O. sulphurea* and ants is a defensive mutualism, and that elevation affects the net outcome of this ant-plant interaction.

Abstract in Spanish is available in the online version of this article.

Key words: ant-plant mutualism; commensalism; geographic variation; insect abundance; temperate region; tropical region.

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ANT-PLANT DEFENSIVE MUTUALISMS INVOLVE THE EXCHANGE OF RESOURCES AND SERVICES between ants and plants, whereby plants offer food rewards (*e.g.*, food bodies, extrafloral nectar) or shelter (*e.g.*, domatia) to attract ants, which in turn defend the plant against herbivores, pathogens, and encroaching vines (Janzen 1966). Costs and benefits for both partners result from the interaction among three actors: protective species (ants), protected species (plants), and their natural enemies (*e.g.*, herbivores; Bronstein 1998). The effects of ants on herbivory rates and plant fitness may therefore depend on the abundance, activity, behavior, and composition of mutualists and herbivores (Barton 1986); changes in these may alter the net result of ant-plant interactions (*i.e.*, the ant-plant interaction outcomes) from mutualism to commensalism or even antagonism (see Rico-Gray & Oliveira 2007). Defensive mutualisms mediated by extrafloral nectaries (myrmecophilic) are particularly variable (Rico-Gray & Oliveira 2007, Chamberlain & Holland 2009, Rosumek *et al.* 2009); their net outcomes can vary due to diet flexibility and activity patterns of ants and herbivores, the defensive abilities of ants species involved, nectar quality and quantity, and the plant species growing around the protected plant (Morris *et al.* 2005, Ness *et al.* 2006, 2009, Rico-Gray & Oliveira 2007, Jezorek *et al.* 2011, LeVan *et al.* 2013, Miller 2014). However, we still know relatively little about the mechanisms responsible for such variation; for example, although several studies have shown that interaction outcomes vary due to changes in the abundance of the species involved in the interaction (*e.g.*, Di Giusto *et al.* 2001), the causes of that abundance shift have not been determined.

An environmental factor that may promote changes in the outcomes of ant-plant interactions is the elevational variation in mountainous regions (Keeler 1979, Rodríguez-Castañeda *et al.* 2011). At higher elevations solar and UV-B radiation tend to increase, while atmospheric pressure and temperature decrease (Körner 2007). These changes may affect both plants and insects in multiple ways. In plants, for example, foliar nitrogen concentration increases and alkaloid and terpene concentration decrease with elevation (Cordell *et al.* 1998, Hengxiao *et al.* 1999, Salmore & Hunter 2001). For insects, their abundance, density, richness, activity, and overall size can change with increasing elevation. Ants, in particular, are less active at higher elevations, and their abundance, density, and richness are lower (Kaspari *et al.* 2000, O'Donnell *et al.* 2007). Similarly, the overall activity of herbivorous insects and their removal and damage of plant tissues

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decrease with increasing elevation (Suzuki 1998, Hengxiao *et al.* 1999). These biotic changes result mainly from the lower temperatures and shorter growth periods characteristic of high elevations (Hodkinson 2005). Depending on whether and how ant and herbivore activity change with elevation, the outcomes of their interactions with plants might also change (Keeler 1979, Koptur 1985, Rodríguez-Castañeda *et al.* 2011).

Young cladodes of the cactus *Opuntia sulphurea* are frequently visited by ants in both a temperate (Villavicencio Nature Reserve, Mendoza, Argentina) and tropical region (La Paz Valley, La Paz, Bolivia). Field observations showed that ants feed on secretions from extrafloral nectaries located at the thorns' base (Fig. 1) and defend plants against herbivorous insects. These observations suggest that this ant-cactus interaction is a defensive mutualism. Our aims were to test two hypotheses regarding the role of the ant-plant interaction as a defence mutualism for *O. sulphurea* and its geographic variation. The first hypothesis is that the interaction between *O. sulphurea* and ants is a defensive mutualism. To evaluate this hypothesis and whether the outcome of the interaction is consistent across the distributional range of *O. sulphurea* we conducted ant-exclusion experiments in two regions located close to the southern and northern distributional extremes of *O. sulphurea* – Villavicencio Nature Reserve, Mendoza, Argentina, and La Paz Valley, Bolivia. If our hypothesis is correct, we expect that plants excluded from ants (treatment) will be more damaged by herbivory, and consequently will have lower growth rate of young cladodes and lower seed production than plants visited by ants (control) in both regions.

The second hypothesis poses that the outcome of the ant-cactus interaction changes with elevation due to changes in activity and composition of ants and herbivores. There are nine possible scenarios for this hypothesis, depending on the elevational variation in ant and herbivore activity (Table 1). For instance, if herbivore activity decreases with increasing elevation and ant activity does not change, the difference between ant-exclusion and control treatments in herbivore damage, plant growth, and reproductive success should be greater at lower elevations (Table 1, scenario 1). Alternatively, if ant activity decreases with increasing elevation while herbivore activity remains unchanged, the difference between ant-exclusion and control treatments in herbivore damage, plant growth, and reproductive success should be greater at lower elevations (Table 1, scenario 6). Other scenarios are possible, depending on the elevational variation in ant and herbivore activity (Table 1). To evaluate this hypothesis we conducted field experiments at two sites located at contrasting elevations in Villavicencio Nature Reserve, Mendoza, Argentina.

TABLE 1. *Alternative scenarios of elevational variation in ant-plant-herbivore interactions. Comparisons are presented for low elevation (lower) and high elevation (upper) sites in ant visitation frequency, herbivore damage, and plant performance (growth and reproductive success). Scenarios in boldface are those predicted by our hypothesis.*

Scenario	Ant visitation frequency	Herbivore damage in ant-exclusion plants	Difference in herbivore damage and plant performance between plants excluded and non-excluded from ants
1	Lower = Upper	**Lower > Upper**	**Lower > Upper**
2		Lower < Upper	Lower < Upper
3		Lower = Upper	Lower = Upper
4	Lower > Upper	Lower > Upper	Lower > Upper
5		Lower < Upper	Lower ≤ Upper
6		**Lower = Upper**	**Lower > Upper**
7	Lower < Upper	Lower > Upper	Lower ≥ Upper
8		Lower < Upper	Lower < Upper
9		Lower = Upper	Lower < Upper

FIGURE 1. Extrafloral nectaries of *Opuntia sulphurea*. (A) New cladode with several nectar drops. (B) New cladode at more advanced stage of development, nectaries are located in each areola. Photography credits: A. M. Alma.

METHODS

STUDY AREA AND PLANTS.—*Opuntia sulphurea* is a short cactus with aligned, spreading cladodes. The variety found in Mendoza, *O. sulphurea* var. *sulphurea*, occurs between 32° and 29° S from 500 to 2000 m of elevation. The variety found in La Paz, *O. sulphurea var. hildemanii*, occurs between 24° and 16° S from 2000 to 3500 m (Kiesling & Ferrari 2005). The most salient difference between *O. sulphurea var. sulphurea* and *O. sulphurea var hildemannii* is the fruit color: yellow in the former, red-violet in the latter. Nevertheless, their growth habit and flowers are very similar (Figure S1). Production of extrafloral nectaries and ant visitation are common in the Cactaceae in general and in *Opuntia* in particular (Oliveira *et al.* 1999, Robbins & Miller 2009, Miller *et al.* 2010, Miller 2014), but to our knowledge there are no specific studies in *O. sulphurea*.

Fieldwork was carried out during the 2012–2013 Austral spring-summer at two distant regions (~1700 km): Villavicencio Nature Reserve, Mendoza, Argentina (68°50′ and 69°10′ W, and 32°25′ and 32°35′ S) and La Paz Valley, Bolivia (68°04′ and 68°04′ W, and 16°38′ and 16°39′ S). In Villavicencio we selected two sites at contrasting elevations: 1235 (hereafter 'lower site') and 1787 m asl (hereafter 'upper site'), and in La Paz we selected one site: 3050 m asl (Huajchilla), ~12 km from La Paz city. We attempted to sample in two sites in each region; however, the plants from the upper site in La Paz were burnt, which prevented us from completing the study in that site.

SAMPLING DESIGN.—To evaluate whether the ant-*O. sulphurea* interaction is a defensive mutualism and whether its outcome varies with latitude and elevation, we conducted ant-exclusion experiments at lower and upper elevation sampling sites in Argentina and Bolivia. We performed ant-exclusion experiments in mature individual plants occurring in the field (hereafter 'natural plants') as well as in young plants obtained by culturing cladodes in pots. The sizes of natural and potted plants were different; whereas natural plants had between 6–70 cladodes, potted plants had only 2–6 cladodes. The use of potted plants allowed us to ensure similar growing conditions of ant-excluded and non-excluded plants (*i.e.*, same soil substrate and environmental conditions) and the exclusions of ants (ants are difficult to exclude from natural plants because of shape and growth plant). Potted plants were vegetatively propagated using healthy cladodes (without damage by pathogens or herbivores) collected nearby the study sites in January and February 2012. Before potting, harvested cladodes were left on the ground at the collection sites during 20 days to promote healing and prevent infections. Then one cladode was planted in each pot filled with humus and a top layer of sand to stimulate root growth and reduce plant stress (Mondragon-Jacobo & Pimienta-Barrios 1999). In August 2012, 25 and 21 pairs of young plants in their pots were dug 15–20 cm into the ground every 4 m along an 84–100 m transect in sites in Villavicencio and La Paz, respectively. Pots within each pair were separated by 20 cm, and one plant of each pair was randomly assigned to either control or ant-exclusion treatment.

We also selected 15 pairs of natural plants with similar size and phenological status at lower and upper sites in Villavicencio. Plants were separated by 1–5 m within pairs and 10–20 m among pairs and arranged along a 150–250 m transect. Plants within each pair were randomly assigned to control or ant-exclusion treatments. Studies on natural plants were conducted only in Villavicencio because of the difficulty of getting materials for ant exclusions in La Paz. To exclude ants from these plants we placed around treatment plants a plastic band of 30 cm of height, with the lower 10–15 cm dug into the ground and the remaining 15–20 cm protruding above the ground. Around plastic bands and pots we applied a ~5–7 cm layer of Tree Tanglefoot®, a plant resin that forms a sticky barrier against ants. We inspected plants regularly to check ant exclusion, adding extra resin when necessary. We also removed any natural bridges providing aerial access of ants to excluded plants, including parts of surrounding plants.

On each study plant we measured the following response variables: ant species composition, ant visitation frequency to young cladodes and ant behavior, herbivore damage, seed production, seed to ovule ratio, and growth rate of young cladodes. In La Paz we recorded all response variables except seed production and seed to ovule ratio because the fruits were lost.

ANT COMPOSITION, ACTIVITY, AND BEHAVIOR.—To determine the composition of ant species visiting extrafloral nectaries, at the onset of the study we collected ant workers at naturally occurring plants not used in the exclusion experiments at each study site. Ants were preserved in 70 percent ethanol and subsequently identified in the laboratory with the aid of taxonomic keys (Fernández 2003, W. Mackay & E. Mackay, unpubl. data).

We assessed ant activity by estimating the frequency of ants visiting young cladodes. For that purpose, we randomly selected 20 control plants from each site (10 natural and 10 potted plants) and counted the number of ants from each species visiting a randomly selected cladode per plant during 1 min. Censuses were performed at sunrise (0600–0700 h), mid-morning (0900–1000 h), and noon (1200–1300 h) every 15 days from October 2012 to February 2013 in Villavicencio, and from December 2012 to February 2013 in La Paz. To test the assumption that ant species composition and ant visitation frequency recorded in the morning/noon were similar to those detected at afternoon/night, we compared data recorded through a 16 h census carried out in summer (0600–2200 h) at the two sites in Villavicencio.

While recording ant visitation frequency, we also recorded ants' responses to the presence of herbivores on *O. sulphurea*, which we characterized as either antagonistic or neutral (Nogueira *et al.* 2012). Behavior was considered antagonistic when the ant pursued, attacked or arched its gaster against the herbivore; and neutral when the herbivore's presence did not affect the ant's behavior (*i.e.*, there was no body contact or other evidence of aggression).

HERBIVORES AND HERBIVORE.—Throughout the study, we collected and indentified the herbivorous insects found in *O. sulphurea* to the lowest possible taxonomic level. Herbivore activity was estimated using herbivore damage on ant-excluded plants.

To estimate herbivore damage in cladodes, one to five new cladodes per plant were photographed in February 2013 (5 months after the start of ant exclusions), when they had fully developed. Because not all plants produced new cladodes during the study period, the damage caused by herbivores to young cladodes was estimated for a subset of the studied plants (Villavicencio: 89 of 100 potted plants and 58 of 60 natural plants in the lower and upper sites, respectively; La Paz: 32 of 40 potted plants, only in the lower site). Herbivore damage included scars, chlorotic and necrotic marks, and any signs of internal damage on the cladodes (Figure S2; Miller 2007). Using a 5 mm × 5 mm grid overlaid with the photographs, we estimated the percent area damaged or consumed by herbivorous insects, averaged among cladodes when plants had multiple cladodes.

PLANT REPRODUCTIVE SUCCESS.—In addition to herbivore damage we measured plant reproductive success, because herbivory itself is a poor indicator of the ant effect on plant fitness. This variable was estimated using two indicators: seed production per plant and seed to ovule ratio (Wiens *et al.* 1987), as plants are likely to develop fewer seeds if they have fewer ovules. To this end, once fruits had developed (in late January), we counted the number of fruits per plant and the number of seeds and ovules (or primordial seeds) in up to three randomly selected fruits per plant.

GROWTH RATE OF NEW CLADODES.—To estimate growth rate, we photographed one cladode in each control and ant-excluded plants in the lower and upper sites of Villavicencio and the lower site of La Paz. Digital photographs were taken biweekly, since new cladodes were detected (October 2012) until they finished growing (February 2013). In each photograph we measured the greatest width and length of the new cladode with Image J software; with the resulting growth curves we calculated the average time in which cladodes reached their final size (70 and 78 days at Villavicencio and La Paz, respectively). With these data we calculated the growth rate for length (G_L) and width (G_W) as:

$$G_L = (L_{\text{final}} - L_{\text{initial}})/t$$

$$G_W = (W_{\text{final}} - W_{\text{initial}})/t$$

where L_{initial}, L_{final}, W_{initial}, and W_{final} are the greatest length and width at the beginning and the end of the growth season, respectively, and t is the time elapsed between the initial and final measurements. The cladodes that dried from unknown causes were excluded from the analysis.

STATISTICAL ANALYSIS.—We used *t*-tests to assess whether ant visitation frequency was similar between potted and natural plants separately for the upper and lower sites in Villavicencio; because visitation frequency was higher in natural plants (see results), the remaining variables were analyzed separately for potted, and natural plants. Ant relative abundance was compared among sites and species with a two-way ANOVA and Duncan a *posteriori* test.

To determine whether the ant-cactus interaction is a defensive mutualism, we compared the herbivore damage, plant reproductive success and growth rate among plants with and without ants using Mann–Whitney *U*-test. The variation in ant and herbivore activity between the lower and upper sites was analyzed by a *t*-test or a Mann–Whitney *U*-test, depending on whether the data satisfied the normality and homogeneity assumptions. To evaluate the effect of elevation on the outcome of the interaction we used Mann–Whitney *U*-test, using as response variables the differences in herbivore damage, plant reproductive success and growth rate between plants with and without ants.

We calculated Cohen's *d* (Cohen 1988) as a standardized estimator of the magnitude of effects for each test performed. Cohen's benchmark values are $d < 0.2$, $0.2 \le d < 0.8$ and 0.8 to infinity, for small, medium, and large effects respectively.

RESULTS

ANTS AND HERBIVORES ASSOCIATED WITH *OPUNTIA SULPHUREA*.—We found 80–100 percent of natural and potted plants in Villavicencio and La Paz had at least one ant-visitor during the study period. Six species of ants from three subfamilies visited extrafloral nectaries of *O. suphurea* in Villavicencio: *Camponotus mus*, *Brachymyrmex* sp., *Camponotus* sp. (Formicinae), *Pheidole* sp. (Myrmicinae), *Forelius* sp.1, and *Dorymyrmex* sp.1 (Dolichoderinae). We recorded the same ant species in lower and upper sites in Villavicencio, and the frequency of ants that visited the extrafloral nectaries was strongly correlated between morning/noon and afternoon/night (Spearman's $r = 0.74$, $P < 0.0001$). In La Paz, we recorded four species of ants from two subfamilies: *Camponotus compositor* (Formicinae), *Forelius* sp. 2, *Dorymyrmex* sp. 2 and *Linepithema* sp. (Dolichoderinae). *Camponotus mus* was the most frequent visitor species in Villavicencio, followed by *Brachymyrmex* sp., especially at the upper site. The visitation frequency of the other species was much lower at both study sites and growth conditions (Fig. 2A

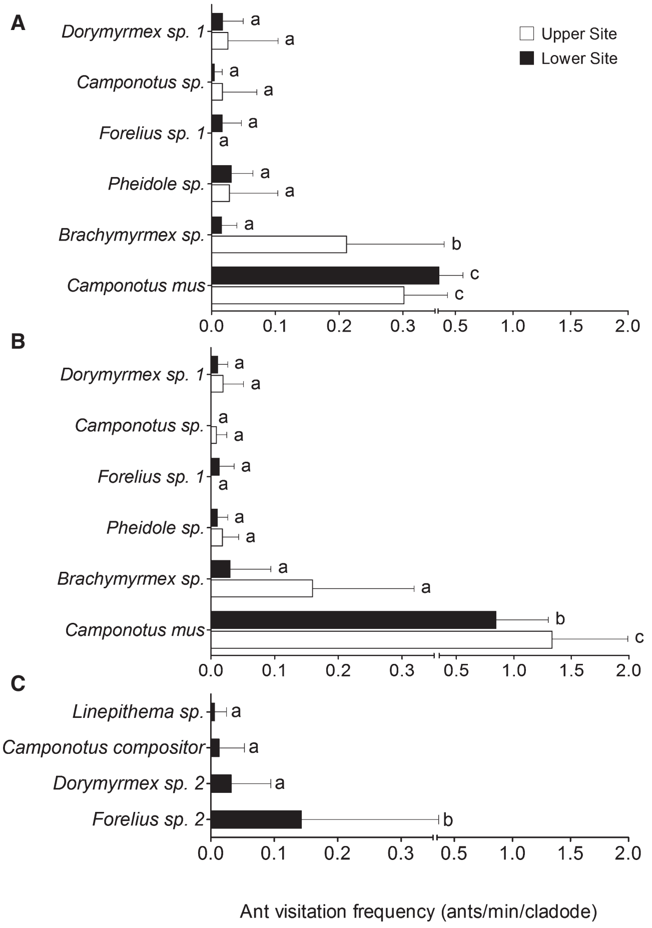

FIGURE 2. Ant visitation frequency (mean + SD) at extrafloral nectaries of *Opuntia sulphurea* for potted (A) and natural plants (B), in the lower and upper sites of Villavicencio Nature Reserve, Mendoza, Argentina, and (C) the lower site of La Paz, Bolivia. Different letters indicate significant differences among species (potted and natural plants of Villavicencio: two-way ANOVA, Duncan's post-hoc test, df = 5, $F = 4$, $P = 0.002$, and df = 5, $F = 3.4$, $P = 0.007$, respectively; potted plants of La Paz: one-way ANOVA, Duncan's post-hoc test, df = 3, $F = 14.94$, $P < 0.0001$).

and B). In La Paz, *Forelius* sp. 2 was the most common visitor, and the other species were infrequent (Fig. 2C).

One assumption of the sampling design was that the composition and frequency of ants visiting the extrafloral nectaries of *O. suphurea* are similar between mature natural plants and young potted plants. Our results partially support this assumption, since almost the same ant species were observed in both conditions (except *Camponotus* sp., which occurred only on potted plants at the lower site of Villavicencio; Fig. 1A and B); however, the frequency of ants was 2.9 and 2.2 times higher in natural plants at the upper and lower sites (upper site: $t = -4.32$, $P < 0.0004$, Cohen's $d = 1.94$; lower site: $t = -3.02$, $P < 0.0098$, Cohen's $d = 1.34$).

Other insects visiting *O. sulphurea* besides ants in Villavicencio included hemipterans (*e.g.*, *Narnia* sp., Membracidae), thysanopterans (*e.g.*, thrips), dipterans, leaf-cutter ants (*e.g.*, *Acromyrmex lobicornis*), one species of lepidopteran (*Cactoblastis doddi*), and coleopterans. Our casual observations indicate that all these taxa are potentially herbivores. For example, we observed nymphs and adults of *Narnia* sp. sucking juice from adult cladodes, thrips and coleopterans feeding on flowers, *Acromyrmex lobicornis* scraping young cladodes free of other ants, dipterans and hymenopterans sucking extrafloral nectar from ant-free cladodes, and caterpillars of *Cactoblastis doddi* burrowing in both young and adult cladodes. The dipterans and coleopterans observed at La Paz had the same feeding patterns than those observed at Villavicencio.

A total of 16 ant-herbivore interactions were identified throughout the study in Villavicencio, nine at the upper site and seven at the lower site. Ants behaved neutrally in eight cases and antagonistically in one case in the upper site, neutrally in three cases and antagonistically in four cases in the lower site. In La Paz, we recorded nine ant-herbivore interactions throughout the study. Ants behaved neutrally in three cases and antagonistically in six; however, the involved ant species had an extremely low visitation frequency (0.01 ants/min/cladode).

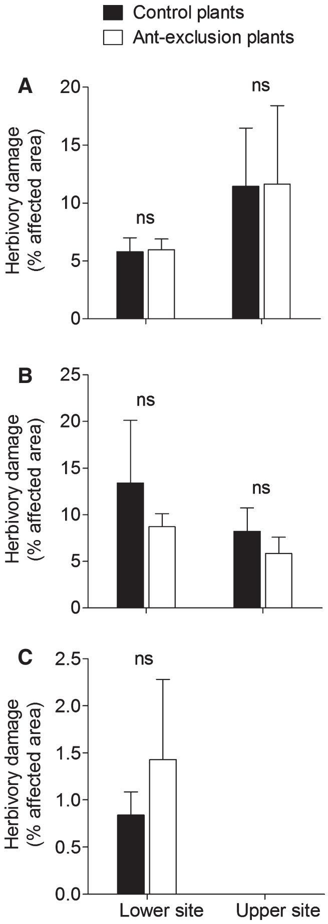

FIGURE 3. Herbivore damage (mean + SE) in potted (A) and natural (B) *Opuntia sulphurea'* plants excluded and not excluded from ants (exclusion and control) in the lower and upper sites in Villavicencio, Mendoza, Argentina, and (C) potted plants in the lower site in La Paz, Bolivia (Mann–Whitney test; ns = not significant, $P > 0.05$).

DEFENSIVE MUTUALISM AND ITS ALTITUDINAL VARIATION.—We hypothesized that the interaction between *O. sulphurea* and the ants is a defensive mutualism. However, herbivore damage, plant reproduction (seed production and seed to ovule ratio), and growth rate were similar between ant-excluded and control plants. The lack of significant effects of ant-exclusion occurred at all sites, geographic regions, and growth conditions (Figs. 3, 4, and Figure S3). In all cases, the magnitude of the difference between control and ant-exclusion treatment plants was small to moderate (Table S1). Despite the general lack of statistical significance, there was a consistent trend of greater seed production and seed to ovule ratio in the absence of ants, except for natural plants in the lower site.

Under our second hypothesis (elevational variation in the outcome of the ant-cactus interaction) we expected a decrease in ant and/or herbivore activity and an effect of this variation on interaction outcomes. Results do not support the prediction about a decrease in ant and/or herbivore activity. In Villavicencio, ant visitation frequency (mean ± SD) increased with elevation for natural plants (upper site: 1.60 ± 0.67; lower site: 0.98 ± 0.51 ants/min/cladode; $t = 2.34$, $P = 0.03$, Cohen's $d = 1.04$). The same trend occurred for potted plants, although the effect of elevation was moderate and not significant (upper site: 0.56 ± 0.36, lower site: 0.45 ± 0.23 ants/min/cladode; $t = 0.85$, $P = 0.41$, Cohen's $d = 0.36$). Damage in ant-excluded plants (an estimate of herbivore activity) did not vary significantly with elevation for natural plants (Mann–Whitney U-test, $U = 251$, $P = 0.07$, Cohen's $d = 0.49$; Fig. 3B), but increased for potted plants ($U = 324.50$, $P = 0.0031$, Cohen's $d = 0.28$; Fig. 3A). On average (± SD), herbivore damage affected 11.62 ± 30.41 percent of the total area of young cladodes in the upper site, and 5.97 ± 3.96 in the lower. Available data for ant visitation frequency in natural plants in La Paz followed the same trend as in Villavicencio, since ant visitation frequency was 0.50 ± 0.35 and 0.28 ± 0.20 ants/min/cladode in upper and lower sites, respectively; although the effect of elevation was not statistically significant, the effect size was large ($t = 1.64$, $P = 0.12$, Cohen's $d = 0.77$).

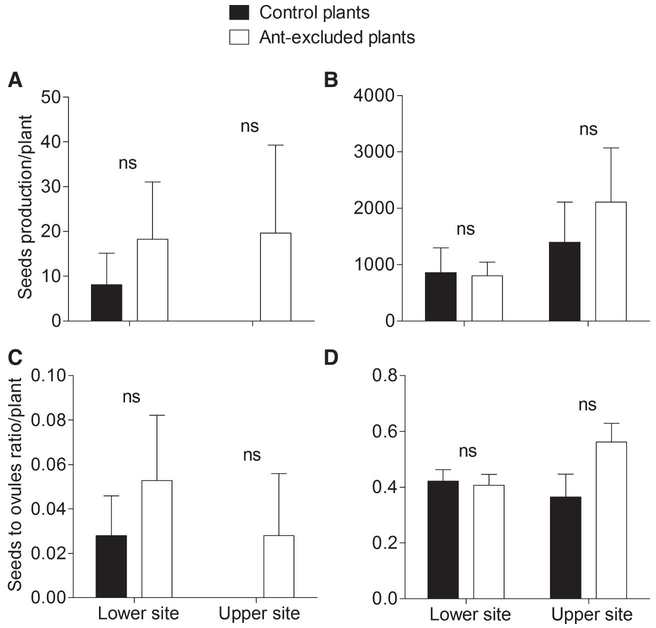

FIGURE 4. Mean (+SE) seed production per plant (upper panels) and seed to ovule ratio (lower panels) in potted (A and C) and natural *Opuntia sulphurea'* plants (B and D) excluded and not excluded from ants (exclusion and control) in lower and upper sites in Villavicencio, Mendoza, Argentina (Mann–Whitney test; ns = not significant, $P > 0.05$).

According to the observed elevational variation in ant and herbivore activity (increased at higher elevation for ants and unchanged for herbivores in natural plants, and unchanged for ants and increased at higher elevation for herbivores in potted plants; scenarios two and nine, respectively, Table 1), we predicted a greater difference between control and ant-excluded plants in herbivore damage, plant reproduction, and growth rate at the upper site. However, the differences between control and ant-exclusion treatment were similar among sites for all variables (natural plants: herbivore damage: $U = 268$, $P = 0.14$, Cohen's $d = 0.10$; seed production: $U = 197$, $P = 0.14$, Cohen's $d = 0.32$; seeds/ovules: $U = 185$, $P = 0.05$, Cohen's $d = 0.74$; growth rate: $U = 45$, $P = 0.56$, Cohen's $d = 1.04$; Figure S4; potted plants: herbivore damage: $U = 527$, $P = 0.13$, Cohen's $d = 0.04$; seed production: $U = 637$, $P = 0.99$, Cohen's d = 0.11; seeds/ovules: $U = 637$, $P = 0.99$, Cohen's $d = 0.07$; growth rate: $U = 60$, $P = 0.43$, Cohen's $d = 0.64$; Figure S5).

DISCUSSION

We tested whether the interaction between *O. sulphurea* and its ant visitors is a defensive mutualism. Contrary with our expectations, plants excluded and non-excluded for ant did not differ significantly in terms of herbivore damage, seed production, seed to ovule ratio, and growth rate. Furthermore, effect sizes of ant-exclusion were relatively small, suggesting that the effect of ant-exclusion on these response variables was irrelevant or weak. Finally, there was a trend toward greater seed production and seed to ovule ratio in the absence of ants. These results were consistent between Villavicencio and La Paz, two extremes in the distribution of this cactus, indicating that ants do not benefit *O. sulphurea*, and that they may even represent a cost for this plant species.

Different factors might explain the absence of ant protection against herbivores in *O. sulphurea*. First, we studied the ant-*O. sulphurea* interaction for only one reproductive season (spring-summer 2012–2013), and it is possible that the benefit of ant defenses can be detected only over a longer period (Heil *et al.* 2001, Mody & Linsenmair 2004, Grangier *et al.* 2008). For example, Grangier *et al.* (2008) found that the rate of herbivory was similar between control and ant-excluded plants in a short-term experiment, whereas ant-excluded plants suffered a greater rate of herbivory than control plants in longer-term experiments. Second, ants seem to lack protective abilities. Ants did not behave aggressively against the herbivores. Furthermore, in some cases big ant species defend better than small ones (Rico-Gray & Oliveira 2007); for example, *Schomburgkia tibicinis* produce more fruits and has less damage in its inflorescence when it is visited by bigger ant species (Rico-Gray & Thien 1989). Among the most frequent ant species visitors we found *Brachymyrmex* sp. and *Forelius* sp. 2 which are small ant species (Fernández 2003). Hence, the size and the behavior of the ant visitors may explain the lack of their defensive abilities. Third, herbivores seemed to avoid ants. The number of ant-herbivore encounters was extremely low (16 ant-herbivore interactions in Villavicencio and 9 in La Paz), as ants were absent from most cladodes in which we found herbivores; in those in which we did find ants, herbivores were in the opposite face of the cladode where ants were feeding. This pattern suggests that herbivores avoid ant attacks through spatially partitioning their shared plant resources (Robbins & Miller 2009). Finally, the natural herbivory rate in this plant species is so low that herbivore abundance may be insufficient to result in significant benefits of the ant presence for the plant (*e.g.*, Agrawal 1998). Other defences in *O. sulphurea,* such as thorns, gloquids, a thick epidermis, and secondary compounds (Moran 1980, Kiesling & Ferrari 2005), might thus be more effective than ant protection (Koptur 1985, Eck *et al.* 2001).

It is at least possible that the lack of difference between control and ant-exclusion treatments has resulted from a failure in our attempts to exclude ants. In potted plants, ant exclusions were 100 percent effective from September 2012 to February 2013; while in natural plants exclusions were 100 percent effective during the first 3 months of the study. By December and January ants were excluded 33 percent and 17 percent of natural plants, respectively, and by February ants were present in all natural plants. However, we think it is unlikely that this failure of ant exclusions toward the end of the study had a significant effect on our results, for at least three reasons. First, even though our experimental exclusion failed toward the end of the study, it worked well during the period of high vulnerability of cladodes to herbivory, the first months of growth (Bentley 1977, Grangier *et al.* 2008). Second, other studies with other species of *Opuntia* have reported significant benefits of ant defences against herbivory with partial exclusions (*e.g.*, Miller 2007, Miller *et al.* 2010). Third, results for potted plants, where ant-exclusion treatments worked better, were similar to those of natural plants, suggesting that the effect of exclusion failure may have been insignificant.

Our second hypothesis posited that elevational variations in ant and herbivore activity should lead to variation in the outcome of the ant-cactus interaction. However, our results do not support this hypothesis: even though ant visitation frequency increased with elevation, and herbivore damage did not change in natural plants and increased in potted plants, we found no differences in herbivore damage, plant reproductive success and plant growth between low and high elevation sites. These results are not surprising given our finding of not significant benefits of ant defences for *O. sulphurea*. In addition, ant visitation frequency may have been too low in our study sites to constitute a defence. For example, mean ant visitation frequency in a study with a comparable species to *O. sulphurea* in the Sonoran Desert was approximately 4.4 ants per new stem (Pickett & Clark 1979); in contrast, in the upper site from Villavicencio (the site with the greatest ant visitation frequency in our study) visitation frequency was 1.6 ants per cladode. Thus, although ant visitation frequency increased with elevation (more than twofold for natural plants), this change may have been insufficient for ants to defend plants from herbivore attacks. This possibility is particularly relevant if we consider the unaggressive behavior of the ant species.

In sum, our results indicate that the ant-cactus interaction studied here does not result in detectable benefits for the plant; furthermore, the trend toward greater seed productive output in the absence of ants suggests that the ant-cactus interaction may even represent a cost for the plant. These results are unlikely to have resulted from methodological limitations of our study, as our results were consistent among methodological approaches (exclusion experiments with potted and natural plants), environmental conditions (higher and lower elevations), and geographical regions for a wide range of plant response variables. Thus, this ant-cactus interaction is probably a commensalism rather than a defensive mutualism.

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SUPPORTING INFORMATION

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

TABLE S1. *Results from Mann–Whitney test examining the effect of ant-exclusion in herbivory, seed production, seed to ovule ratio, and growth rate.*

FIGURE S1. Photographs of *Opuntia sulphurea var. sulphurea* and *Opuntia sulphurea var. hildemanii.*

FIGURE S2. Photographs of different types of herbivore damage.

FIGURE S3. Growth rate of cladodes and natural plants in lower and upper sites in Villavicencio.

FIGURE S4. Difference in herbivory damage, growth rate, seed production per plant, and seed to ovule ratio in natural *Opuntia sulphurea'* plants in the lower and upper sites in Villavicencio.

FIGURE S5. Difference in herbivory damage, growth rate, seed production, and seed to ovule ratio in potted plants, in the lower and upper sites in Villavicencio.

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