Floral visitation by the Argentine ant reduces pollinator visitation and seed set in the coast barrel cactus, *Ferocactus viridescens*

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Abstract Mounting evidence indicates that trade-offs between plant defense and reproduction arise not only from resource allocation but also from interactions among mutualists. Indirect costs of plant defense by ants, for example, can outweigh benefits if ants deter pollinators. Plants can dissuade ants from occupying flowers, but such arrangements may break down when novel ant partners infiltrate mutualisms. Here, we examine how floral visitation by ants affects pollination services when the invasive Argentine ant (*Linepithema humile*) replaces a native ant species in a food-for-protection mutualism with the coast barrel cactus (*Ferocactus viridescens*), which, like certain other barrel cacti, produces extrafloral nectar. We compared the effects of floral visitation by the Argentine ant with those of the most prevalent native ant species (*Crematogaster californica*). Compared to *C. californica*, the Argentine ant was present in higher numbers in flowers. Cactus bees (*Diadasia* spp.), the key pollinators in this system, spent less time in flowers when cacti were occupied by the Argentine ant compared to when cacti were occupied by *C. californica*. Presumably as a consequence of decreased duration of floral visits by *Diadasia*, cacti occupied by *L. humile* set fewer seeds per fruit and produced fewer seeds overall compared to cacti occupied by *C. californica*. These data illustrate the importance of mutualist identity in cases where plants balance multiple mutualisms. Moreover, as habitats become increasingly infiltrated by introduced species, the loss of native mutualists and their replacement by non-native species may alter the shape of trade-offs between plant defense and reproduction.

Keywords Extrafloral nectar · Invasion · *Diadasia* · Mutualism · *Crematogaster*

Introduction

The degree to which plants benefit from the allocation of resources to somatic versus reproductive function can be influenced by interactions between plants and their mutualists (Strauss and Irwin 2004). When plants increase anti-herbivore defenses, for example, pollinator mutualisms can be negatively affected via toxic nectar (Adler 2001) or less attractive flowers (Galen 1999). Likewise, plants that maximize attractiveness to pollinators can be more vulnerable to herbivory (Gomez 2003; Irwin et al. 2003; Strauss et al. 2004). Because interactions between plants and their mutualists can have opposing effects, the costs and benefits of supporting mutualisms are likely to affect patterns of resource allocation.

Ant–plant protection mutualisms provide an interesting case study of the balance between the benefit of herbivore removal and the cost of pollinator harassment. In general, ant–plant interactions benefit plants (Chamberlain and Holland 2009), including cases where the tending ants are introduced species (Ness and Bronstein 2004). Herbivore removal by ants is a valuable service (Messina 1981; Sipura 2002) that improves plant fitness (Oliveira et al.

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1999; Palmer et al. 2010; Styrsky and Eubanks 2010). However, if ants enter flowers, their presence can deter pollinators (Visser et al. 1996; Lach 2007, 2008a; Romero et al. 2011) and negatively affect plant reproduction (Ness 2006). Plants exhibit different strategies to minimize floral visitation by ants; these include repellent floral chemicals (Junker et al. 2007; Agarwal and Rastogi 2008; Willmer et al. 2009; Ballantyne and Willmer 2012) and extrafloral nectaries on leaves or stems (Wagner and Kay 2002). Extrafloral nectar (EFN) draws ants onto plants (Bentley 1977), but can also distract ants from flowers, thus minimizing contact between ants and pollinators (Wagner and Kay 2002; Chamberlain and Holland 2008).

The extent to which mechanisms such as EFN production reduce floral visitation by ants depends on the ant species occupying the plant (Ness 2006; Ness et al. 2006; Junker et al. 2007; Lach 2007, 2008a). When introduced ants displace native ants within an ant–plant mutualism, they may not demonstrate the same aversion to floral chemicals that native ants exhibit (Lach 2003; Ness and Bronstein 2004). Moreover, in situations where production of floral nectar is positively correlated with increased EFN allocation (Rudgers 2004; Goss et al. 2011; Chamberlain and Rudgers 2012), increased allocation to reproductive function may inadvertently increase floral visitation by ants. Introduced ants can differ from native ant mutualists in their response to EFN (Lach and Hoffmann 2011), and thus their presence may shift ant–plant mutualisms away from reciprocally beneficial interactions (Traveset and Richardson 2006; Kiers et al. 2010).

EFN production may either satiate ants and distract them from flowers, or increase the density of ants on plants and thus the likelihood that they will interfere with legitimate pollinators (Holland et al. 2011). In cases involving invasion by nectar-craving ants, plants with high levels of EFN production may be susceptible to increased floral visitation by introduced ants and a greater degree of pollinator interference (Lach 2003). Over evolutionary time, plants might be expected to adjust their EFN allocation in accordance with the costs associated with tissue damage by herbivores and pollinator interference by attendant ants (Rudgers 2004).

In this study, we examine the importance of pollinator interference in an EFN-producing plant, the coast barrel cactus (*Ferocactus viridescens*) in coastal Southern California. This cactus produces EFN attractive to ants, including the invasive Argentine ant (*Linepithema humile*), and ant protection discourages insect herbivores from feeding on cacti (Ludka 2009). In addition to patrolling extrafloral portions of cacti, ants also visit flowers, where they could negatively affect pollinators and reduce seed set, as in the case of ant protection mutualisms involving *Ferocactus wislizeni* (Ness 2006). Ant–pollinator interactions might

be especially likely on cacti occupied by the Argentine ant, which is a species that favors carbohydrate-rich resources (Way 1963; Holway et al. 2002) and often forages for nectar in flowers (Junker et al. 2011), where it tends to displace legitimate floral visitors (Blancafort and Gomez 2005; Lach 2007, 2008a).

Here, we collate 3 years of data on floral visitation by ants and 4 years of data on seed production for cacti occupied either by the Argentine ant or by *Crematogaster californica*, the most prevalent native ant in the system. We combined a 1-year survey of bee pollinators with reproductive data for the same set of cacti. Using these data, we test whether or not floral visitation by ants affects pollinator visitation and, if so, how floral visitation affects the reproductive success of cacti. This system provides a rare opportunity to examine how a novel mutualist partner affects the balance between plant protection and pollinator interference. In addition, relatively few studies on mutualisms make explicit comparisons between native and introduced ants with respect to their performance as mutualist partners (Ness and Bronstein 2004). Because barrel cacti are typically occupied by a single ant species at a time (Morris et al. 2005; Ness 2006; Ness et al. 2006), it is possible to link floral visitation by ants to variables directly related to plant fitness through interactions between ants and pollinators.

Materials and methods

Study system

The coast barrel cactus (*F. viridescens*) is a patchily distributed and uncommon species restricted to well-drained slopes in coastal sage scrub habitats in coastal San Diego County, CA, and northwestern Baja California, USA (Ingram 2008). We studied *F. viridescens* along a 15-km stretch of coastline in San Diego County from Kate O. Sessions Memorial Park (32.814°N, 117.238°W) in the south to the Torrey Pines State Reserve (32.923°N, 117.258°W) in the north. A diagrammatic map of focal cacti is included in Online Resource 1. *Ferocactus viridescens* bears modified spines on its crown that produce EFN during the winter, spring, and summer. Cacti bloom from April to June and produce fruit from July into early fall.

EFN produced by the coast barrel cactus attracts a variety of ant species, including the invasive Argentine ant (*L. humile*). In the spring and early summer, nearly all cacti with flowers are tended by ants. In areas invaded by the Argentine ant, native ant diversity is greatly reduced and mature cacti are tended almost entirely by *L. humile* (Ludka 2009). In uninvaded areas, the native ant, *C. californica*, is the most prevalent native ant occupying mature

cacti (Ludka 2009). Cacti were usually tended by only one ant species during a single survey, and we excluded from all analyses cacti that were occupied by >1 ant species in a season or cacti that were occupied by ants during <50 % of survey visits. Focal cacti occupied by a given ant species in 1 year were generally occupied by the same species in following years.

Within the study region, we use multiple invaded sites and adjacent uninvaded sites as a natural experiment to compare the frequency and consequences of floral visitation by *L. humile* and *C. californica* (Online Resource 1). Within the mosaic of invaded and uninvaded sites in our study region, we selected cacti that were as spatially interspersed as possible between areas with and areas without *L. humile*. Several lines of evidence indicate that invaded and uninvaded sites were well matched in respects other than the presence or absence of *L. humile*. For example, cacti in both types of sites did not differ in size (Argentine ant: 2,972 cm^3 vs. *Crematogaster*: 2,736 cm^3), blooming period, number of floral buds, or number of flowers (Table 1). Although fruit production is not strictly a function of environmental variation, we further observed that cacti in invaded and uninvaded sites produced similar quantities of fruit over a 4-year period (Table 1). Lastly, the results of our pollinator survey indicated that the major categories of pollinators did not differ in their visitation frequency between invaded and uninvaded sites (see "Results").

Our study examined 63 cacti in 2007, 63 cacti in 2008, 62 cacti in 2010, and 58 cacti in 2011. These focal cacti were surveyed for ants, pollinators, and reproductive performance. Most focal cacti were censused each year, but in some years certain individuals were not included because of interannual variation in whether individuals produced flowers, died, or were subject to other factors (see Online Resource 2). In 2007, 2008, and 2010, we conducted weekly surveys of focal cacti to document blooming phenology, the number of floral buds produced, the number of flowers produced, the number of fruits matured, and the identity of attendant ant species. In 2011, we visited cacti in late spring and again in early fall to document the number of flowers produced, the number of fruits matured, and the identity of attendant ant species.

Floral visitation by ants

In weekly surveys for each of 3 years, we identified the occupying ant species, the number of workers present on each cactus, and whether or not ants were present in flowers. Because extrafloral nectaries are concentrated around the crown of the plant, ants could be easily counted and identified in the field. All surveys were conducted when ambient temperatures were approximately 30 °C (±4.4 °C); both focal ant species are active at these temperatures. We used contingency table analyses to compare ant presence in flowers and non-parametric Mann–Whitney tests to compare ant abundance in flowers. For the 2010 survey data, we used univariate logistic regression to test for a relationship between the abundance of ants on cacti (time averaged across weekly surveys) and whether or not they were present in flowers (during at least one weekly survey).

We used an experimental assay to test the potential repellency of cactus flowers to each attendant ant species. This assay was developed to examine the potential for cactus flower petals to repel ants from flowers (Ness 2006; Chamberlain and Holland 2008). The design of this assay reflects the tendency of ants that forage in cactus flowers to crawl through and stand on the petals as they consume floral nectar. Although, in general, ants can be repelled by volatile organic compounds emanating from other portions of the flower (e.g., pollen; see Ballantyne and Willmer

Table 1 Reproductive traits of coast barrel cacti (*Ferocactus viridescens*) tended by native or invasive ants

Identity of occupying ant	Year surveyed	Blooming period (days)	Number of floral buds	Number of flowers	Number of fruits
Linepithema humile	2007	–	–	–	7.7 ± 1.0
	2008	–	–	–	8.25 ± 0.9
	2010	40.1 ± 1.6	23.6 ± 2.0	18.9 ± 1.8	11.8 ± 1.0
	2011	–	–	–	11.1 ± 1.0
Crematogaster californica	2007	–	–	–	8.3 ± 1.7
	2008	–	–	–	6.5 ± 1.1
	2010	40.1 ± 3.4	28.7 ± 4.9	20.2 ± 3.8	14.3 ± 1.6
	2011	–	–	–	9.5 ± 1.7

Cacti were measured for inherent differences in bud, flower, or fruit production as well as flowering phenology between sites in which cacti were occupied by *L. humile* or by *C. californica*

No significant differences existed in these reproductive characters within each year ($p > 0.05$) in comparisons between cacti occupied by the two ant species

Table entries are mean ± SE

2012), we have not observed foraging ants in contact with the male or female floral reproductive structures in barrel cactus flowers. We collected *L. humile* and *C. californica* from individual cacti growing in invaded areas ($n = 15$ sites) or uninvaded areas ($n = 9$ sites); individual collecting locations were separated by at least 100 m and spanned the latitudinal gradient of our study region. After transporting ants and fresh petals to the laboratory, we immediately performed behavioral assays at room temperature under ambient lighting. For each trial, we wiped one-half of a Petri dish (8 cm diameter) with a single petal handled with forceps; we treated both the lid and the bottom of each dish. We then placed a single worker ant into the dish, closed the lid and superimposed petal-treated hemispheres. We allowed each ant to acclimate to its Petri dish for 30 s and then recorded ant location within the treated versus untreated hemispheres for 300 s. We replicated the assay on four workers of *L. humile* or *C. californica* collected from each site (for a single site, we used only two *C. californica* workers). Mean values for each site were used as replicates in the analysis, and we used each ant, Petri dish, and cactus petal once. To test for repellency or attraction to flower petals, we used a paired *t* test to compare the mean time spent in each hemisphere of the dish (Ness 2006; Chamberlain and Holland 2008). This assay has been used to provide evidence of floral repellency for at least one species of ant (*Crematogaster opuntiae*) that visits EFN-bearing cacti (Ness 2006).

Pollinator surveys

In 2010, we conducted pollinator surveys on focal cacti (Online Resource 2) to determine if the presence of ants in flowers has any effect on the type of pollinators that visit flowers, the frequency of their visits, and the length of their visits. Each cactus was surveyed weekly from April to June, and data on pollinator visitation were collected if a cactus had at least one flower open during each survey. In surveying an individual cactus, we spent 5 min watching for pollinators and recorded the following: identity of pollinator, duration of each floral visit from landing to takeoff, the number of ants present on the cactus, and the number of ants present in flowers. A putative pollinator was considered to have visited a flower if it remained in the flower for at least 5 s. The vast majority (96.3 %) of putative pollinators in our system were bees, which we divided into three categories: cactus bees [*Diadasia* spp. (*australis* complex)], honey bees (*Apis mellifera*), and small native bees [numerous species including *Anthophorula* spp. (Apidae), *Halictus tripartitus* (Halictidae), *Lasioglossum* spp. (Halictidae) and *Macrotera tristella* (Andrenidae)]. Differences in pooled pollinator abundance and visitation duration between cacti occupied by each ant were analyzed with a two-sample *t* test. Using a one-way ANOVA, we also compared visitation frequency of each pollinator category.

Cactus reproduction

In each of our four study years, we estimated seed set for our focal cacti (Online Resource 2). In the fall of each year, we haphazardly collected one to three ripened fruits from each plant. We extracted all the seeds from each fruit and placed them in a drying oven for 1 week to remove moisture. We then weighed all the seeds from a single fruit, as well as a haphazardly selected set of 100 seeds from the same fruit, in order to estimate the number of seeds in each fruit (as in Ness 2006). We estimated total seed production as the product of seed mass and fruit production. We then analyzed the effect of the attendant ant species on cactus seed production and estimated seed set using a generalized linear mixed effects model with a Penalized Quasi-Likelihood method (glmmPQL) and Poisson distribution link function. Occupying ant species was treated as a fixed factor and year as a random effect (R v.2.10.1). In this analysis, we nested individual cacti within year to account for individual plants that were included more than once across years. We also analyzed the effect of observed bee visitation on seed and fruit production with Mann–Whitney tests and a two-way ANOVA. In the ANOVA analysis, occupying ant species and the presence of bees were treated as fixed factors.

Results

Floral visitation by ants

Compared to the native *C. californica,* the Argentine ant occurred in higher numbers in flowers. Of those cacti that had ants in flowers, the Argentine ant was more abundant in flowers compared to *C. californica* in 2007 (Fig. 1a; Mann–Whitney test: $U = 527.5$, $p = 0.024$), 2010 ($U = 6208.5$, $p = 0.0009$), and averaged across all years ($U = 771.5$, $p = 0.022$). In some years, the proportion of cacti on which *L. humile* was observed visiting flowers exceeded that for *C. californica*. *L. humile* occurred more often in flowers compared to *C. californica* in 2010 (Fig. 1b; G test: $G = 4.53$, $p = 0.03$) and averaged across years (G test: $G = 4.23$, $p = 0.04$). In 2007, the trend was similar to that of 2010 but was not statistically significant (G test: $G = 2.5$, $p > 0.05$). In 2008, ant presence in flowers was low for both ant species (Mann–Whitney test: $U = 731$, $p > 0.05$); this spring was unusually dry and followed a winter with below average rainfall and low ant occupancy on cacti.

As the abundance of the Argentine ant increased on cacti, the likelihood that ants were found in flowers also increased (simple logistic regression: $z = 2.850$, $n = 32$
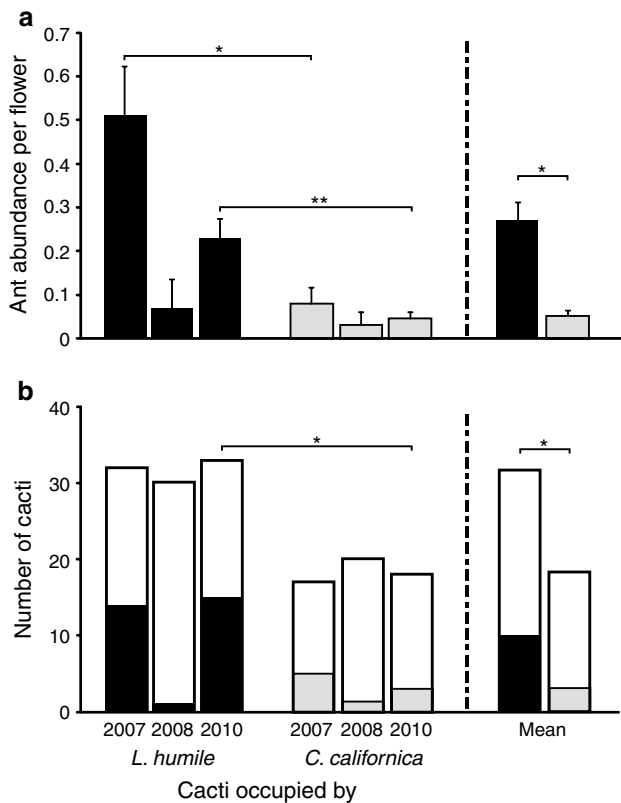
Fig. 1 **a** Abundance (mean ± SE) of the Argentine ant (*Linepithema humile*) (*black*) and *Crematogaster californica* (*gray*) in flowers for each year (2007, 2008, 2010) and across years (mean) for coast barrel cacti (*Ferocactus viridescens*) that had ants in flowers. To calculate mean abundance, we averaged values across years from the same individual. **b** Number of cacti for which the Argentine ant (*black*) or *C. californica* (*gray*) were observed in flowers as a proportion of the total number of cacti surveyed in each year (height of each *bar*). The *open portion* of each *bar* indicates the number of cacti for which ants were not observed in flowers. **p < 0.01, *p < 0.05. These data were collected from 31, 30, and 32 cacti occupied by *L. humile* and 16, 20, and 17 cacti occupied by *C. californica* in 2007, 2008, and 2010, respectively

cacti, $p = 0.008$). Cacti in which *L. humile* was observed at least once in flowers were neither larger, nor did they have more flowers, compared to cacti on which the Argentine ant was never observed in flowers (two-sample *t* tests: $p > 0.05$ for both comparisons). A comparable analysis could not be conducted on *C. californica* because this species much less often occurred in flowers (Fig. 1a). When we evaluated ant responses to flower petals, we found that *C. californica* (paired $t = 0.502$, $df = 8$, $p = 0.63$) and *L. humile* (paired $t = 1.787$, $df = 14$, $p = 0.096$) appeared neither repelled by nor attracted to flower petals (Fig. 2).

Pollinator surveys

The total number of floral visits by putative pollinators did not differ based on the identity of the attendant ant species.
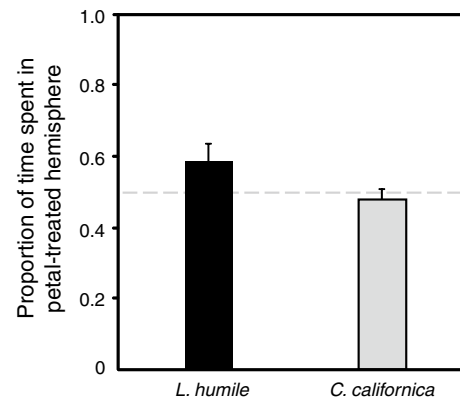

Fig. 2 The mean (± SE) proportion of time that the Argentine ant (*L. humile*) (collected from $n = 15$ sites) and *C. californica* (collected from $n = 9$ sites) workers spent in proximity to the petal-treated side of an 8-cm-diameter Petri dish. *Dashed line* indicates equal visitation between each hemisphere of the dish

The most commonly observed floral visitors were cactus bees, *Diadasia* spp., which we observed visiting 60.4 % (29/48) of cacti in the 2010 pollinator survey. The numbers of visits made by *Diadasia* did not differ between cacti occupied by the Argentine ant and cacti occupied by *C. californica* (Fig. 3a; two-sample *t* test: $t = 0.332$, $df = 46$, $p > 0.05$). However, the duration of floral visits by *Diadasia* was 62 % longer when bees visited cacti occupied by *C. californica* compared to when they visited cacti in areas with *L. humile* (Fig. 3b; two-sample *t* test: $t = 3.024$, $df = 23$, $p = 0.006$).

Compared to *Diadasia*, other bees visited fewer cacti. We observed *A. mellifera* visiting flowers of 27.1 % (13/48) of the cacti in the survey, and, as with *Diadasia*, the species of ant in control of the cactus had no effect on whether *A. mellifera* was observed (χ^2 test: $p > 0.05$) or on the number of floral visits made by *A. mellifera* (two-sample *t* test: $t = 1.63$, $df = 11$, $p > 0.05$). We observed small native bees on 18.8 % (9/48) of the cacti in the survey, and these bees occurred more often on cacti occupied by *C. californica* (7/16) than on cacti occupied by *L. humile* (2/32) (Online Resource 2; χ^2 test: $\chi^2 = 7.54$, $df = 1$, $p = 0.006$). On those cacti where we observed small native bees, however, the number of floral visits did not differ for cacti occupied by *L. humile* versus those occupied by *C. californica* (two-sample *t* test: $t = 0.42$, $df = 7$, $p > 0.05$). Comparisons of overall rates of visitation on those cacti visited by each type of bee revealed that *A. mellifera* visited cactus flowers less often compared to *Diadasia* and small native bees, which did not differ from one another in their rate of visitation (one-way ANOVA: $F_{2,48} = 4.086$, $p = 0.0230$; Fisher's PLSD with $\alpha = 0.05$). In this latter analysis, data were separated according to bee groups but pooled across attendant ant species.
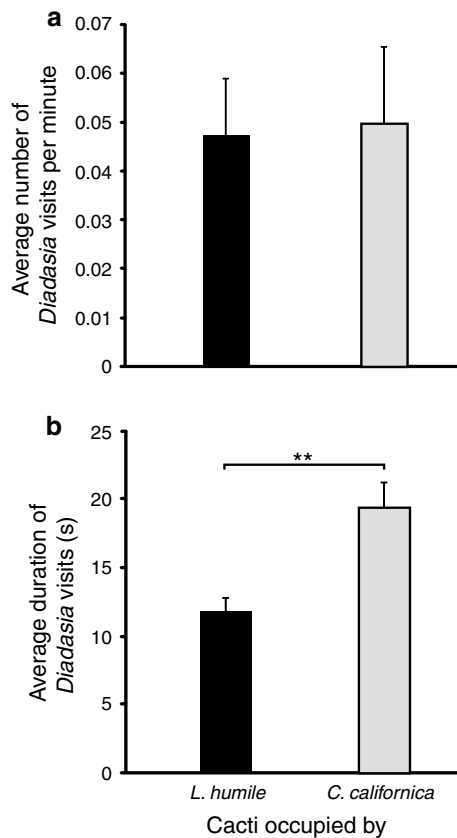
Fig. 3 **a** Number and **b** duration of visits (both mean ± SE) made by cactus bees (*Diadasia* spp.) in 2010 to flowers on cacti occupied by the Argentine ant (*L. humile*) (*black*) or by *C. californica* (*gray*). We conducted 203 surveys on 30 cacti occupied by *L. humile* and 12 cacti occupied by *C. californica*. **p < 0.01

Table 2 The effects of ant species identity and year on plant reproductive characters in the coast barrel cactus

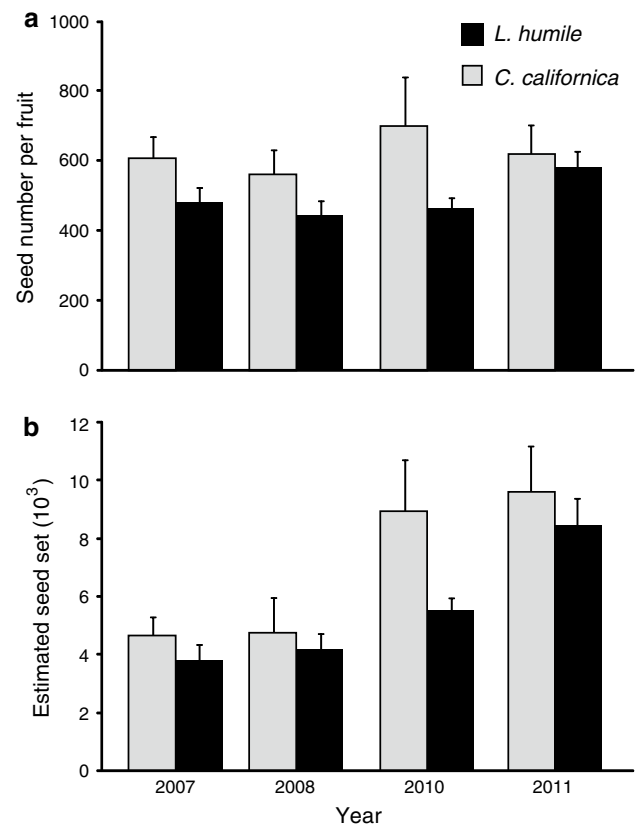| Plant reproductive measure	Model effect	df	t	p
Seed number per fruit	Ant	1,57	2.313	0.02
	Year	1,24	1.4	0.3
Fruit set	Ant	1,57	0.629	0.53
	Year	1,24	4.753	0.04
Estimated total seed set	Ant	1,57	2.835	0.005
	Year	1,24	4.393	0.04

Fig. 4 **a** The mean ± SE number of seeds per fruit was significantly greater ($p = 0.02$; see Table 2) for cacti occupied by *C. californica* (*gray*) compared to those occupied by the Argentine ant (*L. humile*) (*black*). **b** The mean ± SE estimated total seed set by cacti (product of the seed number per fruit and number of fruit) was also significantly greater ($p = 0.005$; see Table 2) when *C. californica* (*gray*) occupied plants as opposed to the Argentine ant (*black*). Sample sizes were 9 and 24 cacti (2007), 20 and 29 cacti (2008), 18 and 34 cacti (2010), and 14 and 27 cacti (2011), for cacti occupied by either *C. californica* or *L. humile*, respectively

Cactus reproduction

Over the 4 years of our survey, cacti occupied by the Argentine ant produced fewer seeds per fruit compared to cacti occupied by *C. californica* (Table 2). Cacti occupied by *L. humile* experienced a 6–33 % reduction in seed number per fruit, depending on the year, compared to cacti occupied by *C. californica* (Fig. 4a). Relatively low rainfall in 2007 [35.8 % of 30-year average precipitation (274 mm)] and 2008 (62.7 % of 30-year average precipitation) may be responsible for significant interannual variation in cactus fruit set (Table 2). Estimated seed set showed qualitatively similar and significant patterns (Fig. 4b). Data on seed number per fruit also revealed that cacti for which we observed bees visiting flowers set more seeds compared to cacti for which we observed no bee visits. For cacti occupied by *C. californica*, for example, individual plants for which we recorded at least one floral visit by a bee produced significantly more seeds per fruit (857 seeds ± 177 SE)

compared to cacti (225 seeds ± 37) for which we did not observe bees visiting flowers (Mann–Whitney test: $U = 37$, $p = 0.01$). In contrast, for Argentine ant occupied cacti, for which the duration of floral visits by

cactus bees was shorter, the number of seeds per fruit did not differ between cacti for which we did not observe bees versus those for which we did (443 ± 32 vs. 511 ± 65 seeds; Mann–Whitney test: $U = 84$, $p = 0.393$). On the other hand, neither the identity of the occupying ant species (two-way ANOVA: $F_{1,43} = 1.68$, $p = 0.20$) nor the observed presence of bees (two-way ANOVA: $F_{1,43} = 0.27$, $p = 0.60$) affected the number of fruits produced by individual cacti. There was no interaction between the ant occupation or observed bee presence (two-way ANOVA: $F_{1,43} = 1.21$, $p = 0.28$).

Discussion

Here, we show how floral visitation by the Argentine ant potentially reduces seed production in the coast barrel cactus. Compared to cacti occupied by *C. californica*, the most prevalent native ant in this system, cacti occupied by *L. humile* had higher numbers of ants in flowers, were visited for shorter durations by cactus bees, and produced fewer seeds per fruit. The characteristics of this system qualitatively match predictions of plant–pollinator interactions most likely to be disrupted by invasive ants to the detriment of the plant mutualist, enumerated by Lach (2003). First, the Argentine ant often attains high abundance (Holway 1998), craves carbohydrates (Newell and Barber 1913; Way 1963), exhibits enhanced colony growth on carbohydrate-rich diets (Grover et al. 2007; Kay et al. 2010), and displaces pollinators from flowers (Blancafort and Gomez 2005; Lach 2007, 2008a, b). Second, the most widespread pollinator in the system spends less time in flowers in the presence of *L. humile* (Fig. 3). Lastly, the coast barrel cactus appears unable to repel ants from flowers (Fig. 2), produces nectar attractive to the Argentine ant, and, like its congeners (Ness 2006), can experience pollen limitation presumably because of floral visitation by ants (Fig. 4).

Patterns of floral visitation by the two focal ant species indicate that *L. humile* typically occurred in higher numbers compared to *C. californica* and in some years visited flowers on more cacti (Fig. 1). Previous studies have found few instances of floral repellency in other ant–cactus systems (Ness 2006; Chamberlain and Holland 2008). Ness (2006) reported that *Crematogaster opuntiae*, a congener of *C. californica*, was repelled by chemicals of *F. wislizeni* petals; nonetheless, neither of the ant species we tested appeared to show aversion to the petals of *F. viridescens* (Fig. 2). Interestingly, we found that the likelihood of floral visitation by *L. humile* increased with the number of ants present on individual cacti (not including those present in flowers). The Argentine ant's strong preference for carbohydrates, coupled with its lack of evolutionary history with the coast barrel cactus, could explain why the presence of

EFN enhances floral visitation (via attracting ants onto the plant) for this species.

Floral visitation by the Argentine ant provides the opportunity for interactions to take place between ants and pollinators (Online Resource 3). These interactions can include interference, exploitation, and modification of floral resources (Lach 2007). Although we do not know the relative importance of these different mechanisms, we did observe the Argentine ant consuming floral nectar and harassing individual pollinators (Online Resource 3), including *Diadasia*. Irrespective of the mechanisms involved, the species of ant in control of cacti did not influence the number of visits made by different types of bees but rather the duration of those visits (Fig. 3). Bees facing threats of predation commonly reduce time spent visiting flowers (Romero et al. 2011), and it seems plausible that bees would make similar decisions in the presence of aggressive ants. Because *Diadasia* are key cactus pollinators (Sipes and Tepedino 2005; Blair and Williamson 2008), the effect of Argentine ant floral visitation on *Diadasia* visit duration may be especially disruptive. Our pollinator survey, for example, revealed that *Diadasia* made up 66 % of all bee visits to barrel cacti. Moreover, members of the *D. australis* complex are cactus specialists (Sipes and Tepedino 2005), whereas the other bees observed visiting coast barrel cactus were all generalists. As with *Ferocactus wislizeni* (Ness 2006), the coast barrel cactus appears to rely primarily on bees for pollination services. In areas not invaded by *L. humile*, for example, cacti on which we observed bees set more than twice as many seeds per fruit compared to cacti where we saw no bees (see "Cactus reproduction" in "Results").

Plants occupied by *L. humile* had flowers that were visited for less time by cactus bees, and these cacti produced fewer seeds per fruit and fewer seeds overall compared to cacti occupied by *C. californica* (Fig. 4). This finding is consistent with previous studies demonstrating that increased duration of pollinator visits is positively related to amounts of pollen deposited onto visited flowers (e.g., Thomson and Plowright 1980). Variation in fruit production across years (Table 2) presumably resulted from differences in the timing and amount of precipitation; for example, spring seasons in 2007 and 2008 followed winters with below average rainfall. Our seed set results appear to reveal a negative effect of reduced pollinator visitation on the female component of cactus fitness. The shorter duration of *Diadasia* visits conceivably could enhance the male component of fitness by increasing pollen transfer (Traveset and Richardson 2006), but this aspect of cactus reproductive success was not measured.

Although floral visitation by the Argentine ant commonly occurs (Blancafort and Gomez 2005; Lach 2007, 2008a), we know of only one other published study that

reports negative consequences to the host plant. Blanca-fort and Gomez (2005) found reduced fruit set and seed set for the shrub *Euphorbia characias* in an area invaded by the Argentine ant compared to an adjacent area occupied by native ants. The present study examined cacti distributed over a relatively large spatial scale with individual plants spatially interspersed among multiple invaded and uninvaded areas. Interestingly, both Blancafort and Gomez (2005) and the present study identified a single type of pollinator that appeared to respond negatively to floral visits by *L. humile*. Cases where the displacement of floral visitors by the Argentine ant fails to reduce seed set involve systems where pollen is not limiting (Lach 2007, 2008a). Barrel cacti may be especially prone to suffering negative effects of floral visitation by ants given the results of the present study and those of Ness (2006).

This study illustrates how invasive mutualists may alter the shape of trade-offs between plant defense and reproduction. The principal native ant in this system infrequently visits cactus flowers and thus appears to have little opportunity to affect pollinators. For these reasons, *C. californica* provides protective services but presumably has little if any direct effect on plant reproduction. Although the Argentine ant also protects cacti from herbivores (Ludka 2009), it does so at a direct cost to plant reproduction. If this cost exceeds the cost of herbivory, then one might expect that *F. viridescens* would adjust its EFN allocation over time to reduce the likelihood of floral visitation by ants. This type of selective pressure seems conceivable given that many of the *F. viridescens* in the US portion of this plant's range now occur in areas invaded by the Argentine ant. Our results illustrate the importance of considering trade-offs between defense and reproduction when evaluating the costs and benefits of protection mutualisms (Ness 2006; Chamberlain and Holland 2008; Holland et al. 2011). An improved understanding of such trade-offs will be particularly important as mutualisms become increasingly infiltrated by non-native species and influenced by changing climatic conditions (Traveset and Richardson 2006; Kiers et al. 2010).

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