

# An Integral Projection Modeling Approach to Understanding Demographic Effects of Multispecies Mutualisms

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## Abstract

Mutualisms are widespread species interactions with diverse and dynamic consequences. They are considered more context dependent than other species interactions, meaning there are many different factors which change the outcomes of interactions between mutualists, including partner diversity. Partner diversity has become a central focus in the field of mutualisms, expanding previous work from primarily pairwise to multispecies mutualisms. It has been shown that pairwise studies are poor predictors of the effects of multispecies mutualistic interactions. The diversity of partners in a multi-species mutualism causes varied demographic effects on the population of the focal mutualist which can be explained by several mechanisms: portfolio effect, complementarity, and sampling effect.

I use the plant-ant multi-species mutualism in which, the cactus *Cylindropuntia imbricata* (tree cholla) produce extrafloral nectar and the ants, *Crematogaster opuntiae*, *Liometopum apiculatum*, *Forelius pruinosus*, and rarer species, provide defense from various herbivores and seed predators. I used 18 years of data collected from plant demographic censuses, which includes data such as size, survival, reproductive status, flowers produced, and ant partner for all plants in 8 30×30 m plots at the Sevilleta National Wildlife Refuge in central New Mexico. With this data I parameterize a series of Bayesian hierarchical generalized linear vital rate models to determine the impacts of different partners on the focal mutualists. I found that different ant partners had different impacts on the vital rates of the tree cholla. Specifically, *C. opuntiae* tended plants had advantages in both growth and survival when small, and large *L. apiculatum* tended plants had floral viability advantages. With these models I constructed an Integral Projection Model in which I could vary the presence of each partner, creating different “diversity scenarios”, to determine under which diversity scenario the focal mutualist experienced the highest plant fitness, and which mechanism(s) may explain the effects of partner diversity. I found that the all scenarios which included the partner *L. apiculatum* resulted in the highest possible fitness for the tree cholla. Results further suggest that diversity benefits in this system are driven by sampling effect

27 , meaning *L. apiculatum* ants are the "best" partner. I also found that partner diversity benefits the focal mutualist in this system in the form of portfolio effect by buffering the tree cholla from the effects of inter-annual variation. This study highlights how partner diversity can increase 30 the overall benefits a focal mutualist receives. It also highlights the importance of a mechanistic understanding to explain the benefits of this diversity across systems.

## Introduction

Mutualisms are species interactions where all participants receive net benefits, leading to higher individual fitness and increased population growth rates. They are among the most widespread species interactions (Axelrod and Hamilton, 1981; Bronstein, 1994; Chamberlain et al., 2014; Frederickson, 2013; Leigh, 2010) but can deteriorate into commensalism or parasitism under conditions that elevate costs or dampen benefits (Bahia et al., 2022; Mandyam and Jumpponen, 2014; Rodriguez-Rodriguez et al., 2017; Song et al., 2020; Thrall et al., 2007). Mutualisms are considered more context dependent than other species interactions (Chamberlain et al., 2014; Frederickson, 2013), meaning the magnitude and sign of interaction strength are often determined by environmental conditions and species' identities Leigh (2010); Noe and Hammerstein (1994).

Mutualism is defined at the level of a species pair (+/+ ) but these interactions are embedded within multi-species communities, and growing evidence suggests that pairwise interactions are poor predictors of the net effects of multi-species mutualism (Afkhami, 2014; Bascompte, 2009; Dattilo et al., 2014; Palmer et al., 2010). A focal mutualist may interact with multiple guilds of partner types (e.g., plants that interact with pollinators, seed dispersers, soil microbes, and ant defenders) or with multiple partner species within the same guild (e.g., plants visited by multiple pollinator species). Within a mutualist guild, partner species often differ in the amount or type of goods or services they provide, making partner identity an important source of contingency in mutualism (Stanton, 2013). Whether and how partner diversity modifies the demographic effects of mutualistic interactions remain open questions within relevance in applied settings (Rogers et al., 2014).

There are multiple mechanisms by which partner diversity can influence the net benefits accrued by a focal mutualist, mirroring the mechanisms by which, at a larger scale of organization, biodiversity can influence ecosystem function Barrett et al. (2015); Ushio (2020); Yeung et al. (2006). First, when there is a hierarchy of fitness effects (a consistent ranking of best to worst mutualists), a more diverse sample of the partner community may be more likely to include the

best partner Frederickson (2013). This can lead to an apparent benefit of diversity driven by a sampling effect Batstone (2018). However, if partner associations are mutually exclusive (a focal mutualist may engage with only one partner at a time), then partner diversity may impose opportunity costs, leading to negative effects of a diverse mutualist assemblage relative to exclusive association with the single best partner (Miller, 2007). Second, even within a single mutualist guild, the benefits conferred by alternative partner species can vary in type, and not just degree Bronstein et al. (2006); Stachowicz and Whitlatch (2005); Stanton (2013). This can lead to a positive effect of partner diversity through complementarity of alternative functions Batstone (2018). Interference or synergies between partners can make their combined effect different than the expected from the sum of complementary functions Afkhami (2014). Third, partner species and herbivores can have species-specific responses to environmental variation, either spatially (Ollerton et al., 2006) or temporally (Alarcón et al., 2008). Multiple partners can therefore act as a 'portfolio' that stabilizes fitness benefits and protection across spatial or temporal heterogeneity, leading to positive effects of partner diversity through the portfolio effect Batstone (2018); Horvitz and Schemske (1990); Lázaro et al. (2022).

Partner diversity can have different effects depending on whether partners are present all at once or sequentially (partner turnover) (Barrett et al., 2015; Bruna et al., 2014; Dattilo et al., 2014; Djiéto-Lordon et al., 2005; Ness et al., 2006; Ushio, 2020). Sequential associations are likely when alternative partners engage in interference competition for access to a shared mutualist Batstone (2018); Kiers et al. (2003); Trøjelsgaard et al. (2015); Wulff (2008). Turnover can happen at different timescales, from minutes to years (Horvitz and Schemske, 1986; Oliveira et al., 1999). The frequency of partner turnover can impact the level of benefits received by the focal mutualist, particularly if the benefits continue to accumulate with successive turnover (e.g., when sequential partners provide complementary functions) or if they saturate over time (Fiala et al., 1994; Sachs et al., 2004). Directionality of turnover can also influence effects of partner diversity if partner identity changes consistently across ontogeny of a focal mutualist (Dejean et al., 2008; Fonseca et al., 2003; Noe and Hammerstein, 1994). For example, plant susceptibility to enemies can

change across life stages (Barton and Koricheva, 2010; Boege and Marquis, 2005), so the benefits of defensive mutualism with ants are greatest when more defensive partner species align with more vulnerable life stages (Dejean et al., 2008; Djiéto-Lordon et al., 2005).

Defensive ant-plant mutualisms – where plants provide food and/or housing to ants that in turn defend them from enemies – are widespread interactions that offer valuable model systems for the ecology and evolution of mutualism (Bronstein, 1998; Bronstein et al., 2006). Extrafloral nectar (EFN) bearing plants can serve as dietary resources that promote ant abundance and colony size (Byk and Del-Claro, 2011; Ness et al., 2006, 2009). Presence of defensive ant partners is often linked to reductions in herbivory (Rudgers, 2004; Trager et al., 2010) and demographic advantages for the plant partner (Báez et al., 2016). Defensive ant-plant mutualisms are commonly multi-species, where a guild of ant partner species share, and often compete for, a plant mutualist (Agrawal and Rutter, 1998; Beattie, 1985; Bronstein, 1998; Trager et al., 2010). Ant partners can vary in their ability to deter herbivores (Bruna et al., 2014), and visitation by low quality ant partners can prevent visitation by higher quality partners, consequently causing a reduction in fitness through missed opportunity costs (Fraser et al., 2001; Frederickson, 2005). Susceptibility to herbivory can also vary significantly throughout the life stages of the plant (Boege and Marquis, 2005), suggesting that the order and timing of successive partners is important to the effectiveness of ant partners. Temporal dynamics of ant visitation therefore can have important impacts on the fitness of the plant partners (Barton and Koricheva, 2010; Boege and Marquis, 2005; Fonseca et al., 2003). Herbivore identity and pressure can vary inter-annually (Wetzel et al., 2023), much like mutualist identity and presence, meaning the threat plants face varies just as much as the protection they receive due to temporal stochasticity. Recent studies have begun to investigate how ant partner diversity affects plant fitness (Afkhami, 2014; Dattilo et al., 2014; Fiala et al., 1994; Gaume et al., 1998; Ludka et al., 2015; Palmer et al., 2010). However, little is known about the combined effects of partner identity, directional partner turnover, and temporal stochasticity, particularly because the long-term data required to detect portfolio effects are rarely available.

This study examined the consequences of partner diversity in a food-for-protection mutualism between the tree cholla cactus (*Cylindriopuntia imbricata*), a long-lived EFN-bearing plant, and multiple species of ant partners. Previous studies have shown that herbivory by specialized insect herbivores negatively affects plant fitness Miller et al. (2009), and ant defense reduces herbivore damage Miller (2007). Tree cholla are tended by two common ant species (*Liometopum apiculatum* and *Crematogaster opuntiae*) and several infrequent species, all of which are ground-nesting. These ant species locally co-occur but individual plants are typically tended by only one species that patrols the plant around-the-clock and maintains control of the plant's nectar resources for an entire growing season (Donald and Miller, 2022; Ohm and Miller, 2014). Switches between partner species, or between vacancy and ant occupancy, commonly occur from one growing season to the next (Miller, 2007). Prior experiments suggested a hierarchy of mutualist quality, with *Liometopum apiculatum* providing strong fitness benefits and *Crematogaster opuntiae* having net negative fitness effects because herbivore deterrence is outweighed by deterrence of pollinators (Miller, 2007; Ohm and Miller, 2014). However, those studies did not integrate the demographic effects of ant defense across the plant life cycle, nor did they account for inter-annual fluctuations in the herbivore populations.

We used a unique long-term data set that allows us to explore mutualistic associations with multiple partner species and how the demographic effects of alternative partner species varied across plant size structure and nearly 20 years of inter-annual fluctuations. We used this observational data set, contextualized by previous experiments, to ask whether and through which mechanism(s) partner diversity affects the fitness benefits of ant visitation for the focal plant partner. Specifically, we asked:

1. What are the demographic effects of association with alternative partners and how do these effects fluctuate across years?
2. What are the frequency and direction of partner turnover across the plant life cycle?
3. What is the net effect of partner diversity on plant fitness, and what mechanism(s) explain(s)

138 this effect?

We used a hierarchical Bayesian statistical approach to estimate demographic vital rates for hosts in different states of ant occupancy and to quantify state-dependent partner turnover from the long-term data. We then used a stochastic, multi-state integral projection model (IPM) that combines diverse effects on vital rates and pathways of partner turnover to quantify effects of partner diversity on plant fitness.

## 144 Methods

### Study System

This study was conducted in the Los Piños mountains, a small mountain chain located on the Sevilleta National Wildlife Refuge, a Long-term Ecological Research site (SEV-LTER) in central New Mexico, USA. This is an area characterized by steep, rocky slopes, and perennial vegetation including grasses (*Bouteloua eriopoda* and *B. gracilis*), yuccas, cacti, and junipers. Tree cholla cacti are common in high Chihuahuan desert habitats, with their native range spanning the southwestern USA (Benson, 1982). These arborescent plants produce cylindrical segments with large spines. In the growing season (May to August in New Mexico), the plants initiate new vegetative segments and flower buds at the ends of existing segments. While most plants produce new segments every season, only those which are reproductively mature produce flower buds. Like other EFN-bearing cacti, tree cholla secrete nectar from specialized glands on young vegetative segments and flower buds (Ness et al., 2006; Oliveira et al., 1999). Flower buds produce more and higher-quality EFN than vegetative segments, making reproductive cholla valuable mutualist partners.

159 Tree cholla EFN is harvested by various ant species. At SEV-LTER, cholla are visited primarily by two species of formicoid, ground-nesting ants, *Crematogaster opuntiae* and *Liometopum apiculatum*, as well as other rarer species, including *Forelius pruinosus* and unidentified species of *Aphaenogaster* and *Camponotus*. *L. apiculatum* are the most frequent visitors with 25% – 60%



of tree cholla tended by these ants, followed by *C. opuntiae* visiting between 5% – 20% of cacti (Donald and Miller, 2022) depending on the year. Between 30%–80% of cacti remain vacant in any given year. These ants rarely co-occur on a plant, likely due to interspecific competition (Miller, 2007): staged introductions of *C. opuntiae* to *L. apiculatum*-tended plants, and vice versa, provoke aggressive responses by resident ants (A. Campbell, *personal observation*). Each cholla is visited by a single ant species for the duration of a season, and the species of the visitors can change from one season to the next. At the beginning of the growing season, when EFN production begins, the ground-nesting ants will begin visiting tree cholla. They will visit the cholla every day during the season around the clock, with the most activity around sunrise or sunset (Ohm and Miller, 2014). Smaller cholla are less likely to be visited because they produce very little EFN, so larger cholla, especially flowering individuals, are generally more highly tended (Miller, 2014). In late August, the tree cholla stop producing EFN and the ants vacate until the next growing season.

There are a variety of insect herbivores and seed predators which attack the cholla (Mann, 1969). An unidentified weevil of the genus *Gerstaekeria* feeds on vegetative and reproductive structures and implants their larvae within the plant tissue for the winter. A cactus bug, *Narnia pallidicornis*, (Hemiptera: Coreidae) feeds on all cholla parts with a preference for the reproductive structures (Miller et al., 2006). A seed predator, *Cahela ponderosella*, (Lepidoptera: Pyralidae) attacks developing fruits pre-dispersal and oviposits in open flowers mid-growing season where larvae burrow into the ripening ovary. These predators can have significant negative impacts on the fitness of individual cholla and depress population growth (Miller et al., 2009). There is experimental evidence that tree cholla tended by *L. apiculatum* and *C. opuntiae* experience less herbivory than plants from which ants were excluded (Miller, 2007).

### *Data Collection*

This study is based a long-term demographic data set spanning 2004 to 2023 at SEV-LTER. From 2004 to 2008, we censused 134 plants distributed across three spatial blocks. This initial census group was discontinued in 2009, when we established six 30 × 30-meter plots and tagged all

189 tree cholla within those plots. Two additional  $30 \times 30$ -meter plots were added in 2011, and this  
group of eight plots has since been censused annually through 2023 (with the exception of 2020  
due to the covid shutdown). For all plants, in May or early June of each year we recorded plant  
192 survival since the last survey and, for survivors, we recorded the height (cm), maximum crown  
width (cm), and crown width perpendicular to the maximum (cm). Size measurements were  
used to calculate plant volume ( $cm^3$ ) based on the volume of an elliptical cone. We recorded  
195 reproductive effort as counts of viable and aborted flowerbuds. We recorded the ant species  
present (or vacancy if no ants present). Occurrences of more than one ant species on one plant  
were rare (QUANTITY), and in these cases we classified the plant as being occupied by the more  
198 abundant species. Plots were searched for new recruits each year, and these were added to the  
census. In total, the data set included # unique individuals and # plant-year observations. These  
data were used to fit vital rate models (survival, growth, reproduction) as functions of plant size  
201 and ant occupancy state.

We used additional, smaller data sets from previously published studies to estimate seed and  
seed bank parameters. Ohm et al. (Ohm and Miller, 2014) provide data on the number of seeds  
204 per fruit for plants tended by *L. apiculatum*, *C. opuntiae*, or no ants (experimental exclusion). Miller  
et al. (Miller et al., 2009) provide data on seed entry to the seed bank and seedling germination  
and survival rates.

### 207 *Multi-state Integral Projection Model*

Integral Projection Models describe population dynamics in discrete time, with functions that  
relate vital rates to continuous state variables. While IPMs are a natural choice for populations  
210 with continuous size structure, they can also be modified to accommodate a combination of  
continuous and discrete state variables, as we do here. We constructed a multi-state IPM that  
stitches together population structure associated with plant size and ant state, allowing us to  
213 determine the individual fitness effects of each ant species and the composite effects of multiple  
partners, with their transition dynamics modeled explicitly.

Given the low frequency of ant species other than *L. apiculatum* and *C. opuntiae* (QUANTIFY  
 216 THIS) we combined observations of all other ants into an “other” category, such that our models  
 included four possible ant states: vacant, *L. apiculatum*, *C. opuntiae*, and “other”. The “other”  
 category was made of unidentified ant species and ant species which occurred at relatively low  
 219 frequencies compared to *C. opuntiae* and *L. apiculatum* (such as unknown species belonging to  
 the genus *Aphenogaster* and unknown species belonging to the genus *Camponotus*). Ant state is  
 included as a predictor variable in sub-models where there are biologically realistic pathways  
 222 through which ants could impact the outcome of that process. Ant partners defend cacti from  
 herbivores, and prior experimental work indicates that ant tending can reduce vegetative tissue  
 loss and floral abortion. Therefore, ant state was included in sub-models for survival, growth,  
 225 and flowerbud viability. In contrast, we have no reason to expect that ant tending can directly  
 influence the probability of flowering and flowerbud production, independent of its influence  
 on plant size. Therefore, these sub-models included plant size but not ant state as predictor  
 228 variables.

Following previous studies, we modeled the tree cholla life cycle using continuously size-  
 structured plants where  $n(x, a)_t$  gives the number of plants of size  $x$  and ant state  $a$  in year  $t$ ,  
 231 plus two discrete seed banks ( $B_t^1$  and  $B_t^2$ ) corresponding to 1 and 2-year old seeds. Seed bank  
 dynamics are given by:

$$B_{t+1}^1 = \delta \sum_{a=1}^A \int_L^U \kappa(a') P(x'; \boldsymbol{\tau}^P) F(x'; \boldsymbol{\tau}^F) V(a; \boldsymbol{\tau}_a^V) n(x, a)_t dx$$

$$B_{t+1}^2 = (1 - \gamma_1) B_t^1$$

Functions  $P(x'; \boldsymbol{\tau}^P)$  and  $F(x'; \boldsymbol{\tau}^F)$  give the probability of flowering and the number of flower-  
 buds produced, respectively, by plants of size  $x'$  in year  $t$ . The proportion of flowerbuds that  
 237 remain viable through fruit set ( $V(a; \boldsymbol{\tau}_a^V)$ ) and the number of seeds per fruit ( $\kappa(a')$ ) are depen-  
 dent on ant state  $a$  but not size. The vector  $\boldsymbol{\tau}$  gives year-specific deviates (with mean zero) that  
 modify the intercepts of the functions in which it appears. This vector appears in functions for  
 240 which we can estimate temporal stochasticity from the long-term data; superscripts indicate the

corresponding vital rate and subscripts indicate that deviates are specific to plants in ant state  $a$ .

Seed production is integrated over the size distribution, from the lower  $L$  to upper  $U$  size limits,

and summed over all possible ant states ( $A$ ) giving total seed production. Seeds are multiplied

by the probability of seed dispersal and survival ( $\delta$ ) to give the number of seeds that enter the

one-year old seed bank. Plants can recruit out of the year-one seed bank with probability  $\gamma_1$  or

transition to the two-year seed bank with a probability of  $1 - \gamma_1$ . Seeds in the two-year seed bank

are assumed to either germinate with probability  $\gamma_2$  or die.

For the above-ground part of the life cycle, the number of plants of size  $x'$  and ant state  $a'$  in

year  $t + 1$  ( $n(x', a')_{t+1}$ ) is given by survival/growth transitions from size  $x$  and ant state  $a$  in year

$t$ , plus germination out of the seed banks:

$$n(x', a')_{t+1} = (\gamma_1 B_t^1 + \gamma_2 B_t^2) \eta(x') \omega \rho(a') +$$

$$\sum_{a=1}^A \int_L^U S(x, a; \tau_a^S) G(x', x, a; \tau_a^G) \epsilon(x, a, a'; \tau^\epsilon) n(x, a)_t dx$$

The first term estimates the number of individuals recruiting from a one or two-year seed bank to a plant of size  $x'$  and ant state  $a'$  based on the recruit size distribution  $\eta(x')$  and the proportion of seedlings which survive from germination (late summer) to the census (May)  $\omega$ .

This term is multiplied by  $\rho(a')$ , which gives the probability that a new recruit has ant state  $a'$  at

its first appears in our census ( $\sum_{a'}^A \rho(a') = 1$ ). The second term represents all possible transitions

from size  $x$  and ant  $a$  to size  $x'$  and ant  $a'$ , conditioned on survival. Survival ( $S(x, a; \tau_a^S)$ ) and

growth ( $G(x', x, a; \tau_a^G)$ ) are both dependent on initial size and ant state. As above, these functions

accommodate inter-annual variability through year-specific intercepts that can vary by ant state

( $\tau_a$ ). Ant transition function  $\epsilon(a', a, x; \tau^\epsilon)$  gives the probability that a plant with size  $x$  and ant

state  $a$  transitions to ant state  $a'$  in the next census. This function accomodates inter-annual

variability through year-specific intercepts which are stable across ant states ( $\tau$ ).

## Statistical modeling and parameter estimation

We parameterized the IPM using a series of generalized linear mixed models (GLMMs) in a hierarchical Bayesian framework to serve as vital rate sub-models. Vital rate models included spatial and temporal random effects associated with plot and year variation, respectively, and included plant size ( $\log(\text{cm}^3)$ ;  $x, x'$ ), ant partner state ( $a, a'$ ), or both as fixed-effect predictor variables. In addition to vital rate models describing plant demographic performance, we also fit a sub-model to predict transition between ant states conditional on plant size and previous ant state. All models were fit using Stan and Rstan (CITE). Unless otherwise mentioned, all models use vague priors.

*Growth.* The growth sub-model ( $G(x', x, a; \tau_a^G)$ ) gives the probability of future size given the fixed effects of previous size  $x$  and previous ant partner  $a$  and random effects of plot  $w$  and year  $u$ . We fit this model to size data at the end of the transition year  $y^G$  using the location-scale parameterization of the student  $t$  distribution, because in preliminary analyses we found that size transition data were more fat-tailed than a Gaussian distribution could accommodate. Specifically, the model was:

$$y^G \sim \text{StudentT}(\hat{n}u, \hat{G}, \hat{\sigma})$$

$$\hat{G} = \beta_0^G + \beta_1^G \times x + \beta_2^G \times a + \beta_3^G \times x \times a + \beta_4^G \times x^2 + \beta_5^G \times a \times x^2 + u + w$$

$$\hat{\sigma} = \beta_0^\sigma + \beta_1^\sigma \times x$$

$$\hat{v} = \beta_0^v + \beta_1^v \times x$$

where  $u \sim N(0, \sigma_{yr \times a}^2)$  is the year random effect with year specific variance of  $\sigma_{yr}$  with ant  $a$  in year  $t$ , and  $w \sim N(0, \sigma_{plot}^2)$  is the plot random effect with plot specific variance of  $\sigma_{plot}$ . Ants are included as predictors here because ant partners defend cholla from herbivores, preventing the loss of limbs and therefore shrinking.

*Survival.* The survival model ( $S(a, x; \tau_a^S)$ ) estimates the probability of survival  $y^S$  from year  $t$  to year  $t + 1$ , with fixed effects of the previous size of the cholla  $x$  and ant partner  $a$  in year  $t$  and random effects of plot  $w$  and year  $u$ , using a Bernoulli distribution:

$$y^S \sim \text{Bern}(\hat{S})$$

$$\text{logit}(\hat{S} = \beta_0^S) + \beta_1^S \times x + \beta_2^S \times a + \beta_3^S \times x \times a + u + w$$

where  $u \sim N(0, \sigma_{yr \times a}^2)$  is the year random effect with year specific variance of  $\sigma_{yr}$  and ant state  $a$  in year  $t$ , and  $w \sim N(0, \sigma_{plot}^2)$  is the plot random effect with plot specific variance of  $\sigma_{plot}$ . A Bernoulli distribution was chosen here because the only two possible outcomes are survival or death. Ants are included as predictors here because ant partners defend cholla from herbivores and predators, decreasing the likelihood of mortality due to either of these.

*Reproduction.* The reproduction model ( $P(x'; \tau^P)$ ) estimates the probability of reproducing  $y^P$  in year  $t + 1$ , with fixed effects for the size  $x'$  in year  $t + 1$  and random effects of plot  $w$  and year  $u$ , using a Bernoulli distribution:

$$y^P \sim \text{Bern}(\hat{P})$$

$$\text{logit}(\hat{P}) = \beta_0^P + \beta_1^P \times x' + u + w$$

where  $u \sim N(0, \sigma_{yr}^2)$  is the year random effect with year specific variance of  $\sigma_{yr}$ , and  $w \sim N(0, \sigma_{plot}^2)$  is the plot random effect with plot specific variance of  $\sigma_{plot}$ .

*Flowers.* The total flowers model ( $F(x'; \tau^F)$ ) estimates the total flowers produced by a plant  $y^F$  in year  $t + 1$ , with fixed effects of size  $x'$  in year  $t + 1$  and random effects of plot  $w$  and year  $u$ , using a Negative Binomial distribution:

$$y^F \sim \text{0TruncatedNegativeBinom}(\hat{F}, \hat{\phi})$$

$$\log(\hat{F}) = \beta_0^F + \beta_1^F \times x' + u + w$$

$$\log(\hat{\phi}) = \beta_0^\phi$$

285 where  $u \sim N(0, \sigma_{yr}^2)$  is the year random effect with year specific variance of  $\sigma_{yr}$ , and  $w \sim N(0, \sigma_{plot}^2)$  is the plot random effect with plot specific variance of  $\sigma_{plot}$ .

*Viability.* The viability model ( $V(a; \tau_a^V)$ ) estimates the proportion of flowers produced by a plant which are viable (not aborted)  $y^V$  in year  $t + 1$ , with fixed effects of the ant partner of the cactus  $a$  in year  $t$  and random effects of plot  $w$  and year  $u$ , using a Binomial distribution:

$$y^V \sim \text{Binom}(\hat{V})$$

$$\text{logit}(\hat{V}) = \beta_0^V \times a + u + w$$

288 where  $u \sim N(0, \sigma_{yr \times a}^2)$  is the year random effect with year specific variance of  $\sigma_{yr}$  and ant state  $a$  in year  $t$ , and  $w \sim N(0, \sigma_{plot}^2)$  is the plot random effect with plot specific variance of  $\sigma_{plot}$ . Ants are included as predictors here because they defend the cacti from seed predation which can lead to floral abortion.

*Ant Transitions.* The ant transition rates model ( $\epsilon(x, a, a'; \tau^\epsilon)$ ) estimates the probability of a cactus being visited by an ant partner  $a'$   $y^\epsilon$ , with fixed effects of the previous size of the cholla  $x$  and the previous ant partner  $a$  in year  $t$  and random effects of plot  $w$  and year  $u$ , using a Multinomial distribution:

$$y^\epsilon \sim \text{Multinomial}(\hat{\epsilon})$$

$$\text{logit}(\epsilon) = \beta_0^\epsilon + \beta_1^\epsilon \times x + \beta_2^\epsilon \times a + u$$

291 where  $u \sim N(0, \sigma_{yr}^2)$  is the year random effect with year specific variance of  $\sigma_{yr}$ . Ant partners are included as predictors here because partners may choose to return to the same cholla repeatedly or choose new ones, therefore the previous partner may be a good indicator of the next partner.

*Recruit Size Distribution.* The recruit size model ( $n(x, a')$ ) estimates the size distribution of all recruits  $y^I$  from a given year  $t + 1$ , with no fixed or random effects, using a Normal distribution:

$$y^I \sim N(\hat{\eta}, \hat{\sigma})$$

$$\hat{\eta} = \beta_0^\eta$$

294 where  $\hat{\sigma}$  is estimated with a non-informative prior.

*Germination.* With germination data from Miller et al., 2007<sup>1</sup>, we fit two Bayesian generalized linear models for the probability of germinating from a seed in the first year ( $\gamma_1$ ) or the second year ( $\gamma_2$ ) in year  $t + 1$ , with no fixed or random effects, using a Binomial distribution:

$$y^{\gamma_1} \sim \text{Binomial}(\hat{\gamma}_1)$$

$$y^{\gamma_2} \sim \text{Binomial}(\hat{\gamma}_2)$$

$$\text{logit}(\hat{\gamma}_1) = \beta_0^{\gamma_1}$$

$$\text{logit}(\hat{\gamma}_2) = \beta_0^{\gamma_2}$$

*Pre-Census Survival.* With data collected in a 2005-2006<sup>2\*\*\*\*\*</sup> recruit census, we fit a Bayesian generalized linear model for the probability of a seedling surviving to May ( $\delta$ ) of year  $t + 1$  (accounting for missed mortality events), with fixed effects of the previous size  $x$  and random effects of the transect  $m$ , using a Bernoulli distribution:

$$y^\delta \sim \text{Bern}(\hat{\delta})$$

$$\text{logit}(\hat{\delta}) = \beta_0^\delta + m$$

where  $m \sim N(0, \sigma_{\text{transect}}^2)$  is the random effect of transect where the recruited individual was analyzed for survival.

*Seeds Per Flower.* With data from Miller 2007<sup>3\*\*\*\*\*</sup>, we fit a Bayesian generalized linear model for the number of seeds produced by every flower on a cholla ( $\kappa(a')$ ) in year  $t + 1$  based on the ant partner  $a'$  in year  $t + 1$ , using a Negative Binomial distribution:

$$y^\kappa \sim \text{0TruncatedNegativeBinomial}(\hat{\kappa}, \hat{\phi})$$

---

<sup>1</sup>CITE

<sup>2</sup>FIX

<sup>3</sup>CITE



$$\hat{\kappa} = \beta_0^\kappa \times a'$$

$$\hat{\phi} = \beta_0^\phi$$

297 Ant partners are included as predictors here because they reduce floral abortion rates and there-  
fore may lead to higher numbers of seeds.

*MCMC Simulations.* To obtain posterior estimates of the demographic parameters, we fit models  
300 using Markov chain Monte Carlo (MCMC) simulations via STAN run through version 4.0.2 of R R  
Core Team (2023); Stan Development Team (2023) For each model, we obtained 3 chains of 10,000  
iterations, each with randomly chosen initial conditions. The first 1,500 iterations were discarded  
303 as burn-in to eliminate transience associated with initial conditions. We did not thin the chains,  
thus all samples were retained. To assess the convergence of our models we assessed between  
and within chain convergence, the resulting figures are included in supplemental documents.  
306 To assess the overall model fit we carried out posterior predictive checks to examine how well  
the fitted model can generate simulated data similar to the real data. Large differences in the  
two indicate a poor model fit and can be assessed visually (figures included in supplemental  
309 documents). All estimated parameters are available in the data. Data and code for all vital rate  
models is included in the supplemental information.

### *IPM Analysis*

312 Analyzing an IPM requires discretizing the composite IPM into a matrix to calculate the domi-  
nant eigenvalue. In a traditional IPM  $x$  is discretized into  $b$  bins, replacing the continuous kernel  
into a  $b \times b$  matrix, in this case there is additional complexity in the form of transitions between  
315 ant partners. Each combination of previous ant state  $a$  and next ant state  $a'$  represents a unique  
set of plants in the population and therefore must be discretized individually, leading to a matrix  
size of  $mb \times mb$  where  $m$  is the number of unique combinations of  $a$  and  $a'$  (how many possible  
318 ant transitions there are). In this model we have two additional discrete states (year one and year  
two seed banks) leading to a final matrix size of  $m(b + 2) \times m(b + 2)$ . We used  $b = 200$  bins.

We extend the integration limits  $L$  and  $U$  to avoid unintentional eviction Williams et al. (2012).

Traditionally, in a deterministic IPM the asymptotic population growth rate  $\lambda$  is estimated as the dominant eigenvalue of the discrete kernel. In a deterministic bayesian IPM, we create 1,000 discrete kernels with a unique set of parameters from our bayesian statistical models to estimate 1,000  $\lambda$  values as a distribution. In a deterministic version of our IPM, this process is repeated separately for every combination of ant partners: complete vacancy; *C. opuntiae* and vacancy; other and vacancy; *L. apiculatum*, *C. opuntiae*, and vacancy, *L. apiculatum*, other, and vacancy; *C. opuntiae*, other, and vacancy; and all ant partners and vacancy. In order to calculate stochastic  $\lambda$  distributions for each ant scenario, every one of the 1,000 parameter-iteration associated  $\lambda$ s actually comes from the mean of 5,000 year-specific estimations each associated with a year random effect which was randomly selected from the 18 years of data.

We compare the distributions of  $\lambda$  across each combination of ant partners to whether sampling effect or complementarity is at play in the system. To compare the distributions of two hypothetical populations, A and B, we subtract the vector of 1,000  $\lambda_A$  estimations of one population from the 1,000  $\lambda_B$  estimations of the other ( $\lambda_A - \lambda_B$ ). If the average of these differences is positive, population A has a higher  $\lambda$  than B, if the average is 0, they are equal, and if the average is negative, population B has a higher  $\lambda$  than A.

To determine if sampling effect is at play in a system, we must first determine if there is a "best" partner, by determining which single ant association is correlated with the highest  $\lambda$  estimation. We can do this by comparing the distributions of each  $\lambda$  and finding the one which is larger than all others. Then we must show that the  $\lambda$  of a population with all possible partners is equal to that of  $\lambda$  of a population with only the "best" partner, by comparing the distributions as described earlier. If it is significantly larger then complementarity is potentially at play (more on this in the next paragraph). If it is significantly smaller, this indicates that rather than positive effects of partner diversity, there are actually important costs of partner diversity that dampen the population growth rate.

To determine if complementarity is at play in a system, we must determine if the partner

scenario which leads to the highest  $\lambda$  is the most partner diverse scenario. We can do this by  
 348 comparing the distribution of  $\lambda$  of a population with all possible partners to the distribution of  
 $\lambda$  of all other populations. If the  $\lambda$  of a population with all possible partners is the largest, it  
 indicates that complementarity is at play. If neither the conditions for sampling effect or com-  
 351 plimentarity, it indicates that rather than positive effects of partner diversity, there are actually  
 costs of diversity.

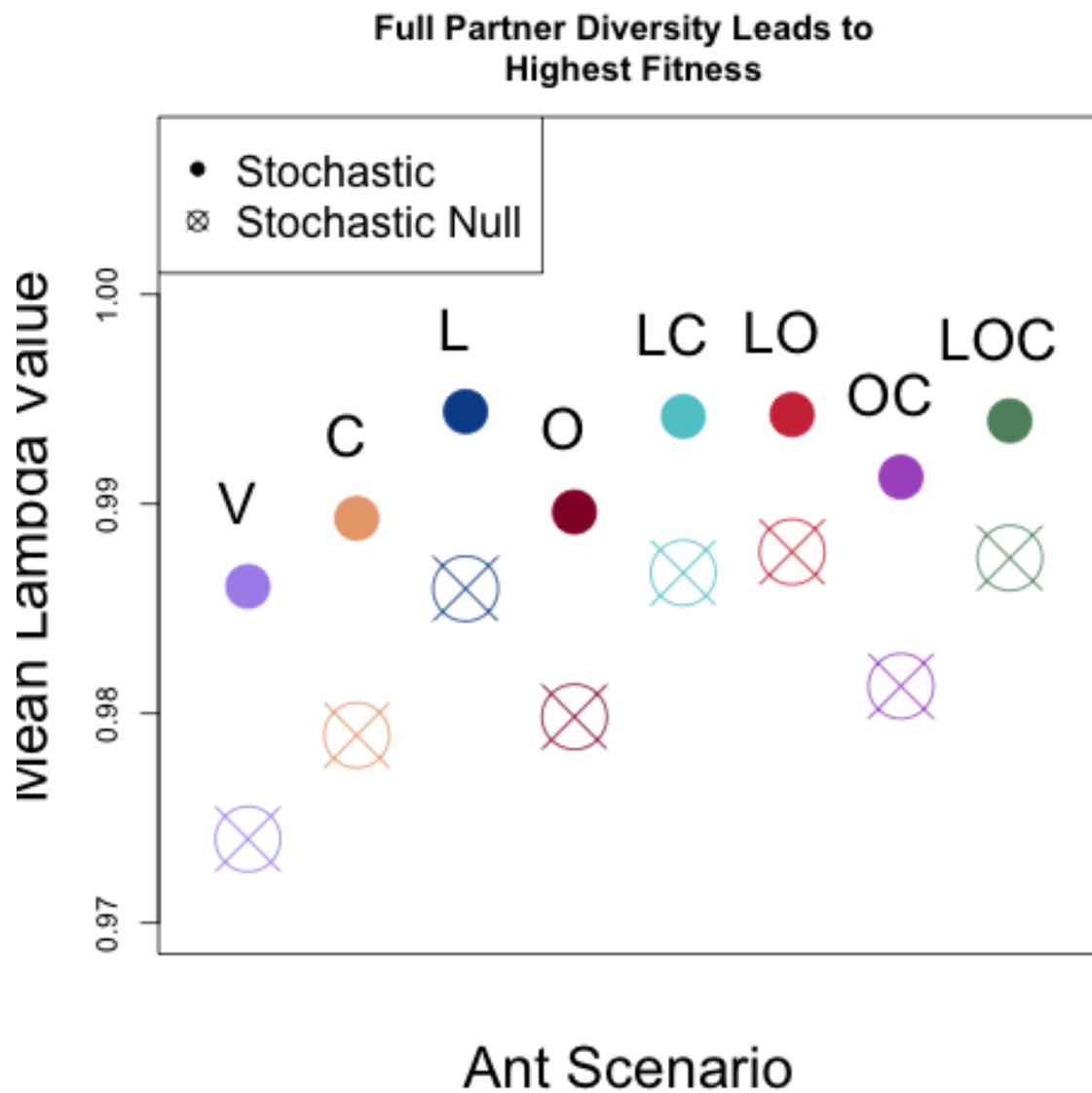
To determine if portfolio effect is at play we must show that partner diversity buffers the  
 354 population from environmental variation. To do this we parameterized a new set of bayesian  
 statistical models in which year random effects were not ant specific, meaning the effects of ant  
 partners were not able to covary with year effects (the scenario in which all ant partners must re-  
 357 spond to annual variation the same way and portfolio effect is not possible). The new estimations  
 of year random effects are  $u \sim N(0, \sigma_{yr}^2)$  with year specific variance of  $\sigma_{yr}$ . With this version of  
 the IPM (now referred to as the stochastic null IPM) we followed the same approach to calculate  
 360 distributions of stochastic  $\lambda$ s for each ant scenario. We can estimate the effects of partner diversity  
 on the focal mutualist by calculating the difference in  $\lambda$  distributions between populations with  
 no partners and populations with all partners present. Then we can compare the difference in  
 363 the effects of partner diversity on the focal mutualist calculated from the stochastic and stochastic  
 null IPMs respectively. If the stochastic difference is greater than the stochastic null difference,  
 this indicates that partner diversity is more beneficial under varying environments and therefore  
 366 portfolio effect is at play.

## Results

### *IPM Analysis*

369 Using the results of the stochastic IPM, we determined if complementarity or sampling effect was  
 at play within this system. We found that populations of tree cholla which have no partners have  
 a mean fitness of  $\lambda = 0.9861$  ???. Populations with only one ant partner present (*L. apiculatum*,

372 *C. opuntiae*, or other ants) have a mean fitness of  $\lambda = 0.9944$ ,  $\lambda = 0.9893$ , and  $\lambda = 0.9896$   
respectively. Populations with two ant partners present (*L.apiculatum* and *C. opuntiae*, *L.apiculatum*  
and other, or *C. opuntiae* and other) have a mean fitness of  $\lambda = 0.9942$ ,  $\lambda = 0.9943$ , and  $\lambda = 0.9913$   
375 respectively. Populations with all ant partners present have a mean fitness of  $\lambda = 0.9940$ . The  
only partner scenarios where  $\lambda > 0.99$  are when *L. apiculatum* ants are present with any other  
ant partner. The means are not the only important result. We used bayesian modeling, so each  
378 of these  $\lambda$  estimations has a distribution rather than a single estimate. By subtracting one of  
these distributions from the other, the difference from 0 tells us how much larger or smaller the  
distributions are with a percent confidence. Any ant partner is shown to lead to an increase in  
381 fitness of the tree cholla in these results. We are between 82% to 100% confident that having any  
ant parnter leads to a higher  $\lambda$  than having no partners.



We are between 84% and 100% confident that any diversity scenario where *L. apiculatum* ants are included as partners leads to a higher  $\lambda$  for the tree cholla than any diversity scenario without these partners. These results indicate that sampling effect, not complementarity, is at play in this system.

Using the stochastic null IPM results, we found that when ant partners responded the same way to inter-annual variation similar patterns were found, though the magnitude of the patterns were somewhat different. We used the results of the stochastic null IPM to determine if portfolio

effect is at play. We found that both the stochastic model and stochastic null model resulted in higher fitness when all ants are present compared to no partners 94% of the time. These results differed in the magnitude of fitness boost recieved from partners. In the stochastic IPM, tree cholla with all ants present resulted in a  $\lambda$  that was 0.008 greater than tree cholla with no ants present; in the stochsatic null IPM, tree cholla with all ants rpresent resulted in a  $\lambda$  that was 0.013 greater than tree cholla with no ants present. We are 52% confidence that the difference is greater when inter-annual effects were not ant dependent (in the stochastic null model). This indicates that portfolio effect is at play, if only weakly evident.

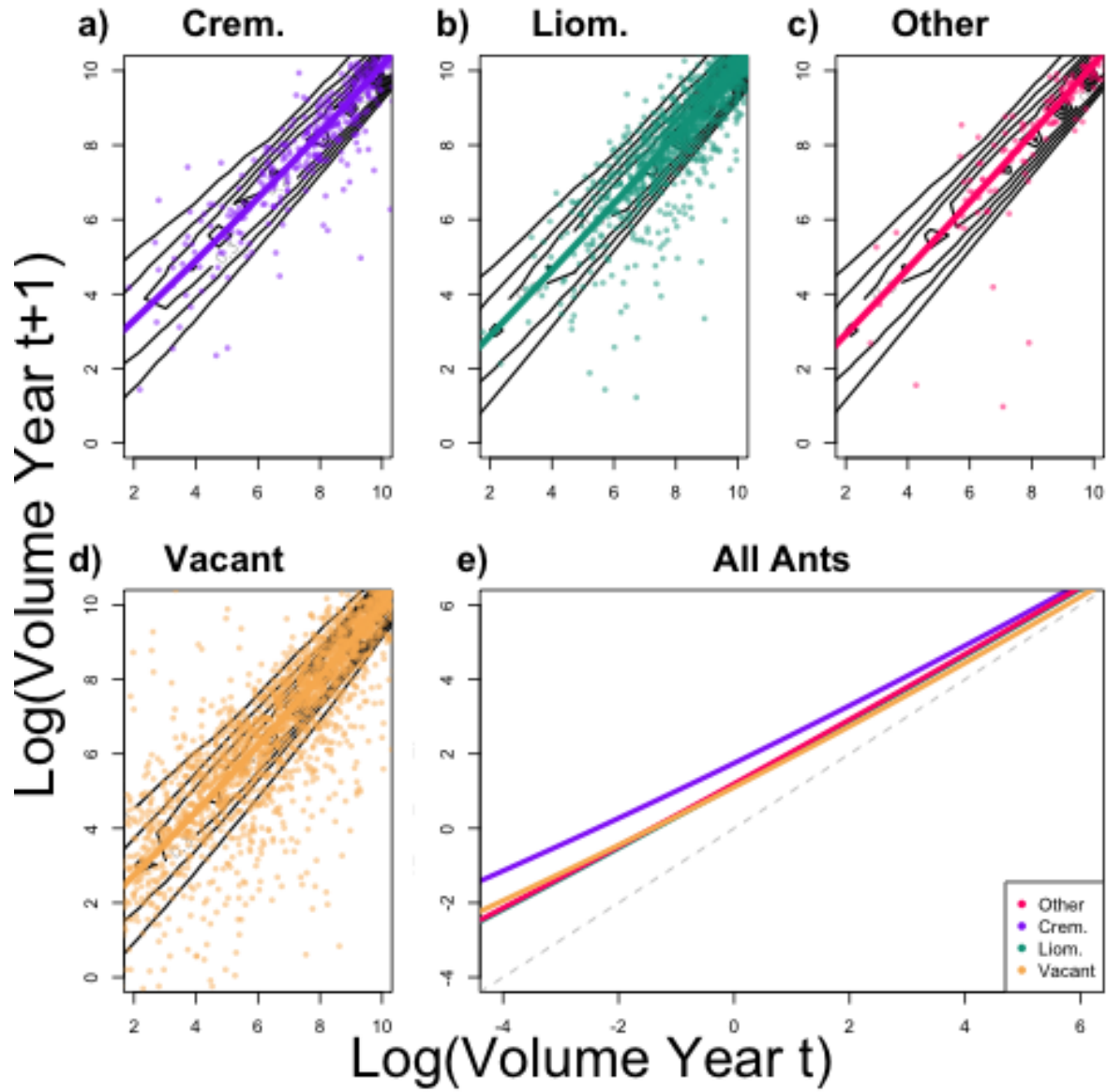


Figure 1: asdasdf

399 *Growth Model.* Tree cholla experience positive mean growth rates across all partners at small to medium sizes with the highest chances of shrinking occurring at the largest sizes 1e. The mean growth rate expected for plants that are  $10 \text{ cm}^3$  or smaller in year  $t$  is  $10.01 \text{ cm}^3/\text{yr}$ , 4.65

402  $\text{cm}^3/\text{yr}$ ,  $7.44 \text{ cm}^3/\text{yr}$ , and  $5.14 \text{ cm}^3/\text{yr}$  when tended by *C. opuntiae*, *L. apiculatum*, other ants, or  
no ants respectively. The mean growth rate expected for plants that are between  $10 \text{ cm}^3$  and  
 $150 \text{ cm}^3$  in year  $t$  is  $2.81 \text{ cm}^3/\text{yr}$ ,  $2.11 \text{ cm}^3/\text{yr}$ ,  $2.49 \text{ cm}^3/\text{yr}$ , and  $1.75 \text{ cm}^3/\text{yr}$  when tended by *C.*  
405 *opuntiae*, *L. apiculatum*, other ants, or no ants respectively. The mean growth rate expected for  
plants that are larger than  $150 \text{ cm}^3$  in year  $t$  is  $1.28 \text{ cm}^3/\text{yr}$ ,  $1.21 \text{ cm}^3/\text{yr}$ ,  $1.29 \text{ cm}^3/\text{yr}$ , and  $1.10$   
 $\text{cm}^3/\text{yr}$  when tended by *C. opuntiae*, *L. apiculatum*, other ants, or no ants respectively. Plants with  
408 *C. opuntiae* ant partners experience the highest mean growth rates across all but the largest sizes,  
where they experience comparable growth to the other tended plants 1a. Plants with *L. apiculatum*  
ants experience the lowest mean growth rates at the smallest sizes and the second lowest at all  
411 other sizes 1b. Plants with other ants experience the second highest mean growth rates across all  
but the largest sizes, where they experience the highest growth rates 1c. Plants with no partners  
experience the second lowest growth rates at small sizes, after which they experience the lowest  
414 growth rates 1d.

We are 89%, 88%, and 70% confident that plants tended by *C. opuntiae* ants experience higher  
mean growth rates across sizes than plants tended by no partners, *L. apiculatum* ants, or other  
417 ants respectively. We are 89%, 65%, and 94% confident that plants with no partners experience  
lower mean growth rates across all sizes than plants tended by *C. opuntiae*, *L. apiculatum*, or other  
ants respectively.



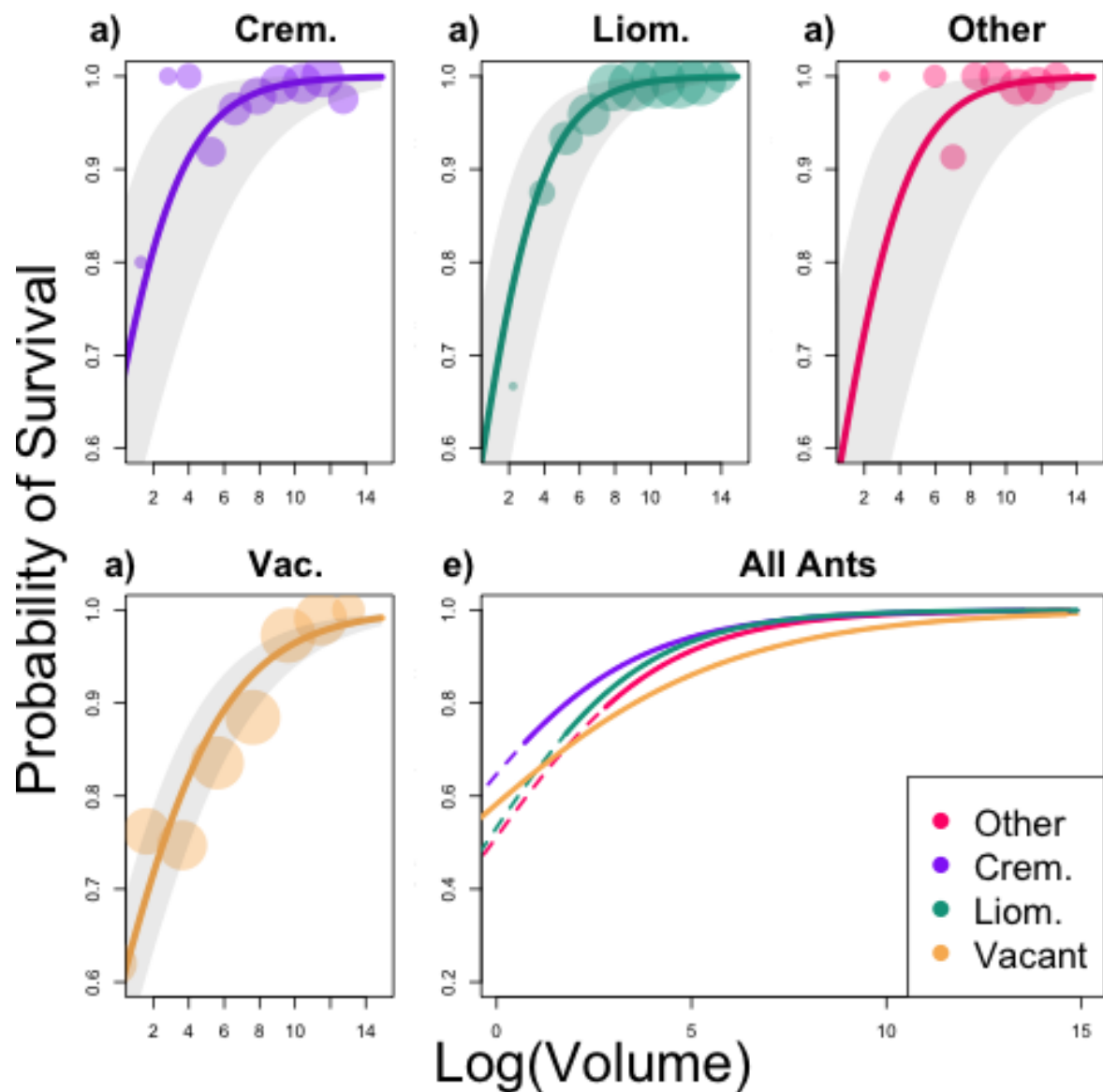


Figure 2: asdasdf

420 *Survival Model.* Tree cholla experience between 7.7% and 99.9% survival rate depending on their  
size and ant partner 2e. Smaller cacti all have lower survival rates, while larger cacti have higher  
survival rates, all nearing 100% when they reach their largest observed sizes. The mean survival  
423 rate expected for plants that are 10  $cm^3$  or smaller in year t is 48%, 37%, 37%, and 47% when  
tended by *C. opuntiae*, *L. apiculatum*, other ants, or no ants respectively. The mean survival rate

expected for plants that are between  $10\text{ cm}^3$  and  $150\text{ cm}^3$  in year  $t$  is 90%, 87%, 84%, and 80% when tended by *C. opuntiae*, *L. apiculatum*, other ants, or no ants respectively. The mean survival rate expected for plants that are larger than  $150\text{ cm}^3$  in year  $t$  is 99%, 99%, 98%, and 95% when tended by *C. opuntiae*, *L. apiculatum*, other ants, or no ants respectively. Plants with *C. opuntiae* ants experience the highest mean survival rates across all sizes 2a. Plants with *L. apiculatum* ants experience the lowest mean survival rates when small and the second highest mean survival rates across all other sizes 1b. Plants with other ants experience the second lowest mean survival rates across all sizes 1c. Plants with no partners experience the second highest survival rates at small sizes, after which they experience the lowest survival rates 1d.

We are 82%, 63%, and 100% confident that plants tended by *C. opuntiae* ants experience higher mean survival rates across all sizes than plants tended by no partners, *L. spiculatum* ants, or other ants respectively. We are 82%, 68%, and 64% confident that plants with no partners experience lower mean survival rates across all sizes than plants tended by *C. opuntiae*, *L. apiculatum*, or other ants respectively.

*Flowering Model.* There is a clear size effect on the number of flowers produced. The mean number of flowers produced by a plant remains at 0 until the plant reaches medium sizes after which the mean number of flower produced increases eponentially to about 40 flowers per plant per year at large sizes. The mean number of flowers produced for a plant that is  $150\text{ cm}^3$  or smaller in year  $t$  is ;1 flower per plant. The mean number of flowers produced for a plant that is larger than  $150\text{ cm}^3$  in year  $t$  is 8.7 flowers per plant.

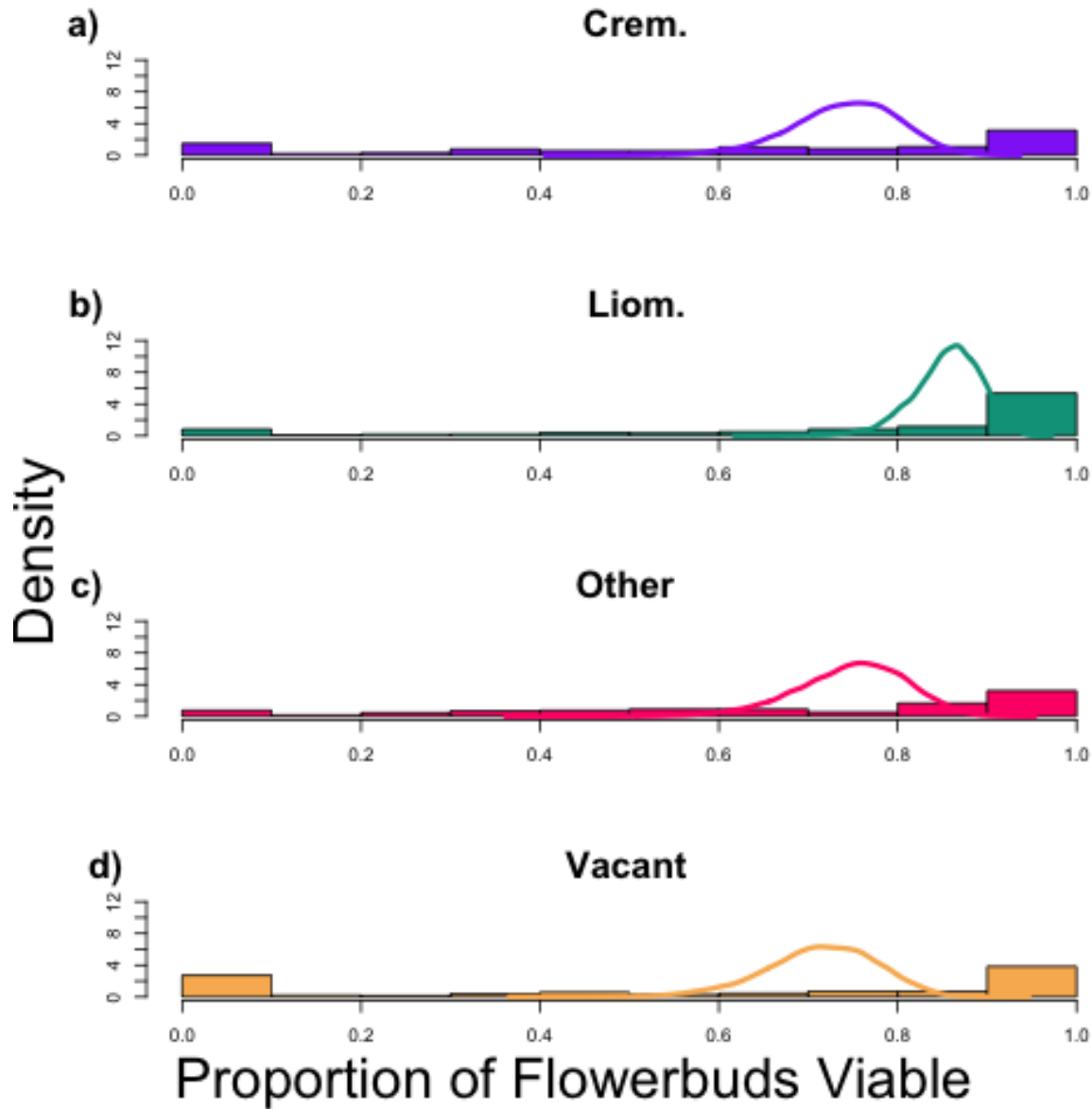


Figure 3: asdasdf

*Viability Model.* Tree cholla that are reproducing in year  $t$  experience between 39% and 96% viability rates of flowers 3. The ant partners make a difference in the mean viability rate of flowers, with *L. apiculatum* tended plants experiencing the highest mean viability rate (at 86%), followed by other tended plants (at 75%), *C. opuntiae* tended plants (at 74%) and vacant plants (at 71%). We are 99%, 98%, and 97% confident that *L. apiculatum* tended plants experience higher

450 viability rates than plants tended by no partners, *C. opuntiae*, or other ants respectively. We are  
95% and 69% confident that vacant plants experience lower viability rates than plants tended by  
*C. opuntiae* ants or other ants respectively.

453 *Reproduction Model.* The probability of a plant reproducing in a given year is highly size de-  
pendent. The mean probability of reproducing remains at about 0% until the plant reaches a  
medium size, after which the mean probability of reproducing increases steadily before reaching  
456 about 100% at large sizes.

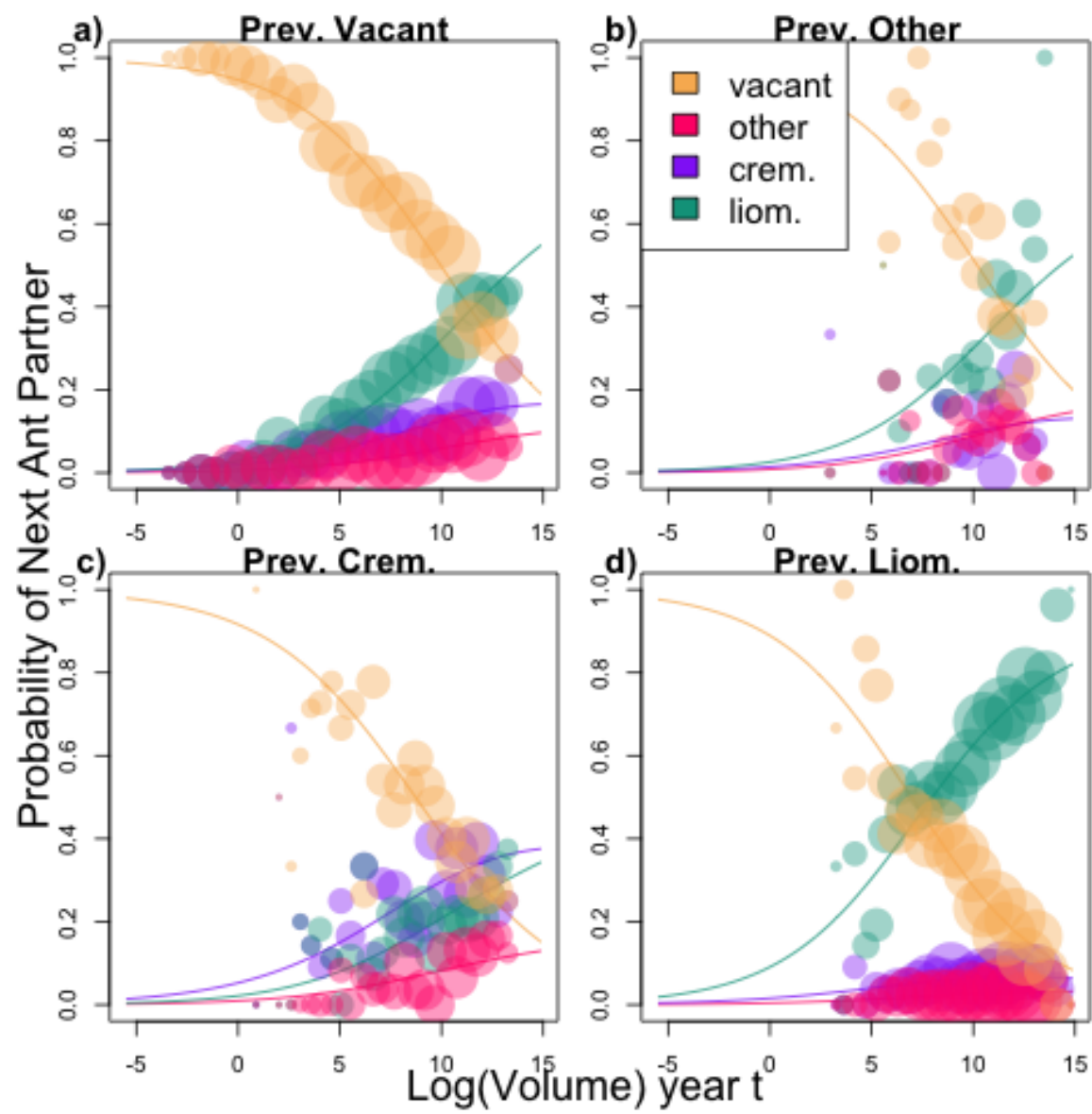
*Seeds Per Flower Model.* Each viable flower on a plant produces between 97 and 257 seeds. This  
number is affected by the ant partner present. *C. opuntiae* tended plants produce a mean of 148  
459 seeds per flower. *L. apiculatum* tended plants produce a mean of 149 seeds per flower. Vacant  
plants produce a mean of 160 seeds per flower. We are 73% and 78% confident that vacant plants  
produce more seeds per flower on average than plants tended by *C. opuntiae* and *L. apiculatum*  
462 ants respectively.

*Precensus Survival Model.* Pre-census seed survival rates fall between 0% and 95% with the mean  
pre-census seed survival at 18%.

465 *Germination Model.* Seeds have a significantly higher probability of germinating in year one than  
in year two. Seeds in year one experience germination rates between 50% and 100% with a mean  
of 62% germination. Seeds in year two experience germination rates between 50% and 98% with  
468 a mean of 58% germination.

New recruits are expected to be between the sizes of  $0.11 \text{ cm}^3$  and  $0.38 \text{ cm}^3$  with a mean size  
of  $0.20 \text{ cm}^3$ .

471 *Ant Transition Model.* All small plants are most likely to be vacant, while large plants are much  
more likely to be occupied by one ant or another. *L. apiculatum* ants are the most likely tender  
in year  $t + 1$  for all large plants that were not previously tended by *C. opuntiae* ants in year  $t$ ,



474 while *C. opuntiae* ants tended plants in year  $t$  are most likely to remain tended by *C. opuntiae* ants  
in year  $t + 1$ . We are 93%, 89%, 86%, and 93% confident that plants that are smaller than  $150\text{ cm}^3$   
477  $\text{cm}^3$  in year  $t$  will be vacant in year  $t + 1$  when they were tended by no partners, *C. opuntiae*, *L.*  
*apiculatum*, or other ants respectively in year  $t$ .

We are 75%, 100%, and 100% confident that plants which were previously tended by *L. apic-*  
*ulatum* and larger than  $150\text{ cm}^3$  in year  $t$  are more likely to be tended by *L. apiculatum* ants in  
480 year  $t + 1$  than be vacant, tended by *C. opuntiae*, or other ants in year  $t + 1$ . We are 35%, 100%,  
and 100% confident that plants which were previously vacant and larger than  $150\text{ cm}^3$  in year  
 $t$  are more likely to be tended by *L. apiculatum* ants in year  $t + 1$  than be vacant, tended by *C.*  
483 *opuntiae*, or other ants in year  $t + 1$ . We are 32%, 100%, and 100% confident that plants which  
were previously tended by other ants and larger than  $150\text{ cm}^3$  in year  $t$  are more likely to be  
tended by *L. apiculatum* ants in year  $t + 1$  than be vacant, tended by *C. opuntiae*, or other ants in  
486 year  $t + 1$ .

We are 37%, 100%, and 100% confident that plants which were previously tended by *C.*  
*opuntiae* and larger than  $150\text{ cm}^3$  in year  $t$  are more likely to be tended by *C. opuntiae* ants in year  
489  $t + 1$  than be vacant, tended by *L. apiculatum*, or other ants in year  $t + 1$ .

<sup>4</sup> Plants which were previously vacant will most likely remain vacant until large. We expect  
that between 81% and 98% of tree cholla which are smaller than  $150\text{ cm}^3$  with no partners in year  
492  $t$  are going to be vacant in year  $t + 1$ . We expect that between 11% and 55% of tree cholla which  
are larger than  $150\text{ cm}^3$  with no partners in year  $t$  are going to be tended by *L. apiculatum* ants  
in year  $t + 1$ . We expect that between 6% and 17% of tree cholla which are larger than  $150\text{ cm}^3$   
495 with no partners in year  $t$  are going to be tended by *C. opuntiae* ants in year  $t + 1$ . We expect that  
between 2% and 10% of tree cholla which are larger than  $150\text{ cm}^3$  with no partners in year  $t$  are  
going to be tended by other ants in year  $t + 1$ .

498 Plants which were previously tended by *C. opuntiae* will most likely be vacant or tended by  
*C. opuntiae* ants in the next year depending on size. We expect that between 74% and 97% of tree

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<sup>4</sup>Or I can do this section like this:

cholla which are smaller than  $150\text{ cm}^3$  and tended by *C. opuntiae* in year  $t$  are going to be vacant  
501 in year  $t + 1$ . We expect that between 8% and 34% of tree cholla which are larger than  $150\text{ cm}^3$   
and tended by *C. opuntiae* in year  $t$  are going to be tended by *L. apiculatum* ants in year  $t + 1$ . We  
expect that between 15% and 38% of tree cholla which are larger than  $150\text{ cm}^3$  and tended by *C.*  
504 *opuntiae* in year  $t$  are going to be tended by *C. opuntiae* ants in year  $t + 1$ . We expect that between  
3% and 13% of tree cholla which are larger than  $150\text{ cm}^3$  and tended by *C. opuntiae* in year  $t$  are  
going to be tended by other ants in year  $t + 1$ .

507 Plants which were previously tended by *L. apiculatum* will most likely be vacant or tended  
by *L. apiculatum* in the next year depending on size. We expect that between 64% and 97% of  
tree cholla which are smaller than  $150\text{ cm}^3$  and tended by *L. apiculatum* in year  $t$  are going to be  
510 vacant in year  $t + 1$ . We expect that between 30% and 82% of tree cholla which are larger than  
 $150\text{ cm}^3$  and tended by *L. apiculatum* in year  $t$  are going to be tended by *L. apiculatum* ants in year  
 $t + 1$ . We expect that between 4% and 7% of tree cholla which are larger than  $150\text{ cm}^3$  and tended  
513 by *L. apiculatum* in year  $t$  are going to be tended by *C. opuntiae* ants in year  $t + 1$ . We expect that  
between 1% and 3% of tree cholla which are larger than  $150\text{ cm}^3$  and tended by *L. apiculatum* in  
year  $t$  are going to be tended by other ants in year  $t + 1$ .

516 Plants which were previously tended by other ants will most likely be vacant or tended by  
other ants in the next year depending on size. We expect that between 82% and 99% of tree  
cholla which are smaller than  $150\text{ cm}^3$  and tended by other ants in year  $t$  are going to be vacant  
519 in year  $t + 1$ . We expect that between 10% and 53% of tree cholla which are larger than  $150\text{ cm}^3$   
and tended by other ants in year  $t$  are going to be tended by *L. apiculatum* ants in year  $t + 1$ . We  
expect that between 4% and 13% of tree cholla which are larger than  $150\text{ cm}^3$  and tended by other  
522 ants in year  $t$  are going to be tended by *C. opuntiae* ants in year  $t + 1$ . We expect that between  
3% and 15% of tree cholla which are larger than  $150\text{ cm}^3$  and tended by other ants in year  $t$  are  
going to be tended by other ants in year  $t + 1$ .

## Discussion

The large, long-lived tree cholla produce EFN which tempts several species of ant partners to protect them from herbivores and seed predators. Many studies have looked at multispecies mutualisms and the how having a variety of partners leads to variation in demographic effects Bascompte (2019); Báez et al. (2016); Ford et al. (2015); Palmer et al. (2010); Stachowicz and Whitlatch (2005). Because these tree cholla interact with only one ant partner at a time, it is a unique system in which to parse out the individual effects of each ant partners, both in isolated settings or in combinations we cannot test in the real world.

We asked what effects the partners which interact with tree cholla (*C. opuntiae*, *L. apiculatum*, and more) have on the vital rates of tree cholla. Using a system of heirarchical bayesian models we found that there were discernable differences in the effects that each partner had on vital processes of the focal mutualist. The different vital rates vary in importance across tree cholla ontogeny. Several of them are negatively impacted by the presence and pressure of herbivores and seed predators Miller et al. (2009, 2006) and positively impacted by the presence of ant partners Miller (2007). The predators and herbivores target new growth and flowers, leading to negative impacts on the growth rates, survival rates, and floral viability rates of tree cholla Agrawal (2004); Louda and Potvin (1995). The presence of the ant partners can reduce those negative effects.

It has been previously hypothesized that their is a heirarchy of partners due to the ability for *L. apiculatum* ants to defend the cacti from seed predators and herbivores Miller (2007). These prior results would suggest that all vital rates that are affected by ant partners would be boosted the most by the presence of *L. apiculatum* ants, this is not what we found. Our results suggest that different partners differ significantly in their effects on vital rates.

Prior to reproduction, the tree cholla experience only growth and survival. *C. opuntiae* tended ants are associated with the highest growth rates and survival rates of plants. This indicates that *C. opuntiae* ants may be good ants for pre-reproductive tree cholla plants. Reproducing plants



experience a probability of reproducing, flower production, and floral abortion. Floral abortion  
552 is heavily affected by seed predators Miller et al. (2008), which the ants defend the cacti, leading  
to increased floral viability. We specifically found that tree cholla experienced the highest floral  
viability rates when tended by *L. apiculatum* ants. This indicates that *L. apiculatum* ants may be  
555 good partners for reproducing plants.

These results together would suggest that complementarity may be the underlying mechanism that explains why partner diversity is beneficial in this system.

558 We have shown that the identity of partners is important to the processes that define tree  
cholla fitness. Now we need to analyze the dynamics of partner turnover which dictate the  
identity of tree cholla partners and therefore the effects of vital rates on the tree cholla. With our  
561 models we were able to identify both the direction, frequency, and distinct patterns of partner  
turnover.

In the literature, it is clear that the frequency of partner turnover can have big effects on  
564 the fitness of the focal mutualist Fiala et al. (1994); Horvitz and Schemske (1986); Oliveira et al.  
(1999); Sachs et al. (2004). In some systems high frequency of turnover is necessary to resiliency  
and leads to higher fitness benefits Trøjelsgaard et al. (2015), while in other systems loyalty is  
567 the most beneficial Batstone (2018). While the purpose of this paper is not to establish which  
would be most beneficial in this system, we were able to identify the pattern. Small plants are  
almost entirely vacant in this system until they grow large enough to begin producing significant  
570 amounts of EFN. Our model shows that once they do produce EFN, plants experience a relatively  
significant amount of turnover. Mid-sized and large plants which were either vacant or tended  
by other ants are most likely to become tended by *L. apiculatum* ants in the next year, thereby  
573 experiencing partner turnover. Plants which were tended by *L. apiculatum* or *C. opuntiae* ants are  
most likely to remain tended by the same partners multiple years in a row. This indicates that  
*C. opuntiae* ants and *L. apiculatum* ants are loyal partners which retain the same plants year after  
576 year with regularity.

As established in previous studies, the direction of partner turnover is important when the

identity of partners impacts the quality of benefits recieved Alonso (1998); Dejean et al. (2008);  
579 Fonseca et al. (2003); Noe and Hammerstein (1994). In our study we found that there are distinct  
patterns to the direction of partner turnover. Specifically, most plants are tended by *L. apiculatum*  
ants at some point, because vacant plants, other tended plants, and *L. apiculatum* tended plants  
582 are all most likely to be tended by *L. apiculatum* ants. This indicates that while *L. apiculatum* ants  
are loyal to their own plants and return multiple years in a row to the same ones, they are also  
strong colonizers.

585 The combination of partner identity, partner turnover, and temporal stochasticity gives us the  
unique power to consider both the fitness of the tree cholla under different partner scenarios (as  
some have done before Palmer et al. (2010)) and a unique set of mechanisms (Batstone (2018))  
588 which explain how the multi-partner interactions lead to fitness differences. We found that the  
combination of accurate partner transitions with partner identity affected the fitness of the tree  
cholla in interesting and dynamic ways. Namely, a best partner emerged in this analysis, which  
591 was surprising given the nature of our vital rate findings. The variation in best partner for  
each vital rate suggested the potential that the different ant partners had some level of unique  
specialty in what they offered, which would support complementarity as the mechanism which  
594 explained the effects of partner diversity Stachowicz and Whitlatch (2005); Stanton (2013). The  
results of our IPM however differ from this prediction.

Using the stochastic IPM we developed, we found evidence of sampling effect rather than  
597 complementarity. We found that *L. apiculatum* was the single best partner, and that all diversity  
scenarios where *L. apiculatum* was present resulted in the highest possible fitness of tree cholla.  
This indicates that despite the fact that *L. apiculatum* partnership does not result in the highest  
600 growth and survival rates, it is still the overall best partner.

Using the stochastic null IPM and the stochastic IPM we compared the fitness boost recieved  
by all ant partners when ants effects varied separately across years and when they did not.  
603 When all ants responded to inter-annual variability the same way (shown in the stochastic null  
IPM) we found that the fitness boost recieved from partners was larger than the fitness boost

received when ants responded to inter-annual variability differently. This indicates that having  
606 multiple possible partners benefits the tree cholla by buffering the potentially negative effects of  
inter-annual variation.

<sup>5</sup> This paper shows the importance of long-term datasets in investigating species interactions  
609 and calls for further use of long-term data. Previously studies have analyzed how partner identity  
and partner turnover impact focal mutualist fitness Barrett et al. (2015); Bruna et al. (2014);  
Dejean et al. (2008); Fonseca et al. (2003); Noe and Hammerstein (1994); Trøjelsgaard et al. (2015).  
612 Separate studies have analyzed how inter-annual variability impacts focal mutualists Alarcón  
et al. (2008); Alonso (1998); Horvitz and Schemske (1990); Lázaro et al. (2022); Ollerton et al.  
(2006). The long term dataset we used gave us the unique ability to consider the combined  
615 effects of partner identity, partner turnover, and temporal stochasticity.

This paper has limitations, specifically surrounding the driving forces behind the ant-plant  
interactions. We revealed the dynamics of partner turnover and showed that different ant part-  
618 ners are correlated with different fitness benefits. As of now, the driving mechanisms behind  
how ant species come to interact with individual plants is still unknown and could be subject to  
future work.

## Acknowledgments

## Data and Code Availability

## Appendix A: Additional Methods and Parameters

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<sup>5</sup> *What this all means more broadly?? I'm currently not sure what to do with this*

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## Tables

## Figure legends

Videos If you have videos, journal style for them is generally similar to that for figures.

Parameter	Median (95% CI)	Prior Distribution
growth xi intercept vacant $\beta_{01}^g$	-5.210899(-5.686865, -5.491787)	sDE
growth xi intercept other $\beta_{02}^g$	-5.8288(-5.956217, 1.766021)	asdf
growth xi intercept <i>C. opuntiae</i> $\beta_{03}^g$	-4.529523(-6.0770390, 0.1222112)	asdf
growth xi intercept <i>L. apiculatum</i> $\beta_{04}^g$	-5.106802(-5.4499944, 0.5453901)	asdf
growth xi size dependent vacant $\beta_{11}^g$	asdf	asdf
growth xi size dependent other $\beta_{12}^g$	asdf	asdf
growth xi size dependent <i>C. opuntiae</i> $\beta_{13}^g$	asdf	asdf
growth xi size dependent <i>L. apiculatum</i> $\beta_{14}^g$	sadf	asdf
growth omega intercept $\omega_0^g$		
growth omega size dependent $\omega_1^g$		
growth alpha intercept $\alpha_0^g$		
growth alpha size dependent $\alpha_1^g$		
1-year germination intercept $\alpha^{\gamma_1}$		
2-year germination intercept $\alpha^{\gamma_2}$		
survival intercept vacant $\beta_{01}^s$		
survival intercept other $\beta_{02}^s$		
survival intercept <i>C.opuntiae</i> $\beta_{03}^s$		
survival intercept <i>L. apiculatum</i> $\beta_{04}^s$		
survival size dependent vacant $\beta_{11}^s$		
survival size dependent other $\beta_{12}^s$		
survival size dependent <i>C. opuntiae</i> $\beta_{13}^s$		
survival size dependent <i>L. apiculatum</i> $\beta_{14}^s$		
flowering intercept $\beta_0^f$		
flowering size dependent $\beta_1^f$		
viability intercept vacant $\beta_0 1^v$		
viability intercept other $\beta_0 2^v$		
viability intercept <i>C. opuntiae</i> $\beta_0 3^v$		
viability intercept <i>L. apiculatum</i> $\beta_0 4^v$	44	