Balancing anti-herbivore benefits and anti-pollinator costs of defensive mutualists

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Abstract. Quantifying costs and benefits of ostensibly mutualistic interactions is an important step toward understanding their evolutionary trajectories. In food-for-protection interactions between ants and extrafloral nectar (EFN)-bearing plants, tending by aggressive ants may deter herbivores, but it may also deter pollinators. The fitness costs of pollinator deterrence are not straightforward for long-lived iteroparous plants, because reproductive vital rates often contribute weakly to fitness relative to growth and survival (vital rates that may be enhanced by ant defense). We used field manipulations of ant and pollinator activity and demographic modeling to examine how pollination costs of ant defense translate to plant fitness, given the benefits of ant defense elsewhere in the plant life cycle. We contrasted the net fitness effects of alternative ant partner species. Our work focused on the tree cholla cactus, *Opuntia imbricata*, an EFN-bearing plant associated with two ant species (*Crematogaster opuntiae* and *Liometopum apiculatum*) that differ in quality of defense against insect herbivores. We found that ant defense imposed pollination costs, despite evidence for ant-repellent floral volatiles and temporal partitioning of ant and pollinator activity. The two partner species similarly reduced pollinator visitation and seed mass, and one (*C. opuntiae*) additionally reduced seed number. We used the experimental data and other long-term demographic data to parameterize an integral projection model that integrated costs and benefits of ant defense over the complete plant life cycle. Model results indicated that the pollination costs of *L. apiculatum* were balanced by beneficial effects on growth, leading to a net fitness effect that was neutral to positive. By contrast, pollination costs outweighed benefits for *C. opuntiae*, the weaker defender, rendering this species a reproductive parasite. Thus, we infer that pollination costs destabilize mutualism with one partner species, but are offset by strong defensive benefits provided by the other, leading to contrasting selective pressures imposed by alternative associations. Accounting for ontogenetic turnover in ant partner identity indicated that most plants avoid the parasitic effects of *C. opuntiae* by associating nonrandomly with *L. apiculatum* at reproductive life stages. Our results highlight the value of a demographic approach to quantifying the costs and benefits of mutualism.

Key words: ant–plant interactions; Crematogaster opuntiae; *extrafloral nectar; herbivory; integral projection model;* Liometopum apiculatum; *mutualism; pollination; Sevilleta National Wildlife Refuge, New Mexico, USA; tree cholla cactus,* Opuntia imbricate.

Introduction

Mutualism is classically defined as an interspecific interaction in which all participants benefit. However, interactions that are recognized as mutualisms often involve combinations of costs and benefits (Bronstein 2001, Bronstein et al. 2006). Identifying and quantifying these costs and benefits are critical for understanding the direction and strength of selection on participation. A large volume of research has documented the prevalence and benefits of mutualism and the degree to which they vary with biotic and abiotic contexts; ant–plant defensive mutualisms are particularly well studied in this regard (Chamberlain and Holland 2009, Rosumek et al. 2009, Trager et al. 2010). Less is known about costs of mutualism and how they are offset (or not) by benefits.

Costs of mutualism may be intrinsic to the interaction (direct costs), arising from trade-offs between participation in mutualism and other life history functions. Direct costs include the physiological costs of housing for partner species (Frederickson et al. 2012) or nutritional rewards such as nectar (Pyke 1991, Rutter and Rausher 2004) or seeds, in the case of some obligate pollination mutualisms (Bronstein 2001). Mutualism may additionally carry indirect ("ecological") costs if it modifies other intra- or interspecific interactions in ways that reduce fitness (Strauss et al. 2002, Miller et al. 2008, Kessler and Heil 2011, Frederickson et al. 2012). For example, some ant defenders of plants can indirectly increase attack by certain herbivores (Frederickson and Gordon 2007, Palmer et al. 2008) or even directly facilitate plant

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enemies such as honeydew-producing insects (Styrsky and Eubanks 2007, Frederickson et al. 2012). Ecological costs of mutualism may also result from a decrease in the frequency of interactions with other mutualists. For example, mutualistic fungal endophytes of plants can reduce colonization of roots by other beneficial microbes (Mack and Rudgers 2008). In food-for-protection mutualisms between ants and plants bearing extrafloral nectar (EFN), the focus of our work, visitation by aggressive ant guards may reduce the frequency of plant–pollinator interactions and, hence, plant reproductive output (Ness 2006, Malé et al. 2012, LeVan et al. 2013). Thus, ignoring the broader community context may yield incomplete inferences about the fitness consequences of ostensibly beneficial pairwise interactions.

In ant–plant protection mutualism, community context includes other animals on which plants rely for reproduction or seed dispersal. Some plants employ ant-deterrent mechanisms to prevent disruption of pollination. These mechanisms include morphological features that promote ant and floral spatial segregation (Raine et al. 2002) or ant-repellent volatile compounds in petals and/or pollen (Willmer and Stone 1997, Ghazoul 2001, Junker et al. 2011, Ballantyne and Willmer 2012). In addition to adaptive plant traits that minimize conflict over pollination, ants and pollinators can both exhibit diel patterns of activity on plants (Nicklen and Wagner 2006); the degree to which these overlap will set the stage for ant–pollinator interactions. Evidence for spatial or temporal segregation of ants and open flowers has led to inferences that plants have effectively resolved conflict over pollination (e.g., Willmer and Stone 1997, Nicklen and Wagner 2006). However, few studies have combined deterrence assays with estimates of pollination costs to determine whether mechanisms that isolate ants from floral visitors actually eliminate reproductive costs of ant defense (Willmer and Stone 1997, Ness 2006, LeVan et al. 2013).

Mutualism often involves guilds of interacting species, and partner identity can be an important source of variation in interaction outcome (Palmer et al. 2003). Although variation among ant species in aggression toward herbivores is well documented (e.g., Ness et al. 2006, Miller 2007), less is known about how the pollination costs of ant mutualism vary with partner identity (Ness 2006, LeVan et al. 2013) or how these costs correlate with benefits across species. For example, Ness (2006) found that the ant guard of barrel cacti most aggressive toward herbivores was also the most aggressive toward cactus pollinators. Too few studies are available to assess the generality of this result, although generality seems plausible because the very traits that promote anti-herbivore defense (high patrolling activity and aggression toward visitors) would also tend to promote pollinator deterrence. Quantifying costs and benefits across species is important for assessing the selective consequences of association with alternative partners.

Antagonistic floral visitors add a layer of complexity to the potential for conflict over pollination. Many plants are vulnerable to attack by nectar robbers, florivores, and pre-dispersal seed predators that oviposit in open flowers. Previous studies have shown that ant defenders can deter antagonistic floral visitors (e.g., Oliveira et al. 1999, Miller 2007). Thus, ant activity on or near flowers may carry a combination of ecological costs (pollinator deterrence) and benefits (floral enemy deterrence), depending on the frequency of different types of floral visitors and the strength of ant interactions with each. We are aware of no previous studies that have disentangled the potentially complex suite of interactions among defensive ants, mutualistic floral visitors, and antagonistic floral visitors. Furthermore, the possibility that ants, themselves, may vector pollen makes matters more complicated, perhaps compensating for deterrence of other insect pollinators. Although ant pollination is thought to be rare (Dutton and Frederickson 2012), it is also rarely tested (Ashman and King 2005, de Vega et al. 2009).

Evidence suggests potential for ants to influence plant reproduction via interactions with floral visitors, but virtually nothing is known about how these effects translate to plant fitness and, hence, selection for participation in mutualism. The link between plant reproduction and fitness is less obvious than it might appear because many ant-associated plants are long-lived iteroparous perennials (Trager et al. 2010) for which seed production may have a weak effect on fitness relative to high-elasticity vital rates (those that make strong proportional contributions to population growth) such as growth and survival. Long-lived plants may tolerate reproductive costs of ant defense without a reduction in fitness if they also experience benefits in terms of growth and survival (Stanton and Palmer 2011). Demographic models are essential for assessing the balance of costs and benefits in the currency of fitness, but their implementation for this purpose is surprisingly rare (Palmer et al. 2010, Rudgers et al. 2012, Yule et al. 2013). Furthermore, because the occurrence of ant defense and the identity of ant partners may change over the lifetime of an individual plant (Young et al. 1997, Fonseca and Benson 2003, Palmer et al. 2010, Miller and Rudolf 2011), assessing the fitness effects of each species in isolation may be too simplistic. Instead, it may be necessary to integrate the demographic effects of alternative partners, weighted by their probabilities of occurrence over the life cycle, as we do here.

Our experimental work focused on the tree cholla cactus, *Opuntia* (=*Cylindropuntia*) *imbricata* [Hawarth] D.C., an EFN-bearing plant that associates with two species of ant partners differing in quality of defense against insect herbivores (Miller 2007). Insect herbivory depresses plant growth and, because growth is a high-elasticity vital rate, population growth (Miller et al.

2009). *O. imbricata* is thought to be an obligate outcrosser that relies on insect pollinators (McFarland et al. 1989). It is also susceptible to pre-dispersal seed predators that oviposit in open flowers. The two ant partners do not co-occur on individual plants, allowing us to quantify their independent demographic effects. However, as in other ant–plant systems, a single cactus may interact with both ant species over its lifetime, and the probabilities of alternative partners vary significantly with plant size and reproductive stage.

We used a combination of field observations, experimental manipulations of ant and pollinator activity, and demographic modeling to examine how pollination costs of ant defense translate to plant fitness, given its benefits elsewhere in the life cycle. To determine the potential for conflict over pollination, we first quantified the ant-repellent properties of flowers and diel activity patterns of ants and pollinators. We then tested whether ant visitation imposes ecological costs and/or benefits via deterrence of pollinators and/or antagonistic floral visitors, and whether these effects differed between partner species. We hypothesized that the partner providing greater defensive benefits also imposes greater pollination costs. Finally, we used a demographic model that integrates ecological costs and benefits into the common currency of fitness to assess the net effects of ant defense. We asked how accounting for partner species turnover influences the net effects of ant defense, relative to the expected effects of each species alone.

EXPERIMENTAL METHODS

Study site and focal organisms

The tree cholla, *Opuntia imbricata*, is native to the deserts and grasslands of the southwestern United States. This arborescent cactus reaches ~2 m in height. Individuals can live ≥30 years and require at least nine years of vegetative growth prior to the onset of reproduction (T. E. X. Miller, *unpublished data*). Our work was conducted at the Sevilleta National Wildlife Refuge, a long-term ecological research (LTER) site in Socorro County, New Mexico, USA. See Miller et al. (2009) for a description of the study area.

Tree cholla secrete extrafloral nectar (EFN) from nectaries located at the base of the spines on young vegetative stem segments and reproductive structures (flower buds and fruits). In our study area, tree cholla are visited primarily by two ant species, the dolichoderine *Liometopum apiculatum* (Mayr) and the myrmicine *Crematogaster opuntiae* (Buren). Other ant species are observed very infrequently (<5% of plants). *L. apiculatum* and *C. opuntiae* co-occur in the environment, often occupying plants <1 m apart, but never co-occur on the same plant, probably an indication of interspecific competition for plant partners (Miller 2007). Associations with these two ant partners change over the course of plant development. The probability of extrafloral nectar secretion and, hence, the probability of ant

tending increase significantly with size and are significantly greater for flowering vs. vegetative plants (Miller 2014). Furthermore, given that a plant is tended by ants, the probability of being tended by *L. apiculatum* as opposed to *C. opuntiae* also significantly increases with size and flowering (Miller 2014).

Field experiments indicated that these species differ in their protective abilities (Miller 2007): *L. apiculatum* provided strong defense (herbivore damage to plants tended by this species was significantly lower than that of ant-free plants), and *C. opuntiae* provided weaker defensive benefits (herbivore damage was not significantly lower than for ant-free plants). Common insect herbivores in this system include a coreid bug (*Narnia pallidicornis*), a cerambycid beetle (*Moneilema appressum*), a weevil (*Gerstaekeria* sp.), and a noctuid moth (*Cahela ponderosella*). Insect exclusion experiments demonstrated that these herbivores depress plant population growth (Miller et al. 2009).

Tree cholla are pollinated primarily by solitary bees in the genera *Lithurge* and *Diadasia* (McFarland et al. 1989). In our study, we also observed visitation by bees in the genera *Halictus* and *Agapostemon*. In central New Mexico, flowering occurs in early June. Individual flowers are open for ~24 h. The moth *C. ponderosella* is a pre-dispersal seed predator and oviposits in open flowers in the early evening or early morning hours. The larvae eat through the petals and into the inferior ovary, leaving a distinctive entrance hole.

Potential for ant–pollinator interactions

Floral aversion experiment.—We conducted an experiment to test whether ants are deterred by floral volatiles. The design followed that of Ness (2006). We marked one-half of a petri dish with floral volatiles by rubbing fresh petals on it and left the other half unmarked. A single worker ant was then placed in the center of the dish. The dish was rotated 180° every 2.5 min and, after 15 min, we recorded whether the ant was in the marked or unmarked half. The experiment was replicated with 16 *C. opuntiae* workers and 17 *L. apiculatum* workers. The workers used were collected from separate plants to increase the likelihood that they belonged to different colonies. We used a binomial generalized linear model to test whether movement of workers to the marked vs. unmarked halves of the dish deviated from random and differed between species. We analyzed these and all subsequent data in R v.3.0.1 (R Development Core Team 2013).

Diel activity patterns.—We quantified diel patterns of ant and pollinator activity during a 24-h census. Reproductive plants tended by either *C. opuntiae* ($n = 6$ plants) or *L. apiculatum* ($n = 6$) were selected two days prior to observations. Plants were observed hourly for 24 h, beginning at 07:00 hours on 31 May 2013 and ending at 07:00 hours on 1 June 2013. For each plant, the number of ants on one haphazardly chosen branch was recorded each hour. Of the 12 plants, three were

randomly selected each hour and monitored for 10 min to measure floral visitation, recorded as the number of bees landing in flowers during that interval. For these observations, *Diadasia* bees constituted an overwhelming majority of all visitors, so we report only *Diadasia* counts.

Ant/pollinator exclusion experiment

Experimental design.—We conducted a field experiment to quantify the effects of ants, pollinators, and their interactions on plant reproductive success, and to contrast these effects between the two partner species. As in many ant–plant systems, it was not possible to randomly assign ant partners to plants; these ant species have very large colonies that forage over large spatial scales (Lanan and Bronstein 2013). This limitation means that partner identity effects could be confounded with other factors that differ between *L. apiculatum*- and *C. opuntiae*-tended plants. We employed a hierarchical experimental design that addresses this problem by assigning treatments to branches within individual plants, allowing us to fit an individual-level random effect in the statistical model and thus better account for plant-to-plant variance that is independent of ant identity.

In late May 2012, we selected 33 cacti naturally occurring within ~3 km². Of these, 17 plants were tended by *L. apiculatum* and 16 were tended by *C. opuntiae*. We selected our sample population with the criterion that all cacti had at least four branches, each with one or more unopened flower buds (maximum nine flower buds, mode three). Within each plant, we randomly applied a factorial combination of ant access or exclusion and pollinator access or exclusion to the four treatment branches. Ant access and exclusion treatments allowed us to assess ant effects on pollinators and plant reproduction. Crossing these treatments with pollinator treatments allowed us to quantify any pollination services provided by ants and to verify that *O. imbricata* is self-incompatible and cannot reproduce in the absence of insect visitors. For pollinator exclusion branches, we covered flower buds with organza mesh to prevent access by flying insects. Pollinator access branches had a sham mesh treatment affixed below the flower buds, providing full access to pollinators, to control for any unintended effects of mesh. For ant exclusion branches, we applied spray-on Tangle-Trap (Contech, Victoria, British Columbia, Canada), a sticky barrier that ants cannot cross, around the branch below the flower buds. Ants present on exclusion treatment branches at the time treatments were applied were removed with an aspirator. For ant access branches, we applied a sham Tangle-Trap treatment along one side of the branch so that ants could still gain access to the flower buds by walking on the other side.

Our experimental design assumed that pollinators perceive ants at the scale of individual branches and could differentiate between branches with vs. without ants on otherwise ant-tended plants. This assumption could make a null result for the ant access treatment difficult to interpret: it could indicate that pollinators perceive ants at the whole-plant level or that pollinators do not respond to ants. To test this assumption, we included an additional 10 plants of similar size (four *L. apiculatum*, five *C. opuntiae*, and one plant that was untended at the time of selection) from which we excluded ants entirely by applying Tangle-Trap near the base of the plants. As described previously, all ants on the plant were removed at the time the treatment was applied. For these plants, we applied pollinator access and exclusion treatments to only two branches. We predicted that if pollinators perceive ants at the branch level, we should see no differences in pollination and seed set between branch-level and plant-level ant exclusion treatments.

For all plants, treatments were maintained from 23 May to 10 June 2012, spanning the entire flowering period. Ant species identity on the experimental plants did not change during this period. At the end of the experiment, we covered all developing fruits with mesh and left them to ripen over the summer. We returned to collect the fruits on 22 September 2012.

Data collection.—We verified that application of Tangle-Trap effectively excluded ants and that the sham treatment did not by recording ant abundance and the presence/absence of EFN droplets on all treatment branches as a proxy for ant activity. Tree cholla will continue to secrete EFN in the absence of ants until the buildup of an EFN droplet on the nectary blocks further secretion. EFN accumulation is less common when ants have access to nectaries.

In the field, pollinator visitation was observed for the two pollinator access branches (ant access and exclusion) on each plant. We observed floral visitors (mostly bees and occasionally flies) during one 10-min period per branch per plant between 09:30 and 15:00 hours over nine days during the flowering period. The numbers of pollinator landings and hovers (close proximity to a flower but not making contact) were recorded. Although only bees landing in flowers can effectively pollinate, hovering in the vicinity of a flower is indicative of potential pollination services as hovering bees are often males waiting to mate with females collecting pollen. During these observation periods, we also counted the number of ants in or on flowers.

In the lab, field-collected fruits were scored for infestation by *C. ponderosella*, the seed-eating moth that leaves a distinctive entrance hole on the crown of the fruit. Fruits were then dried at 60°C until they stopped losing mass, about 48–60 h. After drying, fruits were cracked open and seeds were counted and massed in bulk. For each fruit, we divided the total seed mass by the seed count to estimate mass per seed (g). We examined seed number and mass because both can be affected by pollinator limitation (e.g., Ågren 1989).

Data analysis.—We analyzed the experimental data using generalized linear mixed-effect models (glmer in R package lme4; Bates et al. 2012). All models included individual plant as a random factor, within which each treatment combination was represented once. For all response variables, we first fit a null model with only random plant-to-plant variance. We then fit additional models including fixed effects of ant species, ant exclusion treatment, pollinator exclusion treatment, and their two- and three-way interactions. We calculated likelihood ratios of nested models and used χ^2 tests to determine whether adding treatment terms improved the fit to the data. If more than one treatment model improved fit over the null model, we tested them against each other. When best-fitting models included significant interaction terms, we used 1 df contrasts with Bonferroni adjustment of P values to infer differences among treatment combinations (glht in R package multcomp; Hothorn et al. 2013).

Although we could not randomly assign ant species to plants, our experimental and statistical approaches allowed us to distinguish between true ant effects vs. effects of unmeasured variables that were associated with ant species. For example, an ant access effect in the statistical models would indicate that pollinators responded to ants. On the other hand, a main effect of ant species would indicate that pollinators responded to unmeasured traits that differed between plants tended by either species and not to the ants themselves.

We modeled different response variables with different statistical distributions. The presence/absence of EFN droplets was modeled as a Bernoulli variable. The number of pollinator visits was modeled as a Poisson variable. Infestation by pre-dispersal seed predators was modeled as a binomial process (trials = total number of fruits on a branch; successes = number of moth-infested fruits). For floral visitors, only ant exclusion treatment and ant species were tested (there were no visitation data for the pollinator exclusion branches). Seed counts per fruit differed dramatically between pollinator access and exclusion treatments (*Results*), such that the full data set could not be appropriately analyzed with a single response distribution. We therefore analyzed the two treatments separately, modeling the pollinator-exclusion seed counts (mostly zeros) as a Poisson variable and pollinator-access seed counts as a Gaussian variable (seed counts are a discrete measure, but the pollinator-access data conformed well to the assumptions of a normal model). Mass per seed (in grams) was modeled as a normal variable. Seed count and mass models included an additional random effect of fruit nested within plant, because there usually were multiple, nonindependent observations (fruits) per treatment branch. The analysis of mass per seed was restricted to the pollinator access treatments because very few seeds were produced when pollinators were excluded (*Results*).

We used the additional, complete ant-exclusion plants to test for differences in our response variables between ant-free branches on otherwise ant-tended plants vs. on vacant plants. As before, we fit models to the pollinator visitation, seed count, and seed mass data for ant-exclusion branches. We used likelihood ratio tests to determine whether accounting for presence/absence of ants at the whole-plant level improved the fits of the models.

Experimental Results

Potential for ant–pollinator interactions

Floral aversion experiment.—During the floral aversion trials, 75% of the *C. opuntiae* workers (12/16) and 65% of the *L. apiculatum* workers (11/17) moved to the non-floral side of the petri dishes. The fitted probability of movement to the non-floral side was significantly greater than 0.5 ($z = 2.2$, $P < 0.03$). Accounting for species differences did not improve model fit, indicating that both species equally avoided the floral side of the dish.

Diel activity patterns.—Ants and pollinators had contrasting patterns of diel activity. Tree cholla were tended by *C. opuntiae* and *L. apiculatum* around the clock, but ant activity peaked in the late-evening and early-morning hours (Fig. 1). Pollinator activity showed the inverse pattern, peaking at midday, when ant activity was lowest. We did not observe moth seed predators during the 24-h observation period.

Ant/pollinator exclusion experiment

Treatment effectiveness.—Despite the temporal partitioning and evidence for ant-repellent floral volatiles, we observed workers of *C. opuntiae* and *L. apiculatum* in flowers of ant access branches during pollinator observation periods. Ant abundance in flowers did not differ between species (for *C. opuntiae*, 3.21 ± 0.98 ants/flower, mean $\pm$ SE; for *L. apiculatum*, 2.93 ± 0.76 ants/flower; $t_{24.7} = -0.19$, $P < 0.8$). Application of Tangle-Trap effectively excluded ants. All but two ant exclusion branches had zero ants at the time of our surveys and accumulation of EFN droplets was more frequent on ant exclusion branches (91%) than on ant access branches (12%; $\chi^2 = 115.7$, $P < 0.0001$).

Pollinator visitation.—Ant exclusion significantly increased pollinator visitation, and equally so for *C. opuntiae*- and *L. apiculatum*-tended plants (Fig. 2A). Including ant exclusion treatment in the statistical model significantly improved fit to the data over a null model ($\chi^2 = 6.3$, df = 1, $P < 0.01$), but including ant species ($\chi^2 = 0.74$, df = 1, $P < 0.39$) did not. Further, including a species $\times$ exclusion interaction did not provide a better fit than did ant exclusion alone ($\chi^2 = 0.58$, df = 2, $P < 0.75$). Thus, there was no evidence that the more aggressive guard (*L. apiculatum*) had a more deterrent effect on pollinators. This analysis and Fig. 2A comprise total pollinator visits, including hovering and landing by all bees and flies. We found the same result (main effect of ant exclusion) when we limited the data to only
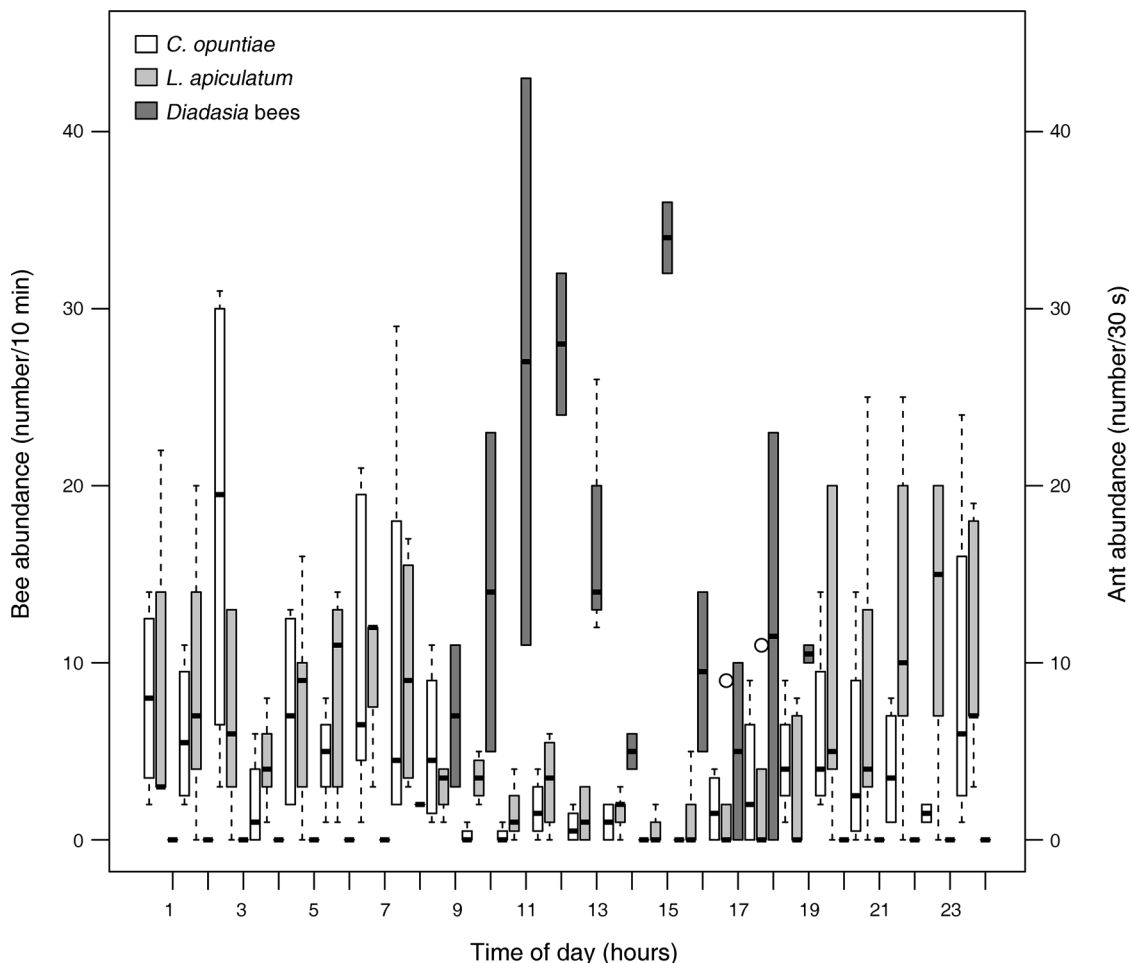
FIG. 1. Temporal patterns of cactus bee and ant abundance censused at hourly intervals on the tree cholla cactus *Opuntia imbricata* over 24 hours beginning at 00:00 hours. Ant data are counts of total ants (for *Crematogaster opuntiae* and *Liometopum apiculatum* separately) on one branch during 30-s monitoring periods, and pollinator data are the number of *Diadasia* bee visits during 10-min monitoring periods. Boxplots show medians (thick black lines), inner quartiles (box borders), 5th and 95th percentiles (whiskers), and outliers (dots).

specialized *Diadasia* bees or only *Diadasia* bees that landed in flowers.

Pre-dispersal seed predation.—Ant activity in flowers had weak effects on attack by *C. ponderosella*, the pre-dispersal seed predator that oviposits into open flowers. Neither the main effect of ant exclusion ($\chi^2 = 0.095$, df = 1, $P < 0.76$; for species, $\chi^2 = 0.037$, df = 1, $P < 0.87$) nor the ant species × exclusion interaction ($\chi^2 = 6.1$, df = 3, $P < 0.11$) provided a statistically significantly improvement over the null model with only plant-to-plant random variance. However, there was a trend toward lower fruit infestation in the presence vs. absence of *L. apiculatum* and higher infestation in the presence vs. absence of *C. opuntiae* (Fig. 2B).

Plant reproductive success.—Pollinator exclusion strongly affected seed production, with the majority of pollinator-excluded fruits (149/174) having zero seeds (Fig. 3A). Although both ant species occurred inside flowers, there was no evidence that either provided

pollination services. There were no significant differences in seed set between ant access and exclusion branches for either ant species in the absence of other pollinators, indicating that neither ant species is an effective pollen vector. This analysis excluded two outlier fruits (one from a *C. opuntiae* plant and one from a *L. apiculatum* plant) with >100 seeds.

In the presence of pollinators, there was a significant interactive effect of ant exclusion and ant species identity on the number of seeds per fruit ($\chi^2 = 10.04$, df = 3, $P < 0.018$). Contrasts indicated that ant access to flowers reduced seed counts on *C. opuntiae*-tended plants ($z = -3.01$, $P < 0.015$), but not on *L. apiculatum*-tended plants ($z = 0.95$, $P < 0.9$; Fig. 3A). On average, *C. opuntiae* access reduced seed count per fruit by 30.5%. Ant access to flowers also reduced seed mass (Fig. 3B). Unlike seed counts, *C. opuntiae* and *L. apiculatum* were consistent in their negative effects on seed mass (only the main effect of ant access improved
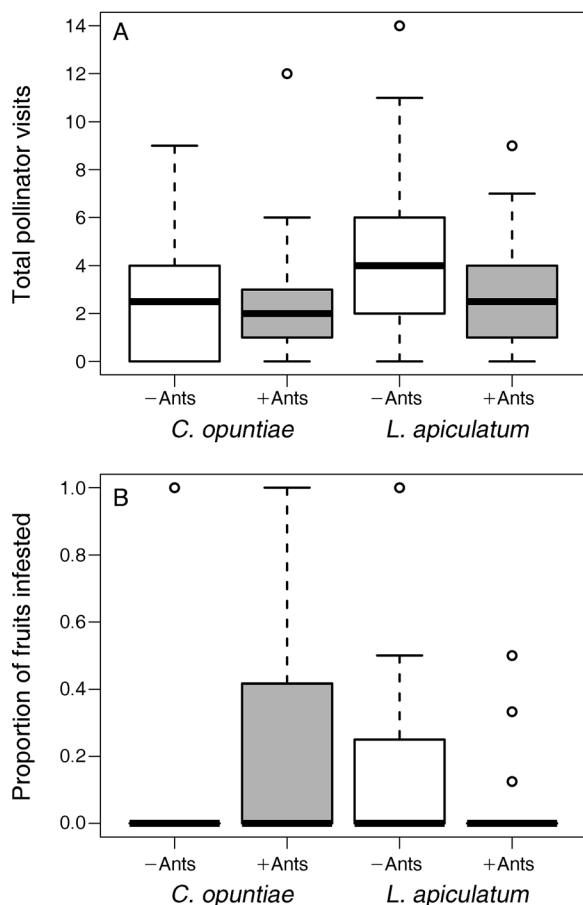
FIG. 2. Effects of ant exclusion (open boxes) and ant access (gray boxes) on floral visitors for *C. opuntiae*- and *L. apiculatum*-tended plants. (A) Total hovers and landings by cactus bees and flies. (B) Proportion of fruits per branch infested by seed-eating moths. Boxplot components are as in Fig. 1.

the fit of the null model: $\chi^2 = 10.01$, df = 1, $P < 0.002$). On average, ant access reduced seed mass by 16.5%.

Whole-plant ant exclusion produced results consistent with branch-level treatments, suggesting that pollinators perceive ants at the branch level. When we analyzed data only from ant exclusion branches, accounting for ant presence or absence on the rest of the plant did not improve models fit to pollinator visitation ($\chi^2 = 0.25$, df = 1, $P < 0.61$), seed number ($\chi^2 = 0.016$, df = 1, $P < 0.9$), or seed mass ($\chi^2 = 0.79$, df = 1, $P < 0.37$).

DEMOGRAPHIC MODEL FOR FITNESS EFFECTS OF ANT DEFENSE

Model construction.—We used a size-structured integral projection model (IPM) to evaluate how benefits and costs of ant visitation translate to net effects on plant fitness. Details of the demographic data collection, parameter estimation, and model construction are provided in the Appendix. Here we provide a brief overview of our approach.

Assessing fitness effects of ants required that we specify their positive and negative effects on demographic vital rates, and then generate fitness predictions relative to the ant-free state. Estimates for positive demographic effects came from previous experimental evidence for the dependence of herbivore damage on ant status (*C. opuntiae*-tended, *L. apiculatum*-tended, or ant-free) and the dependence of plant growth on herbivore damage (Miller 2007, Miller et al. 2009). Together, these data allowed us to quantify the expected growth trajectories of plants in each ant state (*C. opuntiae*, *L. apiculatum*, or ant-free; Appendix). For costs of ant defense, the present study provided estimates for seeds per fruit and seed mass of *C. opuntiae*-tended, *L. apiculatum*-tended, and ant-free plants. Thus, specifying differences in seed number based on ant status was straightforward. The demographic effects of reduced seed mass (Fig. 3B) were less clear, although reduced germination is a reasonable possibility. We modeled two
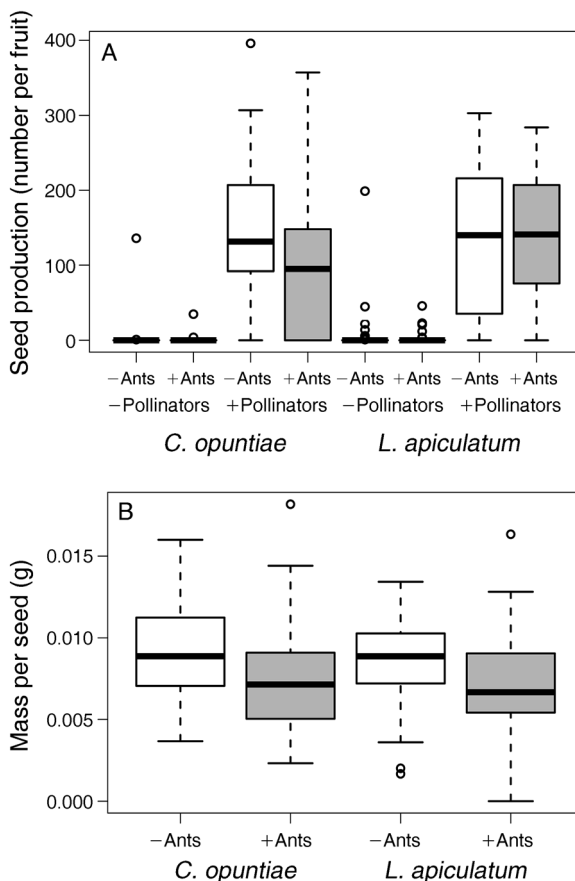

FIG. 3. Effects of ant exclusion (open boxes) and ant access (gray boxes) on (A) seed number per fruit and (B) mass per seed for *C. opuntiae*- and *L. apiculatum*-tended plants. Seed numbers in panel (A) are shown for pollinator exclusion (−Pollinators) and access (+Pollinators) branches. Because few seeds were produced in the absence of pollinators, seed masses in panel (B) are shown for pollinator access only. Boxplot components are as in Fig. 1.

scenarios for the effects of ants on seed mass: (1) reduced seed mass has no demographic consequences, and (2) the proportional reduction in seed mass (16.5%) causes the same proportional reduction in germination. The real consequences of reduced seed mass probably lie between these extremes, given the evidence for effects of seed mass on recruitment in some systems (e.g., Hegland and Totland 2007) but not others (e.g., Eriksson 1999). Although we did not explicitly include positive effects of ants on seed production via deterrence of pre-dispersal seed predators (Fig. 2B), any beneficial effects on seed counts were represented in the data and, hence, in the parameter estimates.

We used the model to examine whether and how growth benefits could offset pollination costs. We numerically generated fitness isoclines by identifying combinations of seeds per fruit and growth (specifically, the intercept of the size-dependent growth function; see Appendix) that yielded equivalent fitness based on ant-free parameter values. We generated isoclines for each of the two scenarios regarding germination effects of seed mass. The isocline analysis indicated whether the observed effects of ants on seed production and growth were consistent with positive, neutral, or negative effects on fitness.

In addition to the isocline analysis, which assumes fixed parameter values, we modeled plant fitness accounting for dynamic transitions in ant associations (i.e., partner turnover). We estimated the fitness of hypothetical plants tended by either *C. opuntiae* or *L. apiculatum*, exclusively, over their life cycle, and the fitness of plants that interacted with both partner species over their life cycle, as real plants typically do. These simulation experiments included ontogenetic variation in ant visitation, based on empirical estimates (Miller 2014), such that the probability of ant visitation increased with size and the onset of flowering. However, only in the latter, two-ant scenario did ant identity change with ontogeny (the probability of *L. apiculatum* increased with size and flowering). For each simulation treatment, we calculated fitness relative to plants that experienced ant-free demographic rates for their entire life cycle. We estimated fitness as R_0, the expected lifetime reproductive rate.

Because we relied on multiple, independent experiments to estimate the demographic effects of ant defense, we required a statistical framework that allowed us to translate uncertainty associated with each data set to uncertainty in quantities derived from them, including fitness, our ultimate response variable. For this reason, we constructed the IPM using Bayesian statistical methods, which are ideally suited for quantifying the propagation of uncertainty. Details of the Bayesian analysis are provided in the Appendix.

Model results.—The model predicted that, for a given reduction in seed output due to pollinator deterrence, plants could match their fitness with a particular increase in growth; the fitness isoclines in Fig. 4 define
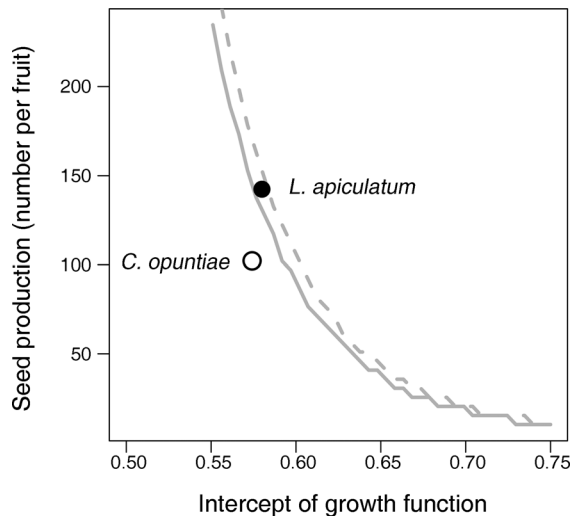

FIG. 4. Fitness isoclines predicted by integral projection model (IPM). Isoclines (gray) show combinations of reproduction (number of seeds per fruit) and growth (intercept of the size-dependent growth function; see Appendix) for which ant-free plants achieve equal fitness (net reproductive rate, R_0). Solid and dashed isoclines show scenarios in which observed negative effects on seed mass have a proportionally equal negative effects on seed germination (dashed) or have no effect on seed germination (solid). Best parameter estimates for *C. optuniae* and *L. apiculatum* are indicated (Appendix).

this relationship. The convexity of the isoclines indicates that a large reduction in seed output could often be matched by a relatively small increase in growth. A negative effect of seed mass reduction on germination increases the compensatory burden, such that the same reduction in seed output requires a greater increase in growth to achieve equal fitness (Fig. 4). Our best estimates for the demographic effects of *C. opuntiae* fell below the ant-free fitness isoclines, regardless of assumptions about the effects of reduced seed mass on germination, suggesting a net negative effect. By contrast, best estimates for *L. apiculatum*-tended plants fell just above or below the ant-free isoclines, depending on assumption about seed mass effects (Fig. 4).

Simulations that included size-dependent transitions in ant status indicated that *C. optuntiae* had a significantly negative fitness effect when it was the only ant in the system, regardless of our assumptions about the effects of reduced seed mass on germination (Fig. 5). Thus, this species' pollination costs clearly outweighed its defensive benefits. The predicted fitness effects of *L. apiculatum*, alone, depended on the consequences of seed mass reductions. Fitness effects of this species were positive when seed mass did not affect germination and negative when it did, although in both cases 95% confidence intervals for the fitness effects included zero (Fig. 5). The consistency of these results with the isocline analysis is not surprising, because flowering plants (the stage at which pollination costs are manifest) were virtually guaranteed to be ant-tended in the dynamic-
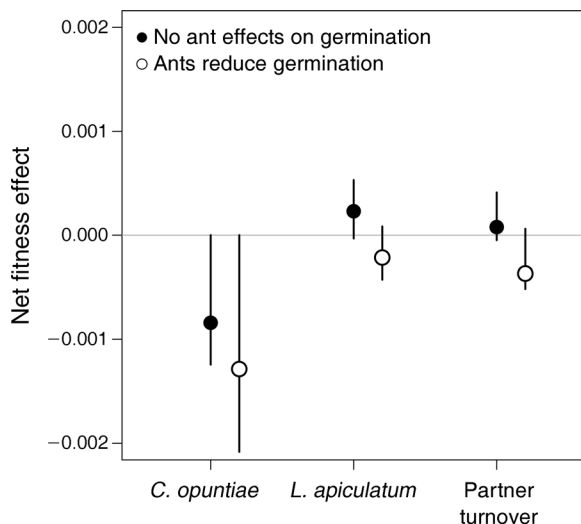
FIG. 5. Net fitness effects of *C. optuniae* alone, *L. apiculatum* alone, and both ant species with partner turnover. Net fitness effect is calculated as the difference in R_0 (expected lifetime reproductive output, i.e., expected number of seeds produced per seed) between the focal treatment and the ant-free treatment. Points and bars show means and inner 95% quantiles of the posterior distribution of the fitness effects, derived from Bayesian parameter estimation (see Appendix). Results are plotted for two scenarios in which the negative effects of ant access on seed mass (Fig. 3B) either have no effect on seed germination (solid points) or reduce seed germination (open points) by the same proportional amount that seed mass was reduced (16.5%, on average).

transition model. Finally, the model in which a plant could interact with either species based on its size and reproductive state (the model that best reflects conditions in the field) predicted fitness effects of ant defense that were highly consistent with the independent effects of *L. apiculatum* (Fig. 5). This suggests that partner turnover allowed plants to avoid costs associated with *C. opuntia*.

DISCUSSION

We found that plant traits and ant and pollinator behaviors minimized potential for conflict over pollination, as in other ant–plant systems (Willmer and Stone 1997, Raine et al. 2002, Nicklen and Wagner 2006). Ants were deterred by floral volatile compounds and they were least active at the time of day when pollinators were most active. Nevertheless, plants experienced ecological costs of defense in terms of pollinator visitation, seed number, and seed mass. Thus, evidence for segregation of ants and pollinators should not be taken as evidence that conflict over pollination is resolved, although presumably it is minimized. A demographic model parameterized for this system showed that plants can absorb a decrease in reproduction (a low-elasticity vital rate) without a reduction in fitness if they experience a sufficient benefit in growth (a high-elasticity vital rate); see Miller et al. (2009). Our

best estimates of costs and benefits indicated that one partner species met or exceeded the beneficial effects necessary to offset costs, whereas the other species did not, rendering it a reproductive parasite. Interestingly, dynamic partner transitions allowed plants to maintain relatively high fitness by avoiding the reproductive parasite at reproductive life stages. Previous observational studies have reported negative effects of ant defenders on plant pollination and reproductive success (e.g., Ness 2006, LeVan et al. 2013), but the implications of these costs for plant fitness, and hence selection on mutualistic traits, have remained elusive. We provide the first experimental evidence for pollination costs of ant defense and the first assessment of whether and how these costs are balanced by benefits elsewhere in the life cycle.

Our experimental design and statistical approach allowed us to rigorously quantify partner-specific effects on pollination despite the inability to randomly assign ant status to plants. We found that *C. opuntiae*, the weaker defender, imposed more severe pollination costs than did *L. apiculatum*, an aggressive defender, in contrast with a previously reported positive correlation across ant species between anti-herbivore and anti-pollinator effects (Ness 2006), a study that also included *C. opuntiae*. The conclusion that *C. opuntiae* imposes a stronger cost than *L. apiculatum* is based on the former species' effect on the number of seeds produced. Otherwise, the two species were consistent in their effects on pollinator visitation (Fig. 2A) and seed mass (Fig. 3B). The lack of effect of *L. apiculatum* on seed number was surprising, given its negative effects on visitation and seed mass. We suspect that the difference in moth seed predation between ant species (Fig. 2B) contributed to this result. Although treatment effects on seed predation were not statistically significant at $\alpha = 0.05$, there was indication that *L. apiculatum*, but not *C. opuntiae*, provided some protection against seed predators, as was found in a previous experiment (Miller 2007). Thus, we speculate that pollinator deterrence by *L. apiculatum* was offset by deterrence of antagonistic floral visitors, yielding a neutral effect on seed number.

Our conclusions regarding the growth benefits of *C. opuntiae* and *L. apiculatum* also warrant further discussion. Estimates for the effects of both partners on herbivore damage came from experiments conducted at a single life stage (mature plants), and we extrapolated the expected ant-mediated increase in growth over the size distribution (Appendix). It is possible that plant-size-specific effects of ants would modify our quantitative results. Furthermore, we suspect that we have underestimated the defensive benefits of *L. apiculatum*. This ant species occurs at high frequency in the study population, occupying, on average, 80% of plants over the study years 2004–2013 (R. Patterson and T. E. X. Miller, *unpublished data*). In populations where this strong defender is rare or absent, insect herbivore abundance and damage are significantly greater (Miller

et al. 2009). We suspect that the rarity of herbivores in the focal population reflects pervasive and effective biotic defense. Experimental ant-exclusion plants embedded in a matrix of well-defended and herbivore-free plants may therefore remain minimally susceptible to herbivore attack; this would lead to the underestimation of defensive benefits. Additionally, as indicated previously, our current results for interactions with *C. ponderosella* (Fig. 2B) are inconsistent with prior work in this system, which indicated that *L. apiculatum*, but not *C. opuntiae*, deterred this pre-dispersal seed predator (Miller 2007). Seed predator attack rates were lower in the present study year than in the previous one (~10% of fruits infested in 2012 vs. 30% in 2005), leading us to hypothesize that this benefit of ant defense may be stronger in years of high seed predation. However, defense against threats that are infrequent may still confer advantages in the long term (Stanton and Palmer 2011). For these two reasons, we think that *L. apiculatum* is a more strongly beneficial mutualist than our best demographic parameter estimates suggest.

The key to the net neutral-to-positive effect of *L. apiculatum*, despite pollinator deterrence, was the balance of component costs and benefits in different parts of the life cycle. There is growing evidence that longevity and life cycle complexity can buffer iteroparous organisms against costs of mutualism. For example, negative effects of fungal symbionts on the survival of perennial grasses can be more than offset by positive effects of symbionts on growth (Yule et al. 2013) or reproduction (Rudgers et al. 2012), resulting in stable mutualism. Similarly, Palmer et al. (2010) showed that long-lived *Acacia* trees can sustain negative effects of ant symbionts on low-elasticity vital rates and still gain net fitness benefits if those costs are balanced by positive effects on high-elasticity rates. All of these studies relied on models that integrate demographic processes over the life cycle to assess the fitness consequences of component costs and benefits. Thus, attention to life history complexity and interplay between models and experiments are emergent themes in our growing understanding of the fitness effects of mutualistic interactions that carry both benefits and costs, as most do.

Attention to life history complexity may be especially important in multispecies mutualisms with potential for partner turnover. Ontogenetic shifts in ant partner identity are intriguingly common across ant defensive mutualisms involving long-lived plants (Young et al. 1997, Fonseca and Benson 2003, Djieto-Lordon et al. 2004, Dejean et al. 2008, Palmer et al. 2010). In our system, flowering plants are significantly more likely to be tended by *L. apiculatum* than are vegetative plants, and longitudinal observations indicate that turnover from *C. optuntiae* to *L. apiculatum* is significantly more likely than the reverse, particularly at the onset of plant reproduction (Miller 2007, 2014). (Indeed, it was difficult to locate a sufficient number of *C. opuntiae*-tended reproductive plants for the present study.) Our results add to a growing body of work suggesting that ontogenetic turnover can influence the lifetime fitness effect of defensive mutualism. Palmer et al. (2010) showed that partner turnover generates complementary sets of benefits over *Acacia* ontogeny, such that plant fitness is maximized under high lifetime partner diversity. Here, too, we find beneficial effects of partner turnover for plant fitness (Fig. 5). However, in contrast to the *Acacia* study, we find no evidence for beneficial effects of diversity per se. Turnover allowed plants to achieve the fitness associated with the superior mutualists, alone, but not to exceed it.

The pattern of partner turnover clearly works in the plants' favor (Fig. 5), raising questions about its underlying mechanisms. Our results suggest the hypothesis that increased interactions with *L. apiculatum* at reproductive life stages reflect adaptive traits with which plants bias their ant associations in favor of the more beneficial/less costly species. We are still exploring potential for partner selection mechanisms, but we think that the nutritional reward—extrafloral nectar (EFN)—plays a key role. The quantity (rate of secretion) and quality (carbohydrate and amino acid content) of EFN increase significantly at the onset of plant reproduction (Miller 2014), making reproductive plants much more valuable, from the ants' perspectives, than vegetative plants. *L. apiculatum* is thought to be competitively superior to *C. opuntiae* (Miller 2007), raising the possibility that *L. apiculatum* outcompetes *C. opuntiae* for access to higher-quality plant partners. There may also be a role for differences in digestive enzymes between ant species, because the EFN of reproductive plants is significantly enriched in disaccharide sugars (Miller 2014), which *L. apiculatum* is better equipped to digest (R. Patterson and T. E. X. Miller, *unpublished data*). Whether or not these plants employ partner selection mechanisms, as in other ant–plant systems (e.g., Heil et al. 2005), remains to be determined. Our results certainly indicate that they are under selection to do so.

The discussion of EFN raises one final caveat to our conclusions. EFN production is probably a direct cost of participation in mutualism with ants (Pyke 1991, Rutter and Rausher 2004). We did not attempt to quantify this direct cost; our approach focused solely on pollinator-mediated costs of ant defense. Accounting for the cost of attracting ants with EFN would amplify the total cost of mutualism by some degree. However, this investment is likely to be independent of ant species (because there is no evidence to suggest that one species extracts more rewards), and therefore should not affect our conclusions about relative fitness effects of the two partners.

Conclusions

Aggression toward herbivores and reductions in herbivory are the criteria most commonly used to assess and compare the effects of ostensibly mutualistic

partners in plant defensive mutualisms. Our results add to previous findings that these indicators fail to reveal ecological costs of defensive "mutualists" (Ness 2006, Mack and Rudgers 2008, Frederickson et al. 2012, Malé et al. 2012, LeVan et al. 2013) and suggest the need to consider mutualisms in their more realistic, multispecies community context. We report evidence for ecological costs of ant defense, despite the operation of mechanisms that minimize them. By evaluating costs and benefits through the lens of the complete life cycle, we were able to quantify their net effects in the currency of fitness and identify the direction of selection on plant associations with alternative partner species. Life cycle longevity and complexity may allow plants to absorb costs, yet accrue net benefits, because not all vital rates contribute equally to fitness. Ontogenetic turnover could further allow plants to avoid costly partners at vulnerable life stages. We anticipate that the continued integration of field experiments with models that account for realistic demographic complexity will advance understanding of the costs and benefits of mutualism.

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SUPPLEMENTAL MATERIAL

Appendix

Integral projection model (IPM) for ant effects on plant fitness (*Ecological Archives* E095-253-A1).