

Do extrafloral nectar resources, species abundances, and body sizes contribute to the structure of ant–plant mutualistic networks?

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Abstract Recent research has shown that many mutualistic communities display non-random structures. While our understanding of the structural properties of mutualistic communities continues to improve, we know little of the biological variables resulting in them. Mutualistic communities include those formed between ants and extrafloral (EF) nectar-bearing plants. In this study, we examined the contributions of plant and ant abundance, plant and ant size, and plant EF nectar resources to the network structures of nestedness and interaction frequency of ant–plant networks across five sites within one geographic locality in the Sonoran Desert. Interactions between ant and plant species were largely symmetric. That is, ant and plant species exerted nearly equivalent quantitative interaction effects on one another, as measured by their frequency of interaction. The mutualistic ant–plant networks also showed nested patterns of structure, in which there was a central core of generalist ant and plant species interacting with one another and few specialist–specialist interactions. Abundance and plant size and ant body size were the best predictors of symmetric interactions between plants and ants, as well as nestedness. Despite interactions in these communities being ultimately mediated by EF nectar resources, the number of EF nectaries had a relatively weak ability to explain variation in symmetric interactions and

nestedness. These results suggest that different mechanisms may contribute to structure of bipartite networks. Moreover, our results for ant–plant mutualistic networks support the general importance of species abundances for the structure of species interactions within biological communities.

Keywords Ant–plant interaction · Interaction frequency · Community structure · Ecological network · Nestedness

Introduction

Non-random structures of ecological networks comprised of interactions among species are widespread among mutualistic communities (Bascompte and Jordano 2007; Vázquez et al. 2009a), including, for example, degree (the number of different species a species interacts with), patterns of interaction frequencies, and nestedness. With frequency of interaction as a surrogate for interaction strength, it has been suggested that mutualistic species tend to exhibit asymmetric interactions with other species (Vázquez et al. 2007), where the effect of one species on another does not match the reciprocal effect. Nestedness, on the other hand, is the non-random pattern whereby generalist species tend to interact with one another, while specialists interact with this generalist core, but do not tend to interact with other specialists (Krishna et al. 2008; Burgos et al. 2009). Non-random structures of communities in general (McCann 2000), and of mutualistic networks in particular (Okuyama and Holland 2008), have implications for the persistence and stability of species and communities as a whole. For example, the resilience of mutualistic networks may be enhanced by species richness (community

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size) and symmetric interaction strengths of highly nested networks (Okuyama and Holland 2008).

Despite our increasingly greater understanding of the structure of mutualistic networks, we have only a limited knowledge of the correlates, or potential mechanisms, underlying the structure of mutualistic networks (Vázquez et al. 2009a). Two factors that may contribute to the structure of ecological communities are body size and the relative abundances of interacting species within communities. First, body sizes of animals have been shown to scale with some properties of mutualistic networks (Stang et al. 2006, 2007; Dalsgaard et al. 2008; Chamberlain and Holland 2009a). In predator–prey food webs, for example, larger predators consistently consume more prey species (Otto et al. 2007). Second, the distribution of species abundances has been shown to be a good null model shaping the structure of ecological networks (Vázquez et al. 2007). That is, abundant species are predicted to interact with more species simply due to more frequent encounters. Abundance has been shown to be a factor shaping structure in many types of ecological networks, including plant–pollinator communities and plant–seed disperser communities (Burns 2006; Stang et al. 2007; Vázquez et al. 2007, 2009b; Stang et al. 2009). A shortcoming of abundance is that it can also affect observed network structure via sampling effects. For example, rare or not easily observed species will be counted proportionally less than abundant species that are easily observed, which may confound observed patterns.

In contrast with body size and abundance, bottom-up resources, recognized as important determinants of food web structure (Sánchez-Pinero and Polis 2000; Shurin et al. 2006), have been given little attention in mutualistic communities (but see Stang et al. 2006, 2009). This is despite the increased recognition of mutualisms as consumer–resource interactions (Holland et al. 2005; Holland and DeAngelis 2010). Resources mediate interactions in many mutualistic networks, such as nectar in plant–pollinator networks (Stang et al. 2006) and fruit in seed dispersal networks (Morales and Vázquez 2008). The clear strength of resources in mediating interactions in other systems (e.g., Bukovinszky et al. 2008) begs for the evaluation of resources to mediate the structure of mutualistic networks.

Using interactions between ants and plants that produce extrafloral (EF) nectar, we examined the influences of ant body size, plant size, species abundances, and EF nectar resources on the nestedness and (a)symmetry of interactions of ant–plant species interactions. Ant–plant interactions are now known to be mutualistic on average (Chamberlain and Holland 2009b; Rosumek et al. 2009). Previous studies have shown that mutualistic ant–plant protection networks are significantly nested, with

asymmetric interactions (Guimarães et al. 2006; Vázquez et al. 2007). However, we have yet to examine factors shaping these non-random structures of mutualistic ant–plant networks. We examine the relative contributions of plant and ant abundance and size, and plant EF nectar resources to explain patterns of (1) (a)symmetry of interactions between plants and ants, and (2) nestedness.

Materials and methods

Study sites

This study was conducted from May to July 2008 in the Ironwood Forest National Monument (IFNM; 32°27′28.3″–32°28′28.1″N, 111°27′02.0″–111°30′33.2″W; ca. 683 m) in the Sonoran Desert of Arizona, USA. The two farthest sites (Sites 1 and 5) are 5.8 km apart. IFNM is a 52,204-ha park with perennial plant communities largely consisting of legumes and cacti. Many of the plant species produce EF nectar on vegetative or reproductive organs; the EF nectar-bearing species can collectively form a large fraction of the plant cover in the Sonoran Desert and elsewhere (Oliveira and Freitas 2004). None of the species in the IFNM have domatia or food bodies, unlike many tropical ant–plant systems.

Quantification of empirical networks

For each of the five sites, we established one randomly positioned 120 × 300 m (3.6 ha) plot. We censused an equal number of individuals (~17) per plant species per plot to minimize biases associated with sample accumulation curves (i.e., unequal sampling effects; Gotelli and Colwell 2001). We censused each plant for 1 min each by day (ca. 1830 hours) and by night (ca. 2030 hours), for a total of 2 min per individual plant. We recorded the abundance of each ant species on all censused plants. Because the same individual plants were censused by day and night, we combined both diurnal and nocturnal censuses. Each site was censused once to avoid inclusion of species with non-overlapping phenologies (Basilio et al. 2006; Medan et al. 2006).

Ecological drivers of network structure

Plant size and ant body size

We measured the size of the ~17 focal individuals of each plant species censused for the ant–plant networks. Plant size was estimated using different methods for plant species with different growth forms. We converted measurements of individual plants to mass (g) separately for woody plants

(Jenkins et al. 2004) and succulents (Price and Enquist 2006). We converted diameter at root collar (DRC) to aboveground dry biomass with the following equations for woody plants: *Acacia constricta* and *A. greggii*: $M = \text{DRC} + (0.128 \times \text{DRC}^2)$; *Prosopis velutina*: $M = -1.25 + 2.546 \times \log_{10} \text{DRC}$. We converted plant height to aboveground dry biomass using the equation $M = \text{height}(a) + b$ for succulents, with species-specific constants for *Ferocactus wislizenii* ($a = 0.316$, $b = 1.614$), *Cylindropuntia leptocaulis* ($a = 0.323$, $b = 2.014$), *C. fulgida* ($a = 0.415$, $b = 1.67$), *C. acanthocarpa* ($a = 0.311$, $b = 2.064$), *C. bigelovii* ($a = 0.401$, $b = 1.295$), *Opuntia engelmannii* ($a = 0.307$, $b = 1.839$), and *Carnegeia gigantea* ($a = 0.461$, $b = 1.481$).

Body size for each ant species was estimated from 1 to 23 workers per ant species as in Chamberlain and Holland (2009a). Only minor workers were measured for dimorphic species (e.g., *Camponotus*). Head capsule length of each worker was measured with a dissecting microscope (to 0.01 mm) equipped with an ocular micrometer, which was calibrated with a stage micrometer. Body mass was then estimated using allometric equations derived by Kaspari and Weiser (1999). The equation $M = aL^b$, where L = head capsule length, was used to convert head capsule length to body mass for each species, with subfamily specific constants for Dolichoderinae ($a = 3.870 \times 10^{-4}$, $b = 2.621$), Formicinae ($a = 6.319 \times 10^{-4}$, $b = 3.493$), and Myrmicinae ($a = 5.1475 \times 10^{-4}$, $b = 3.361$) (Kaspari and Weiser 1999). A mean measure of ant body size was calculated for each species.

Species abundances

Plant abundance was calculated by counting all individuals of each EF nectar-bearing plant species within each of the five plots; thus, plant abundance at each site is known exactly. Ant abundance was estimated using pitfall traps. Pitfall traps were 50-mL centrifuge tubes with a 3-cm diameter opening (LeBrun et al. 2007), and were set for 24 h at all sites. Pitfall traps were filled with approximately 20 mL of 50% propylene glycol.

Extrafloral nectar

Due to the different anatomical locations of EF nectaries on plant species in our study, we used different methods to estimate them on each species; we recognize that number of nectaries does not equate with nectar volume, but is a good first approximation of resources for such a study. For *F. wislizenii* we were able to count the number of EF nectaries directly. For *Acacia constricta*, *A. greggii*, and *Prosopis velutina*, there is mainly just one nectary per leaf; thus, we estimated the number of EF nectaries per plant by

estimating the number of leaves per plant. *Cylindropuntia leptocaulis*, *C. acanthocarpa*, and *Carnegeia gigantea* only had nectaries on buds, flowers, and fruits; we estimated the number of EF nectaries on these three species by multiplying the number of buds, flowers, and fruits per plant by the mean number of EF nectaries per bud, flower, or fruit. As *Cylindropuntia bigelovii* had EF nectaries on new stems only, we estimated the number of EF nectaries by multiplying new stem length (cm) by the mean number of EF nectaries per cm of new stem. EF nectaries on *Cylindropuntia fulgida* are found on areoles of old as well as new stems, and on areoles of buds, flowers, and fruits; buds, flowers, and fruits were largely not present during the study. Thus, for *C. fulgida*, we estimated EF nectary number by multiplying stem length (cm) of old and new stems by the mean number of EF nectaries per cm of stem. For *Opuntia engelmannii*, EF nectaries are on areoles of new stems and buds, flowers, and fruits; thus, we estimated EF nectary number by summing the number of nectaries on new stems (number of new stems \times mean number of EF nectaries/new stem) and on buds, flowers, and fruits (number of buds, flowers, and fruits \times mean number of EF nectaries per bud, flower, or fruit). Lastly, it is important to note that these measures of EF nectaries are proximate measures that do not estimate nectar resources directly (e.g., EF nectar volume).

Statistical analyses

Explanatory variables

We calculated Pearson's correlation coefficients r for each pair-wise combination of explanatory variables. Instead of presenting each pair-wise correlation coefficient, we summarized the correlation coefficients using meta-analysis. We z -transformed all correlation coefficients using the following equation: $Zr = 0.5 \ln[(1 + r)/(1 - r)]$, and weighted each correlation coefficient by its variance, $w = n - 3$. The weighted mean of Zr is defined as $Z_w = \sum w_i z_i / \sum w_i$. We used bootstrapping with 9,999 iterations to calculate the mean and 95% bias-corrected confidence limits of Z_w . Calculations were performed using MetaWin v.2.1 (Rosenberg et al. 2000).

Frequency of interaction

We calculated asymmetry following Vázquez et al. (2007). We define frequency of interaction asymmetry as the difference between the effect of a focal species on its interaction partners, and the effect of the interaction partners on the focal species (Vázquez et al. 2007). Although frequency of interaction is often used to approximate interaction strength in mutualistic networks (Goldwasser and

Roughgarden 1993; Bascompte et al. 2006), limitations exist for using frequency of interaction as a surrogate for interaction strength. First, all interactions are assumed to be non-negative; this assumption is unlikely to always be true. Second, we are assuming the relationship between interaction frequency and total effect is linear; this may not always be true (Vázquez et al. 2005a). Despite these limitations, interaction frequency for weighted network interactions is better than simple presence and absence links.

Interaction frequency between two species is defined using two coefficients: s_{ij} and s_{ji} , the effect of species i on j , and j on i , which define two matrices, $\overrightarrow{S} = |s_{ij}|$ and $\overleftarrow{S} = |s_{ji}|$, respectively. Because interaction strength is difficult to measure directly, we use a matrix of interaction frequencies among species $F = [f_{ij}]$ to approximate \overrightarrow{S} and \overleftarrow{S} . The effect of species i in one group (e.g., ants) on species j in a second group (plants) is defined as $s_{ij} = f_{ij} / \sum_{m=1}^I f_{mj}$, where I is the total number of species in the first group; and s_{ji} is calculated similarly. Values in the matrices \overrightarrow{S} and \overleftarrow{S} go from zero (minimal strength) to one (maximal strength). Symmetry of interaction strength is defined as $d_{ij} = s_{ij} - s_{ji}$, where zero indicates perfect symmetry, and -1 (species i stronger effect on species j than reciprocal) or 1 (vice versa) indicates asymmetry. The asymmetry of interactions of species i , A_i , is the average d_{ij} values, $A_i = \sum_{j=1}^J d_{ij} / k_i$, where k_i equals degree of species i . A_i values approaching 1 mean that the species strongly affects its interaction partners, but experiences weak reciprocal effects.

Nestedness

Nestedness is the tendency for generalists to interact among each other, while specialists interact with the generalist core, but do not tend to interact with other specialists. A nestedness value of 1 represents perfect nestedness (or order), whereas a value of 0 represents perfect disorder. We used the BINMATNEST algorithm developed by Rodríguez-Gironés and Santamaría (2006) within the *bipartite* package (Dormann et al. 2009) of R software v.2.8.0 (R Development Core Team 2008). To test the significance of nestedness for each site, we used three different null models implemented in the *bipartite* package. Null model 1 is that of the nestedness temperature calculator (NTC) program developed by Atmar and Patterson (1993). Null model 2 was proposed by Fischer and Lindenmayer (2002), and null model 3 was proposed by Rodríguez-Gironés and Santamaría (2006). Null model 1 is often associated with Type I error, while null models 2 and 3 are often conservative; null model 3 is thought to be the most robust null model by some (Rodríguez-Gironés and Santamaría 2006).

Null model

We used null model simulations using modified code of Vázquez et al. (2007) to ask if size, abundance, and EFN could explain interaction frequency asymmetry and nestedness. Note that, unlike previous studies (Vázquez et al. 2007), here we use abundance data for both plants and ants collected independently of the frequency of interaction data used to calculate interaction asymmetry; thus, there are not a lack of independence issues. The algorithm creates a binary matrix by assigning plant–ant interactions proportional to species-specific probabilities, with the caveat that each ant species has to interact with at least one plant species, and vice versa. Species specific probabilities were proportional to some combination of ant (relative size, relative abundance) and plant variables (relative size, relative abundance, relative number of EF nectaries). For example, we simulated networks with interaction probabilities proportional to ant abundance and plant size. In another run, interaction probabilities were proportional to ant size and plant size; and so on. Connectance was kept constant between the real and simulated networks by distributing any remaining interactions among the filled cells. We simulated 10,000 networks for each site and each combination of explanatory variables (relative size, relative abundance, relative number of extrafloral nectaries for plants and relative size and relative abundance for ants; $n = 6$ combinations). The algorithm was implemented for use with GNU Octave (<http://www.octave.org>), and is available from J.R.K. (<http://bioserver.cs.rice.edu/~jk4/software/>).

Results

Correlations among factors

Plant species that were large were not necessarily abundant (Fig. 1a, b), and these two variables were not significantly correlated ($\overline{Z}_w = 0.06$, bias-corrected 95% CI -0.10 to 0.38). Larger plant species did, however, have more EF nectaries than smaller plant species (Fig. 1a, c), and these two variables were correlated ($\overline{Z}_w = 0.81$, 0.58 to 0.94). This correlation may be driven in part by use of plant size to calculate EF nectaries per plant, but the relationship is nonetheless real: larger plants have more EF nectaries, or rather, number of EF nectaries increases with plant size. The two largest plant species were *Prosopis velutina* and *Carnegieia gigantea*, which exceeded the next biggest plant species by ~ 10 times. More abundant plants had more EF nectaries (Fig. 1b, c). Larger ant species were not necessarily abundant (Fig. 1), and the relationship between the two variables was significantly negative ($\overline{Z}_w = -0.25$, -0.33 to -0.17).

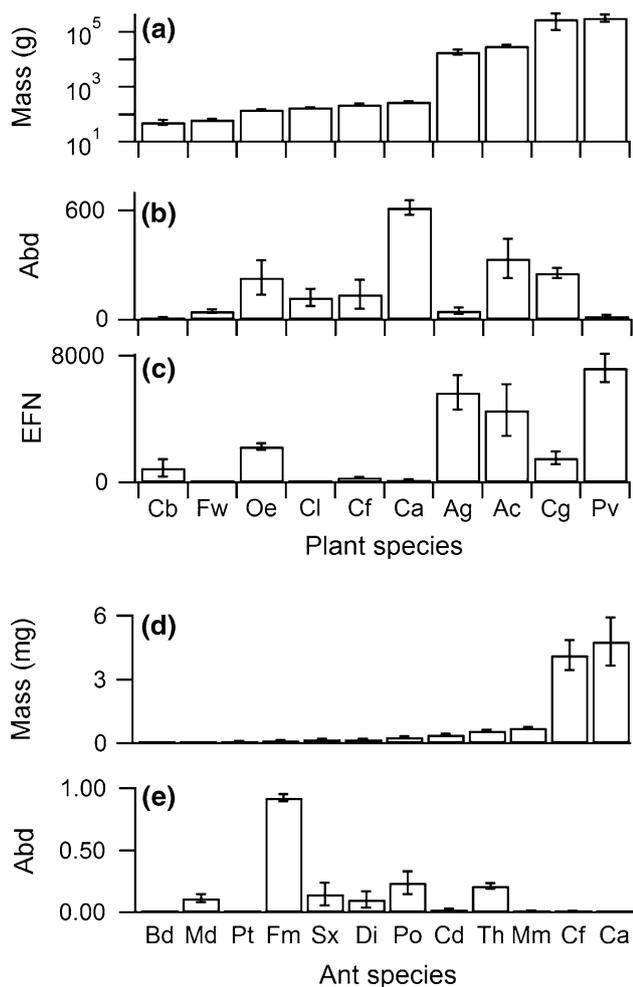


Fig. 1 Comparison of plant species based on distribution of size in **a** mass (g), **b** abundance (*abd*) and **c** number of EF nectaries (*EFN*) and ant species based on **d** mass (mg) and **e** abundance. Means (± 1 SE) are taken across each of the five sites for each species. Plant species: *Ac*, *Acacia constricta*; *Ag*, *A. greggii*; *Cg*, *Carnegiea gigantea*; *Ca*, *Cylindropuntia acanthocarpa*; *Cb*, *C. bigelovii*; *Cf*, *C. fulgida*; *Cl*, *C. leptocaulis*; *Fw*, *Ferocactus wislizeni*; *Oe*, *Opuntia engelmannii*; *Pv*, *Prosopis velutina*. Ant species: *Bd*, *Brachymyrmex depilis*; *Ca*, *Camponotus atriceps/sayi*; *Cf*, *C. fragilis/festinus*; *Cd*, *Crematogaster depilis/opuntiae*; *Di*, *Dorymyrmex insanus/sp.1/sp.2*; *Fm*, *Forelius mccooci*, *Md*, *Monomorium n.sp.desert*; *Mm*, *Myrmecocystus mimicus*; *Pt*, *Paratrechina terricola*; *Po* *Pheidole obtusospinosa*; *Sx* *Solenopsis xyloni*; *Th*, *Tetramorium hispidum*

–0.19; Fig. 1d, e). The most abundant ant species by far was *Forelius mccooci*, the 4th smallest out of 12 ant species. The two largest species, *Camponotus atriceps/sayi* and *C. fragilis/festinus*, were quite rare overall, and ant species with the greatest abundance were medium to small species (Fig. 1).

Interaction frequency asymmetry

Interaction frequency asymmetry (A_i) measures the extent to which a focal species effects on its interaction partners

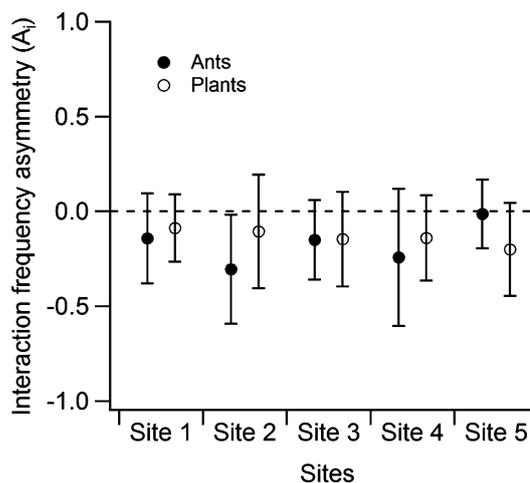


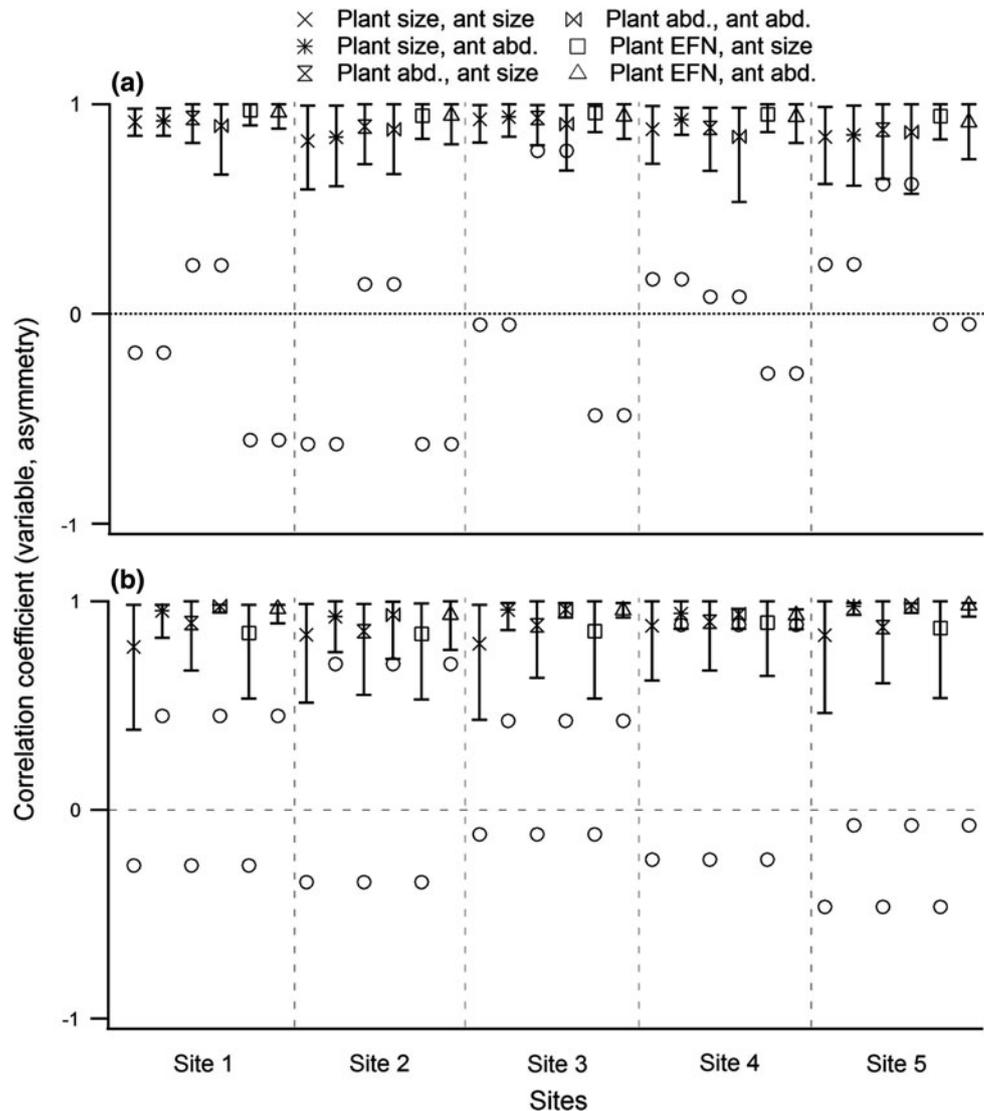
Fig. 2 Observed interaction frequency (a)symmetry (A_i) for ants and plants in networks of ant–plant mutualisms at five sites. Points are mean \pm 95% CIs. As A_i values approach 1, a species strongly affects its interaction partners, but does not experience reciprocally strong effects. As A_i values approach –1, a species experiences strong effects from its interaction partners, but does not have a strong influence on its interaction partners. As A_i values approach zero a species effects on its interaction partners are nearly equal to the reciprocal affects from its interaction partners

equal the reciprocal effects from its interaction partners. In our study, observed asymmetry values for both ants and plants were largely negative and not significantly different from zero (Fig. 2). That is, both ant and plant species experience strong effects from interacting with one another. Asymmetries between ants and plants in their interaction effects on one another largely did not occur, with the exception of ants at Site 2. In other words, ant–plant interactions were by and large symmetric.

Symmetry of interactions was both positively and negatively correlated with different plant–ant variable pairs (Fig. 3). For plants, observed correlation coefficients were only explained by null matrices in two of five sites (Sites 3 and 5; Fig. 3). In both Sites 3 and 5, when null plant–ant interaction networks were built with interactions proportional to either plant abundance and ant size, or plant abundance and ant abundance, observed values could be adequately explained by null matrices. For ants, observed correlation coefficients were only explained by null matrices in two of five sites (Sites 2 and 4; Fig. 3). In both Sites 2 and 4, when null plant–ant interaction networks were built with interactions proportional to either plant size and ant abundance, plant abundance and ant abundance, or plant EF nectaries and ant abundance, observed values could be adequately explained by null matrices. Overall, abundance explained more variation in symmetry than plant size or ant body size or number of EF nectaries.

The difference in interaction frequency between plant and ant pairs is expected to be negatively correlated with

Fig. 3 Comparison of null models based on plant mass, abundance (*abd*), and number of extrafloral nectaries (*EFN*) to predict interaction frequency asymmetry (A_i) for **a** plants and **b** ants separately in networks of ant–plant mutualisms at five sites. The y-axes are Pearson correlation coefficients, ranging from -1 to 1 . Correlation coefficients for observed data (*open circle*) and mean \pm 95% CIs of simulated data (*various symbols*) are shown in each panel. *Dashed lines* indicate a correlation coefficient of zero. If confidence intervals from simulated data overlap observed data, we consider the combination of variables to adequately explain the response variable



the product of the abundance of two interacting species (Vázquez et al. 2007). We extend this question to that of size and number of EF nectaries. Observed correlation coefficients were always positive for plant–ant variables of size–size and EF nectaries–size, and were either positive or negative for size–abundance, abundance–size, abundance–abundance, and EF nectaries–abundance (Fig. 4). The most successful null models at replicating observed correlation coefficients were plant abundance–ant size, and plant abundance–ant abundance, both of which estimated observed values in three out of five sites. Plant size–ant size, EF nectaries–ant size, and EF nectaries–ant abundance all estimated observed correlation coefficients in two of out of five sites. The plant size–ant abundance null model performed the worst, only correctly capturing the observed correlation coefficient in one out of five sites. Taken as a whole, abundance explained the most variation

in difference in interaction frequency among ants and plants.

Nestedness

Nestedness measures the extent to which a community displays a pattern of specialists interacting with proper subsets of generalists, and few if any interactions among specialists. Observed values of nestedness ranged from 0.77 to 0.89 among the five sites, with an average of 0.82 among them (Fig. 5); all nestedness values were significant, or different from random, under null model 1 (all $P < 0.04$), except for Site 5. Nestedness was only significant for null model 2 for Site 1 ($P = 0.04$). Of the five sites, nestedness was only significant under null model 3 for Sites 1 ($P = 0.03$) and 4 ($P = 0.02$). With some exceptions, the studied ant–plant networks display a nested

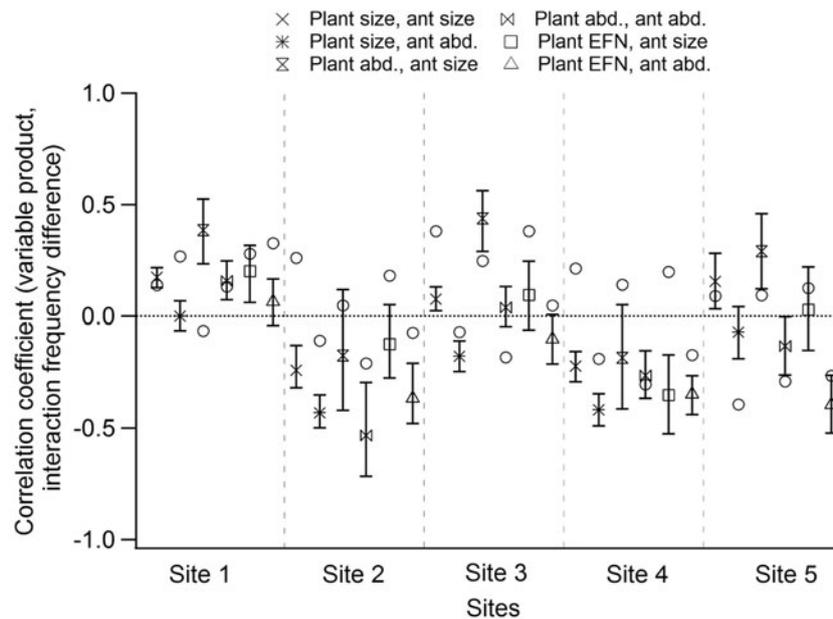
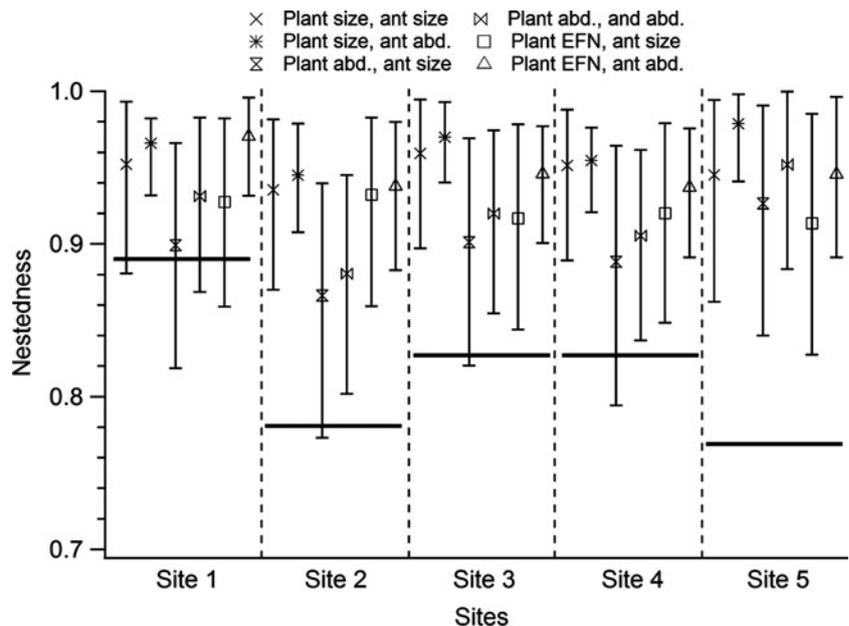


Fig. 4 Comparison of null models based on plant mass, abundance (*abd*), and number of extrafloral nectaries (*EFN*) to predict frequency of interaction difference between plants and ants in networks of ant–plant mutualisms at five sites. The ordinate shows Pearson correlation coefficients, ranging from -1 to 1 . Correlation coefficients for observed data (*open circle*) and mean \pm 95% CIs for simulated data (*various symbols*) are shown. Data are correlation coefficients

between the product of two variables (e.g., plant size and ant abundance) and the difference in frequency of interaction between the ant and plant species. The *dotted line* at zero represents a correlation coefficient of zero, or no correlation between the product of variable pairs and interaction frequency difference. If confidence intervals from simulated data overlap observed data, we consider the combination of variables to adequately explain the response variable

Fig. 5 Comparison of null models based on plant mass, abundance (*abd*), and number of extrafloral nectaries (*EFN*) to predict nestedness in networks of ant–plant mutualisms at five sites. The ordinate shows nestedness values from zero to one, with 1 being perfect nestedness. *Various symbols* and 95% CIs represent simulated data, while *bold horizontal lines* represent observed values. If confidence intervals from simulated data overlap observed data, we consider the combination of variables to adequately explain the response variable



pattern, where generalists tend to interact with one another, and specialists attach to the generalist core.

The plant abundance–ant size model was the most successful at capturing observed values of nestedness, with observed nestedness values falling within confidence limits of the model in four out of five sites. Observed values of

nestedness were encompassed by the confidence limits of the plant size–ant size, plant abundance–ant abundance, and EF nectaries–ant size null models for one out of five sites each. Plant size–ant abundance and EF nectaries–ant abundance null models did not encompass observed values of nestedness at any of the five sites, suggesting that plant

size and ant abundance are not likely important factors in determining nestedness.

Discussion

Non-random structures of mutualistic networks are now known to be widespread among mutualistic communities, yet we are just beginning to understand the factors that give rise to them (Vázquez et al. 2009a). Here, we demonstrated that species abundances, body sizes, and to a lesser extent, extrafloral (EF) nectar resources, can contribute to the non-random structures of Sonoran Desert mutualistic ant–plant networks. Species abundances and body sizes were the best predictors of observed symmetric interactions among individual plant and ant species, as well as nestedness. These results are consistent with results of Vázquez et al. (2005b, 2007, 2009b) and Stang et al. (2006, 2007), which have shown that abundance and body size are important predictors of network structures. We found that species abundance is a better predictor of network structures than body size, supporting abundance as one of the best single predictors of mutualistic network structure.

In this study, we have shown that quantitative interactions between ants and plants in ant–plant mutualistic networks are symmetric (Fig. 2)—ant and plant species exert roughly equal effects on one another, as measured by their frequency of interaction. Symmetric interactions of ants and plants in this study is in contrast with often asymmetric interactions of other mutualistic (plant–pollinator, plant seed–disperser) and antagonistic (fish–worm, mammal–flea) networks (Bascompte et al. 2006; Vázquez et al. 2007). This discrepancy may be explained by differences in methodology. In particular, the methodology of these other studies did not control for the importance of relative abundances in their sampling of species interactions, as our study did in sampling a roughly equal number of individuals of each focal study species (see “Materials and methods”). Vázquez et al. (2007) examined the correlation of abundances of mutualists with patterns of frequency of interaction asymmetry, and found that abundance and asymmetry were almost always positive. In this study, we found that abundance–asymmetry correlations were sometimes positive, but sometimes negative; controlling for relative abundance in our sampling and data collection may contribute to this discrepancy, whereas other studies try to account for variation in relative abundances of species after data collection. In addition, size–asymmetry and EF nectary–asymmetry correlations were positive as well as negative. However, predictions from our null models always resulted in positive correlations among variables and asymmetry (Fig. 3). The difference in interaction

frequency was predicted to be negatively correlated with the product of abundance, size, or number of EF nectaries for plant–ant pairs. This was generally true in this study, but there were often positive correlations among interaction frequency difference and products of factors (e.g., abundance \times body size; Fig. 4). Frequency of interaction may be driven by ant body size more than abundance as body size is correlated with liquid consumption capacity, aggression, and speed, among other life history variables (Nowbahari et al. 1999; Davidson et al. 2004; Hurlbert et al. 2008). Body size correlates with many ecological variables of diverse organisms, and their interactions with the biotic and abiotic environment (Brown et al. 2004). However, because body size is correlated with so many aspects of species, including a strong negative correlation with abundance (Brown et al. 2004), it is difficult to tease apart whether body size, or a correlated variable, is contributing to network structures.

Mutualistic communities are predicted to display a nested structure, where generalists interact among one another, forming a generalist core, to which specialists attach (Krishna et al. 2008). Nestedness was on average 0.82 among the five sites in this study, higher than average nestedness found among four sites in a previous study of ant–plant networks (0.71; Guimarães et al. 2006), but roughly equal to average nestedness for seed dispersal (0.84) and pollination (0.85) networks (Bascompte et al. 2003). Nestedness among our five sites was explained most consistently by a combination of plant abundance and ant body size (Fig. 5). The importance of ant body size for nestedness may be due to the importance of ant body size for patterns of degree in other studies (Chamberlain and Holland 2009a).

Extrafloral nectar resources are known to mediate interactions between pairs of ants and plant species (Rudgers 2004; Chamberlain and Holland 2008). In this study, simulations including EF nectaries sometimes captured observed patterns of symmetry, interaction frequency difference, and nestedness (Figs. 3, 4 and 5). Notwithstanding some explanatory power of EF nectaries for patterns in network structure, size and abundance were often better predictors of network structures in this study. We used number of EF nectary resources as a proxy for the actual EF nectar resource mediating ant–plant networks, that is the actual quantity and quality of the EF nectar. Our inadequate proxy measures of EF nectar resources may contribute to their lack of effects in explaining network structures. Different results may emerge if quantity and quality of EF nectar resources were more accurately examined in whole communities of ants and plants, or other mutualistic networks such as pollinators and plants. In a plant–pollinator network

Stang et al. (2006) showed that number of pollinator species interacting with plant species increased with number of flowers (that is the resource exploited by pollinators of plants). However, in plant–pollinator interactions, flower visitors mostly fly directly to flowers, whereas ants typically have to navigate up a plant's trunk from the base and out to the EF nectaries on the stems. Thus, this intuitive direct link between available niche space and available resources in plant–pollinator interactions may be broken in ant–plant interactions because foraging costs for ants likely increase with increasing plant size.

Our analyses of multiple determinants of network structures suggest that different factors may be important to a different extent for each partite (e.g., plants or ants) of bipartite networks. Plant abundance was an important determinant of plant interaction frequency symmetry, whereas plant size was more important than plant abundance for ant symmetry. Perhaps size variation among species matters less for ants than for plants, as ant size range was two orders of magnitude, whereas plant species varied in size over three orders of magnitude (Fig. 1). Because size variation among ant species is low, perhaps abundance overrides the importance of size in determining their interactions with plants. Alternatively, ant size may not correlate strongly with amount of resources consumed; yet, Davidson et al. (2004) have shown that resource consumption likely does scale with body size. Although single variables in separate studies have been held up as explaining many network structures in bipartite networks, we may need to consider different mechanisms for resource and consumer species of bipartite networks.

Although some emerging trends have surfaced in the search for general patterns in mutualistic network structure, the factors that shape network structure are still not clear. Our results show that abundance and size are strong drivers of symmetric ant–plant mutualistic interactions and nestedness, with abundance as the single most important factor. To our knowledge, this is the first analysis of the relative contributions of abundance, size, and resources to the structure of mutualistic ant–plant species networks. As this study was purely observational, we suggest that experimental studies are needed to tease apart the mechanistic links between explanatory factors and network structures.

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References

- Atmar W, Patterson BD (1993) The measure of order and disorder in the distribution of species in fragmented habitat. *Oecologia* 96:373–382
- Bascompte J, Jordano P (2007) Plant–animal mutualistic networks: the architecture of biodiversity. *Annu Rev Ecol Evol Syst* 38:567–593
- Bascompte J, Jordano P, Melian CJ, Olesen JM (2003) The nested assembly of plant–animal mutualistic networks. *Proc Natl Acad Sci USA* 100:9383–9387
- Bascompte J, Jordano P, Olesen JM (2006) Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science* 312:431–433
- Basilio AM, Medan D, Torretta JP, Bartoloni NJ (2006) A year-long plant pollinator network. *Austral Ecol* 31:975–983
- Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. *Ecology* 85:1771–1789
- Bukovinsky T, van Veen FJF, Jongema Y, Dicke M (2008) Direct and indirect effects of resource quality on food web structure. *Science* 319:804–807
- Burgos E, Ceva H, Hernández L, Perazzo RPJ (2009) Understanding and characterizing nestedness in mutualistic bipartite networks. *Comput Phys Commun* 180:532–535
- Burns KC (2006) A simple null model predicts fruit–frugivore interactions in a temperate rainforest. *Oikos* 115:427–432
- Chamberlain SA, Holland JN (2008) Density-mediated and context-dependent consumer–resource interactions between ants and extrafloral nectar plants. *Ecology* 89:1364–1374
- Chamberlain SA, Holland JN (2009a) Body size predicts degree in ant–plant mutualistic networks. *Funct Ecol* 23:196–202
- Chamberlain SA, Holland JN (2009b) Quantitative synthesis of context dependency in ant–plant protection mutualisms. *Ecology* 90:2384–2392
- Dalsgaard B, González AMM, Olesen JM, Timmermann A, Andersen LH, Ollerton J (2008) Pollination networks and functional specialization: a test using Lesser Antillean plant–hummingbird assemblages. *Oikos*. doi: 10.1111/j.2008.0030-1299.16537.x
- Davidson DW, Cook SC, Snelling RR (2004) Liquid-feeding performances of ants (Formicidae): ecological and evolutionary implications. *Oecologia* 139:255–266
- Dormann CF, Frund J, Bluthgen N, Gruber B (2009) Indices, graphs and null models: analyzing bipartite ecological networks. *Open Ecol J* 2:7–24
- Fischer J, Lindenmayer DB (2002) Treating the nestedness temperature calculator as a “black box” can lead to false conclusions. *Oikos* 99:193–199
- Goldwasser L, Roughgarden J (1993) Construction and analysis of a large Caribbean food web. *Ecology* 74:1216–1233
- Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol Lett* 4:379–391
- Guimarães PR Jr, Rico-Gray V, dos Reis SF, Thompson JN (2006) Asymmetries in specialization in ant–plant mutualistic networks. *Proc R Soc Lond B* 273:2041–2047
- Holland JN, DeAngelis DL (2010) A consumer–resource approach to the density-dependent population dynamics of mutualism. *Ecology* 91:1286–1295
- Holland JN, Ness JH, Boyle AL, Bronstein JL (2005) Mutualisms as consumer–resource interactions. In: Barbosa P, Castellanos I (eds) *Ecology of predator–prey interactions*. Oxford University Press, New York, pp 17–33
- Hurlbert A, Ballantyne F IV, Powell S (2008) Shaking a leg and hot to trot: the effects of body size and temperature on running speed in ants. *Ecol Entomol* 33:144–154

- Jenkins JC, Chojnacky DC, Heath LS, Birdsey RA (2004) Comprehensive database of diameter-based biomass regressions for North American tree species. GTR NE-319. USDA Forest Services Northeastern Research Station, Portland
- Kaspari M, Weiser MD (1999) The size-grain hypothesis and interspecific scaling in ants. *Funct Ecol* 13:530–538
- Krishna A, Guimaraes PR, Jordano P, Bascompte J (2008) A neutral-niche theory of nestedness in mutualistic networks. *Oikos* 117:1609–1618
- LeBrun EG, Tillberg CV, Suarez AV, Folgarait PJ, Smith CR, Holway DA (2007) An experimental study of competition between fire ants and Argentine ants in their native range. *Ecology* 88:63–75
- McCann KS (2000) The diversity–stability debate. *Nature* 405:228–233
- Medan D, Basilio AM, Devoto M, Bartoloni NJ, Torretta JP, Petanidou T (2006) Measuring generalization and connectance in temperate, year-long active systems. In: Waser NM, Ollerton J (eds) *Plant–pollinator interactions: from specialization to generalization*. University of Chicago Press, Chicago
- Morales JM, Vázquez DP (2008) The effect of space in plant–animal mutualistic networks: insights from a simulation study. *Oikos* 117:1362–1370
- Nowbahari E, Fénéron R, Malherbe M-C (1999) Effect of body size on aggression in the ant, *Cataglyphis niger* (Hymenoptera: Formicidae). *Aggress Behav* 25:369–379
- Okuyama T, Holland JN (2008) Network structural properties mediate the stability of mutualistic communities. *Ecol Lett* 11:208–216
- Oliveira PS, Freitas AVL (2004) Ant–plant–herbivore interactions in the neotropical cerrado savanna. *Naturwissenschaften* 91:557–570
- Otto SB, Rall BC, Brose U (2007) Allometric degree distributions facilitate food-web stability. *Nature* 450:1226–1229
- Price CA, Enquist BJ (2006) Scaling of mass and morphology in plants with minimal branching: an extension of the WBE model. *Funct Ecol* 20:11–20
- R Development Core Team (2008) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna
- Rodríguez-Gironés MA, Santamaría L (2006) A new algorithm to calculate the nestedness temperature of presence–absence matrices. *J Biogeog* 33:924–935
- Rosenberg MS, Adams DC, Gurevitch J (2000) *MetaWin*. Statistical software for meta-analysis, 2nd edn. Sinauer Associates, Sunderland
- Rosumek FB et al (2009) Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160:537–549
- Rudgers JA (2004) Enemies of herbivores can shape plant traits: Selection in a facultative ant–plant mutualism. *Ecology* 85:192–205
- Sánchez-Pinero F, Polis GA (2000) Bottom-up dynamics of allochthonous input: direct and indirect effects of seabirds on islands. *Ecology* 81:3117–3132
- Shurin JB, Gruner DS, Hillebrand H (2006) All wet or dried up? Real differences between aquatic and terrestrial food webs. *Proc R Soc Lond B* 273:1–9
- Stang M, Klinkhamer PGL, van der Meijden E (2006) Size constraints and flower abundance determine the number of interactions in a plant–flower visitor web. *Oikos* 112:111–120
- Stang M, Klinkhamer PGL, van der Meijden E (2007) Asymmetric specialization and extinction risk in plant–flower visitor webs: a matter of morphology or abundance? *Oecologia* 151:442–453
- Stang M, Klinkhamer PGL, Waser NM, Stang I, van der Meijden E (2009) Size-specific interaction patterns and size matching in a plant–pollinator interaction web. *Ann Bot* 103:1459–1469
- Vázquez DP, Morris WF, Jordano P (2005a) Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecol Lett* 8:1088–1094
- Vázquez DP, Poulin R, Krasnov BR, Shenbrot GI (2005b) Species abundance and the distribution of specialization in host–parasite interaction networks. *J Anim Ecol* 74:946–955
- Vázquez DP, Melian CJ, Williams NM, Bluthgen N, Krasnov BR, Poulin R (2007) Species abundance and asymmetric interaction strength in ecological networks. *Oikos* 116:1120–1127
- Vázquez DP, Bühlgen N, Cagnolo L, Chacoff NP (2009a) Uniting pattern and process in plant–animal mutualistic networks: a review. *Ann Bot* 103:1445–1457
- Vázquez DP, Chacoff NP, Cagnolo L (2009b) Evaluating multiple determinants of the structure of plant–animal mutualistic networks. *Ecology* 90:2039–2046