Evaluating multiple determinants of the structure of plant–animal mutualistic networks

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Abstract. The structure of mutualistic networks is likely to result from the simultaneous influence of neutrality and the constraints imposed by complementarity in species phenotypes, phenologies, spatial distributions, phylogenetic relationships, and sampling artifacts. We develop a conceptual and methodological framework to evaluate the relative contributions of these potential determinants. Applying this approach to the analysis of a plant–pollinator network, we show that information on relative abundance and phenology suffices to predict several aggregate network properties (connectance, nestedness, interaction evenness, and interaction asymmetry). However, such information falls short of predicting the detailed network structure (the frequency of pairwise interactions), leaving a large amount of variation unexplained. Taken together, our results suggest that both relative species abundance and complementarity in spatiotemporal distribution contribute substantially to generate observed network patters, but that this information is by no means sufficient to predict the occurrence and frequency of pairwise interactions. Future studies could use our methodological framework to evaluate the generality of our findings in a representative sample of study systems with contrasting ecological conditions.

Key words: forbidden links; Monte Desert, Mendoza, Argentina; neutrality; phenotypic complementarity; phylogenetic signal; plant–pollinator network; pollination; Villavicencio network.

Introduction

There is growing interest in the study of networks of interacting plant and animal mutualists. This interest stems from the realization that considering the community context is important to understand the ecological and evolutionary implications of mutualistic interactions (Strauss and Irwin 2004). Studies of mutualistic networks have uncovered some apparently general structural properties, such as the skewed distribution of links per species (many species with few links and few species with many links; Jordano et al. 2003, Vázquez and Aizen 2003), the nested organization of the interaction matrix (Bascompte et al. 2003), and the frequent occurrence of asymmetric interactions (Vázquez and Aizen 2004, Bascompte et al. 2006).

Recent discussion about the potential ecological and evolutionary determinants of these structural patterns has centered on the relative importance of neutrality vs. the so-called "forbidden links." The neutrality hypothesis posits that network patterns result from the fact that individuals interact randomly, so that abundant species interact more frequently and with more species than rare species (Dupont et al. 2003, Ollerton et al. 2003,

Vázquez et al. 2007). The forbidden links hypothesis posits that network patterns result from constraints to interactions imposed by the complementarity in species phenotypes, phenologies, spatial distributions, and phylogenetic relationships (Jordano et al. 2003, Rezende et al. 2007, Santamaría and Rodríguez-Gironés 2007, Stang et al. 2007). For example, two species cannot interact if their phenologies do not overlap, regardless of what their abundance alone predicts.

Available evidence suggests that both neutrality and forbidden links contribute to some extent to determine network structure (Bascompte and Jordano 2007, Vázquez et al. 2009). For example, Vázquez et al. (2007) have shown that relative species abundance partly (but not entirely) explains the observed asymmetry in the strength of pairwise interactions, whereas Stang et al. (2007) showed that information on both abundance and morphological traits of plants and pollinators are needed to predict asymmetry and nestedness in binary networks. Several recent studies have also shown that phenologies and interannual dynamics of plant and animal species influence network structure (Basilio et al. 2006, Alarcón et al. 2008, Olesen et al. 2008, Petanidou et al. 2008). In a similar vein, a recent simulation study has shown that the degree of mixing resulting from the spatial aggregation of plant individuals and the scale of animal movement decisions has strong influences on network structure (Morales and Vázquez 2008). The key unanswered

question is how important each of these processes is in determining network structure. Here we develop a conceptual and methodological framework to answer this question and apply it to investigate the determinants of a plant–pollinator network.

Consider a mutualistic network depicted as an interaction matrix **Y** with *I* rows and *J* columns corresponding to the plant and animal species in the network, respectively, and a positive integer in cell y_{ij} representing the number of interactions recorded between plant *i* and animal *j*. This matrix is a function of multiple interaction probability matrices of the same size as **Y**, determined by relative species abundance (**N**), temporal (**T**) and spatial overlap (**S**), and phenotypic traits of interacting species (**K**). The effects of these factors on **Y** can be constrained by the phylogenetic relationships among plants ($\mathbf{P}_p$) and animals ($\mathbf{P}_a$) (Rezende et al. 2007). In addition, detection probabilities of interactions resulting from sampling effects (**E**) can also influence the observed network (Blüthgen et al. 2008). Thus,

$$\mathbf{Y} = f(\mathbf{N}, \mathbf{T}, \mathbf{S}, \mathbf{K}, \mathbf{P}_p, \mathbf{P}_a, \mathbf{E}). \qquad (1)$$

Below we use this conceptual framework to evaluate the contribution of abundance, spatial and temporal overlap, and phylogenetic relatedness among species on the structure of a plant–pollinator network. Specifically, we address the following questions: (1) To what extent do relative abundance and spatiotemporal overlap predict aggregate network statistics (connectance, nestedness, interaction strength evenness, and the distribution of interaction strength asymmetries)? (2) To what extent do these factors predict pairwise interaction frequencies in the interaction matrix? (3) Is there any detectable phylogenetic signal in the interaction matrix, suggesting that the influence of abundance and spatiotemporal overlap on network structure could have resulted from phylogenetic constraints imposed by the phylogenetic relationships among plants and among animals?

MATERIALS AND METHODS

Study system.—Data come from a plant–pollinator network from the Monte Desert at Villavicencio Nature Reserve (32°32′ S, 68°57′ W, 1270 m above sea level), Mendoza, Argentina. We worked in four 1-ha plots, separated by 1–2 km. Predominant vegetation is a tall shrubland dominated by *Larrea divaricata*, *Zuccagnia punctata*, *Prosopis flexuosa*, *Condalia microphylla*, *Acantholippia seriphoides*, and *Opuntia sulphurea* (Roig 1972; see Plate 1). We give only a summarized description of field methods here; further details can be found in the original publication describing the network (Chacoff et al. 2009).

Plant–pollinator interactions.—Flower visiting insects were observed on plant species in weekly surveys in two consecutive flowering seasons (2006 and 2007) between September and January (2006) or December (2007). We attempted to sample plant–pollinator interactions in the whole community as comprehensively as possible,

recording interactions between 41 plant species and 97 insect species. With these data we constructed a quantitative plant–pollinator interaction matrix **Y** = [y_{ij}], with rows corresponding to plant species and columns to pollinator species; cell entries y_{ij} are integers representing the number of flowers of plant species *i* visited by pollinator species *j* (Fig. 1a). This is the network we want to predict.

Species abundance.—Plant abundance was assessed in weekly surveys along five fixed 50×2 m transects in the four sites, where we recorded the number of individuals of each entomophilous species and, for a subset of individuals, the number of flowers per individual. We also collected three fresh flowers from ten individuals of each species to estimate the number of pollen grains produced per flower. We attempted to obtain nectar from flowers, but we failed for most plant species; flowers in this system usually have very small standing volumes of nectar. With these data we estimated the density of individuals, density of flowers (density of individuals × flowers per individual), and density of pollen (density of individuals × flowers per individual × pollen grains per flower) for each species at each site. Arguably, density of flowers is the most appropriate measure of abundance from the flower visitors' perspective: it is a better estimate of resource abundance than density of individual plants (because there is high variation among plant species in the mean number of flowers per individual) and focuses on the flower as the resource unit for both pollen and nectar. However, because different studies use different measures of abundance, we considered the three measures to evaluate how the choice of a particular measure affects our results.

Because flowers of most species usually last less than a week, and because we were interested in an overall measure of abundance, we summed density across weeks and sites to obtain an overall estimate of abundance of each species. Thus, a plant species could be abundant because it produced many flowers in a short period or because it produced few flowers over a long period. These two forms of abundance were then distinguished by incorporating temporal and spatial structure in our network model, as described below (see *Spatial and temporal overlap*).

Insect abundance was defined as the total number of visits made by a particular insect species to any plant species (that is, the column sum of the interaction matrix), as done in previous studies (see, e.g., Vázquez et al. 2007).

Spatial and temporal overlap.—To quantify spatial and temporal overlap of plant and pollinator species, we first compiled matrices of temporal and spatial occurrences, with species in rows and date or site in columns, and cells with ones for presences and zeros for absences. We thus had one temporal and one spatial occurrence matrix for plants, $\mathbf{O}_p^t$ and $\mathbf{O}_p^s$, and one of each for animals, $\mathbf{O}_a^t$ and $\mathbf{O}_a^s$. We then used matrix multiplication to calculate spatial and temporal overlap matrices between plants and
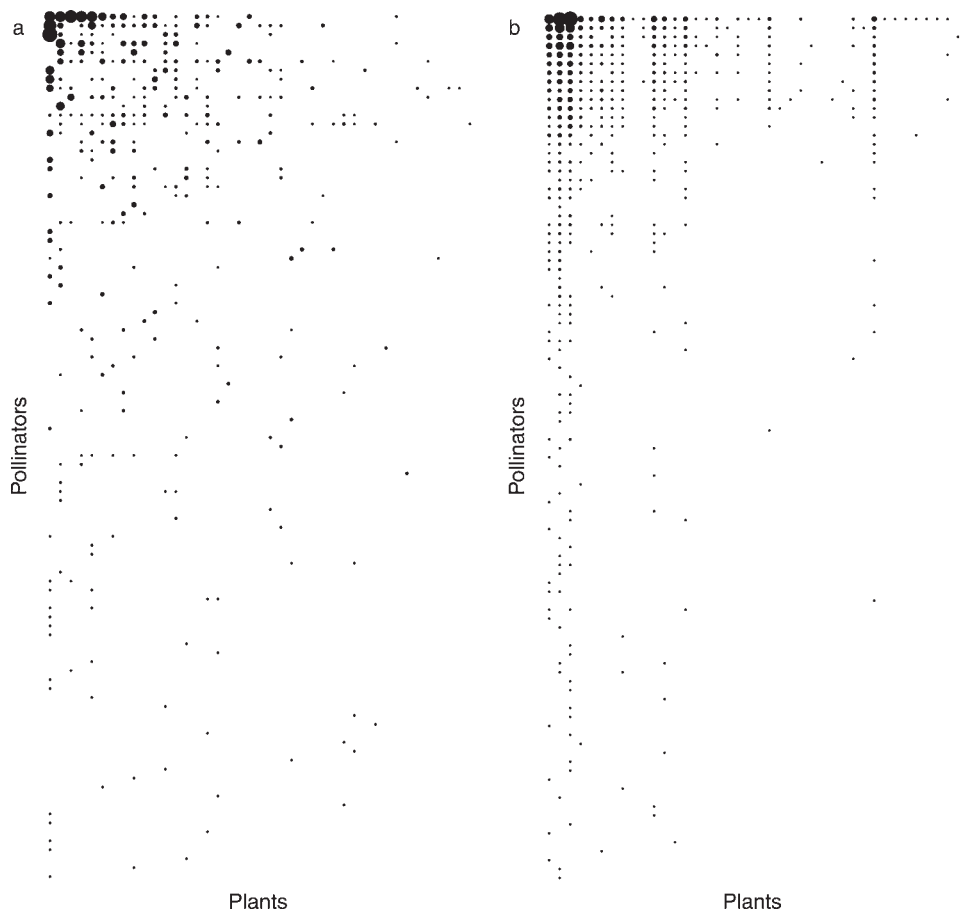
Fig. 1. Plant–pollinator interaction matrices. (a) Observed plant–pollinator matrix in the Monte Desert of Villavicencio Nature Reserve (Mendoza, Argentina). (b) Interaction matrix resulting from one iteration of the randomization algorithm, using the $\overline{\mathbf{NT}}$ probability matrix (resulting from all possible combinations of abundance $\mathbf{N}$ and temporal overlap $\mathbf{T}$) to assign interactions. In each matrix, rows represent pollinator species, columns represent plant species, and circle diameter of a matrix element y_{ij} is proportional to the square root of the number of interactions between pollinator i and plant j.

animals, $\mathbf{S} = \mathbf{O}_p^s \mathbf{O}_a^{s'}$ and $\mathbf{T} = \mathbf{O}_p^t \mathbf{O}_a^{t'}$, where the prime symbol indicates the transpose of a matrix or vector.

Calculation of interaction probabilities.—We calculated interaction probability matrices expected under the assumptions that interactions were determined solely by relative species abundances, temporal overlap and spatial overlap. For relative abundance, interaction probability between a plant species i and a pollinator species j is simply the product of their relative abundances. In matrix notation, the interaction probability matrix expected from relative abundances is $\mathbf{N} = \mathbf{n}_p \mathbf{n}_a'$. For temporal and spatial overlap, we used overlap matrices $\mathbf{T}$ and $\mathbf{S}$ normalized so that their elements added up to one, so as to transform them into probabilities. (For simplicity, we call these normalized matrices $\mathbf{T}$ and $\mathbf{S}$ hereafter.) Thus, the greater the temporal or spatial overlap of two species, the greater their probability of interaction; species with no temporal or spatial overlap had zero probability of interaction. We also calculated combined probabilities as the element-wise multiplication of matrices $\mathbf{N}$, $\mathbf{T}$, and $\mathbf{S}$, again normalizing the resulting matrices so that their elements added up to one. These combined matrices represent the expected probability under the joint influence of more than one of these factors. For example, $\overline{\mathbf{NS}}$ denotes the combined abundance–spatial-overlap probability matrix, and represents the interaction probabilities expected if species interact proportionally to their abundances given that they co-occur at a particular site. Thus, we had seven probability matrices with all possible combinations of relative abundance and temporal and spatial overlap: $\mathbf{N}$, $\mathbf{T}$, $\mathbf{S}$, $\overline{\mathbf{NT}}$, $\overline{\mathbf{NS}}$, $\overline{\mathbf{TS}}$, and $\overline{\mathbf{NTS}}$. In addition, we defined an eighth probability matrix in which all pairwise interactions had the same probability $1/IJ$ of occurrence, where I and J are the numbers of plant and animal species in the network; this probability matrix, termed "Null" (see Figs. 2 and 3), is taken as a benchmark null model for comparison with the other seven probability matrices.

Analysis of aggregate network statistics.—We considered four aggregate network statistics frequently used in the analysis of plant–animal mutualistic networks: connectance, nestedness, interaction evenness, and
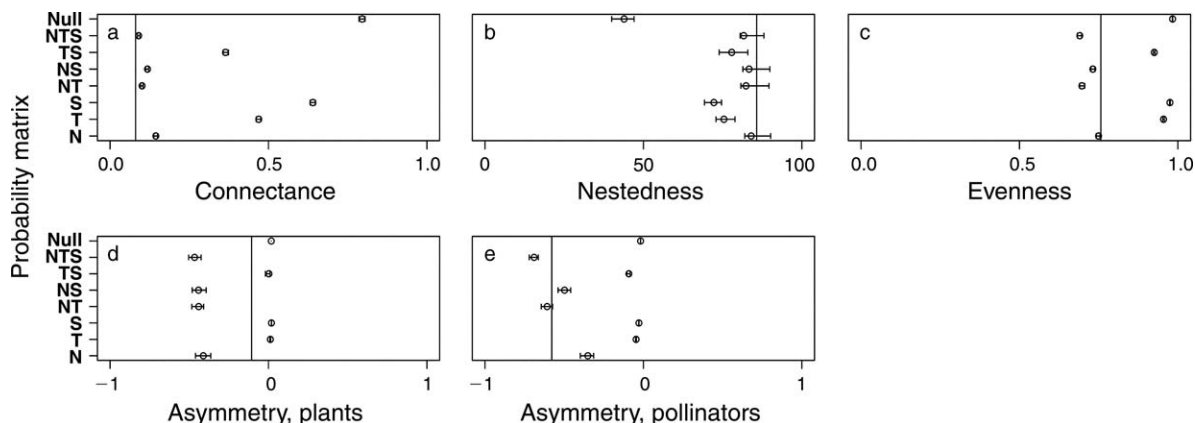
FIG. 2. Comparison between aggregate network statistics observed in the Villavicencio network (Monte Desert at Villavicencio Nature Reserve, Mendoza, Argentina) and those predicted by the probability matrices. In each panel, the vertical line represents the observed value of an aggregate statistic, and the circles represent the value of the statistic expected from each probability matrix, with error bars indicating 95% confidence intervals. Results are shown for the seven probability matrices resulting from all possible combinations of abundance (**N**), temporal overlap (**T**), and spatial overlap (**S**), and for the null probability matrix with homogeneous interaction probabilities across all pairwise interactions (**Null**).

interaction asymmetry. Connectance is the proportion of realized interspecific links, defined as $C = L/(IJ)$, where L is the number of nonzero entries in the binary interaction network and I and J are, as above, the numbers of plant and animal species in the network. Nestedness is the tendency of specialized species to interact with a subset of the interaction partners of more generalized species or, more precisely, the degree of symmetry in the distribution of unexpected absences and presences on each side of the boundary line defining perfect nestedness (Almeida-Neto et al. 2007). Thus, a nestedness value of 1 represents complete lack of symmetry (perfect nestedness), while a value of 0 represents the highest symmetry in the distribution of unexpected presences and absences, with absolute randomness falling somewhere in between 0 and 1. Nestedness was calculated with the BINMATNEST algorithm proposed by Rodrígues-Gironés and Santamaría (2006), implemented in the bipartite package (Dormann et al. 2008) of R statistical software (R Development Core Team 2007); the BINMATNEST algorithm overcomes several limitations of the widely used nestedness temperature calculator developed by Atmar and Patterson (1993). Following Tylianakis et al. (2007), interaction evenness was defined as Shannon's index, $H = p_{ij} \log_2 p_{ij}/\log_2 F$, where F is the total number of plant–pollinator interactions in the matrix (see Eq. 2) and p_{ij} is the proportion of those interactions involving plant i and pollinator j. An uneven network is one with high skewness in the distribution of interaction frequencies. Interaction asymmetry for a given species was defined as

$$A_i = \frac{\sum_j d_{ij}}{k_i}$$

where k_i is the degree of species i (i.e., the number of

species with which i interacts) and d_{ij} is a measure of the symmetry of the strength of the pairwise interaction between i and j (Vázquez et al. 2007); as in previous publications, we used interaction frequency as a surrogate of interaction strength (see Vázquez et al. 2005, 2007, Bascompte et al. 2006).

We used a randomization algorithm implemented in R (see Supplement) to evaluate to what extent interaction probabilities derived from relative abundance and spatial and temporal overlap of species occurrences predicted the observed aggregate network statistics. The algorithm assigned the total number of interactions originally observed in the interaction matrix according to the seven probability matrices defined by all possible combinations of abundance and temporal and spatial overlap (see *Interaction probabilities*), with the only constraint that each species received at least one interaction.

Likelihood analysis of pairwise interaction probabilities.—We used a likelihood approach to evaluate the ability of abundance, temporal overlap and spatial overlap to predict the detailed structure of the interaction matrix. Consider the observed interaction matrix **Y** and a probability matrix **X**, whose entries x_{ij} are the probabilities of occurrence for each pairwise interaction; we want to evaluate whether those probabilities match the observed frequencies of interaction. This evaluation can be done by calculating the likelihood of probability matrix **X** given the data (**Y**). We assumed that the pairwise probability of interaction between a plant i and a pollinator j followed a multinomial distribution. Thus, the likelihood of probability matrix l given the data is

$$L_l = \frac{F!}{\prod_{i=1}^{I}\prod_{j=1}^{J} y_{i,j}!} \prod_{i=1}^{I}\prod_{j=1}^{J} x_{ijl}^{y_{ij}} \qquad (2)$$

where F is the total number of observed interactions (i.e., the sum of the elements of matrix **Y**), and I and J are the total number of animals and plants in **Y**, respectively. We calculated this likelihood using function dmultinomin the stats package of R. We then calculated Akaike's Information Criterion as $AIC_l = \mathbf{L}_l - 2k_l$, where k_l is the number of parameters involved in generating probability matrix l. For ease of comparison, we used the differential AIC, ΔAIC, which is the AIC for probability matrix 1 minus the AIC for the best fitting probability matrix, which by definition has ΔAIC = 0. The number of parameters was defined as the number of factors contributing to generate a particular probability matrix; thus, matrix **N** has one parameter, $\overline{\mathbf{TS}}$ has two, and $\overline{\mathbf{NTS}}$ has three.

Phylogenetic analysis.—Ideally, we would like to incorporate phylogenetic effects into our conceptual framework, constructing a phylogenetic probability matrix as we did with abundance, temporal overlap and spatial overlap. Regrettably, incorporation of phylogenetic effects was not possible because we found no way of predicting pairwise interactions from the plant and pollinator phylogenies alone, without reference to the observed interaction matrix. Alternatively, we evaluated the strength of the phylogenetic signal of the two phylogenies on the interaction matrix with the method developed by Ives and Godray (2006): a linear model approach to fit the phylogenetic variance–covariance matrix to the interaction matrix. Using this method we calculated the independent signals of the plant (d_p) and animal (d_a) phylogenies and the strength of the signal of both phylogenies combined (from all the data, MSE_d; Appendix).

RESULTS

Aggregate network statistics.—No probability matrix predicted connectance and interaction evenness values whose confidence intervals included observed values of these statistics (Fig. 2a, c). However, predictions from probability matrices $\overline{\mathbf{NTS}}$, $\overline{\mathbf{NT}}$, $\overline{\mathbf{NS}}$, and **N** were extremely close to observed connectance and interaction evenness. Observed nestedness was included within the randomization confidence intervals of the same four probability matrices (Fig. 2b). A similar result was observed for interaction asymmetry for pollinators (Fig. 2e), but not for plants (Fig. 2d). In the latter case, observed average asymmetry was close to zero (predominantly symmetric interactions) and similar to that predicted by the null model that assumes that all species have the same probability of interaction. Highly negative asymmetry in pollinators and high symmetry for plants matches previous results for other interaction networks (Vázquez et al. 2007). Thus, with the exception of asymmetry for plants, information on abundance and temporal overlap suffices to simulate interaction networks whose aggregate structure resembles very closely
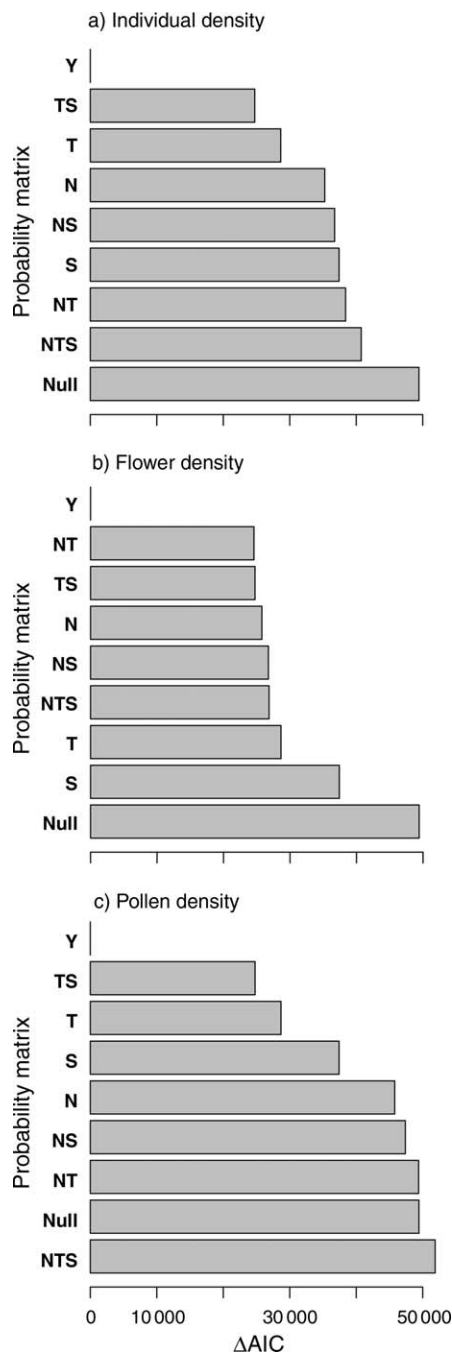

FIG. 3. Likelihood analysis of pairwise interaction probabilities. Results are shown for three abundance measures (see *Methods*): (a) density of individuals, (b) density of flowers, and (c) density of pollen. Each panel shows the ΔAIC values corresponding to each of the seven probability matrices resulting from all possible combinations of abundance (**N**), temporal overlap (**T**), and spatial overlap (**S**). The ΔAIC value for a null probability matrix with homogenous interaction probability across all pairwise interactions (**Null**) and the observed interaction matrix fitted to itself (**Y**) are also shown for comparison.

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the structure of the observed matrix **Y**. This result was the same regardless of the measure of abundance used (i.e., density of individuals, individuals × flowers or individuals × flowers × pollen; result not shown).

Frequency of pairwise interactions.—Taking density of flowers as our measure of plant abundance (which, as explained above, is arguably the most appropriate measure), the combined probability matrix $\overline{\textbf{NT}}$ had the lowest ΔAIC value, with a difference with the next best-fitting probability matrix $\overline{\textbf{TS}}$ of 156, and several orders of magnitude with the null model (Fig. 3b). However, this best-fitting probability model was also orders of magnitude worse than the perfect fit obtained by fitting the interaction matrix to itself. These results suggest that abundance and temporal overlap are useful to predict part of the detailed structure of the interaction matrix, but that we are far from an accurate prediction, with much unexplained variation.

The latter conclusion can be visualized by comparing the observed interaction matrix (Fig. 1a) with an example of a matrix resulting from the randomization procedure with the $\overline{\textbf{NT}}$ probability matrix (Fig. 1b). For example, although the two randomized matrices look roughly similar to the observed matrix in terms of connectance and nestedness, it is obvious that the most frequently observed interactions are not those predicted to occur most frequently by $\overline{\textbf{NT}}$. Thus, although knowledge of abundance and phenology allows us to predict aggregate network properties with high accuracy, we do rather poorly at predicting the detailed occurrence of pairwise interactions.

Unlike results for aggregate network statistics, using a different measure of abundance affects our ability to predict pairwise interactions (Fig. 3a, c): $\overline{\textbf{NT}}$ is now in sixth place, doing particularly badly when abundance is measured as density of pollen, almost as badly as the null model. Notice that because matrices not including abundance are the same between Fig. 3a, b, c, results can be directly compared, indicating that the $\overline{\textbf{NT}}$ probability matrix when abundance is measured as density of flowers provides the overall best fit.

Phylogenetic signal.—The independent phylogenetic signal of the insect phylogeny was weak ($d_a = 0.067$) and its confidence interval overlapped zero (95% confidence limits: [0, 0.286]). The independent plant phylogenetic signal was stronger ($d_p = 0.327$) and did not overlap zero (95% confidence limits: [0.189, 0.532]). The overall strength of the phylogenetic signal for the linear model fitted to the actual data ($\text{MSE}_d = 250.05$) was much closer to that found under the assumption of no phylogenetic covariances (the "star" phylogeny: $\text{MSE}_{star} = 269.29$) than for the assumption of maximum phylogenetic signal (Brownian motion evolution: $\text{MSE}_b = 420.61$). Taken together, these results suggest that only phylogenetic relationships among plants, not insects, impose some structure on the interaction matrix **Y**, but that the overall phylogenetic signal is extremely weak.

DISCUSSION

We have developed a conceptual and methodological framework to evaluate the simultaneous contributions of neutrality, forbidden links and sampling effects on the structure of mutualistic networks. We have used this framework to evaluate the influence of relative abundance (neutrality) and spatiotemporal overlap on a pollination network. Although we have not included information on phenotypic trait matching and sampling effects, if available this type of information can be easily incorporated to our conceptual and methodological framework. For example, Stang et al. (2006, 2007, 2009) have derived interaction probabilities based on rules of phenotypic matching between plants and nectar-feeding flower visitors. From such information it would be straightforward to derive a probability matrix to evaluate the relative contribution of phenotypic matching to the structure of the interaction matrix. Similarly, if detection probabilities of particular interactions could be estimated, then a detection probability matrix **E** could be incorporated into our framework (our implicit assumption above has been that all interactions have a detection probability of one).

One issue we have not been able to solve is how to incorporate phylogenetic information into our framework. This limitation stems from the difficulty of predicting interaction probabilities based on the independent plant and animal phylogenies, with no reference to the observed interaction matrix. Current methods for the detection of a phylogenetic signal in interaction networks (Ives and Godfray 2006) use a linear model approach, fitting the phylogenetic variance–covariance matrix to the interaction matrix, and there is currently no way of deriving an expected probability matrix based on the independent phylogenies alone. We hope our efforts can stimulate others to work out a solution to this crucial problem.

Using our conceptual and methodological framework, we have shown that interaction probabilities derived from abundance and temporal overlap predict very closely the aggregate properties of a plant–pollinator network. In contrast, our likelihood analysis shows that information on abundance and spatiotemporal distribution falls short of predicting the detailed network structure, leaving a large amount of variation unexplained. Thus, although information on abundance and spatiotemporal overlap allowed us to construct networks with the same aggregate features of real-world networks, we failed resoundingly when attempting to delve into the details of pairwise species interactions, which is arguably the ecologically and evolutionarily relevant scale of analysis. Taken together, our results suggest that both relative species abundance and complementarity in spatiotemporal distribution contribute substantially to generate observed network patterns, but that this information is by no means sufficient to predict the detailed structure of the interaction network.

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PLATE 1.   Representative plant–pollinator interactions in the Monte Desert of Villavicencio, Argentina. Clockwise from upper left: bumble bee *Bombus opiphex* (Apidae), a highly generalized pollinator, visiting *Opuntia sulphurea* (Cactaceae); wood-nesting bee *Megachile* sp. A (Megachilidae) visiting *Larrea divaricata*; *B. opiphex* visiting *Capparis atamisquea* (Capparaceae); *B. opiphex* visiting *Dipyrena glaberrima* (Verbenaceae). Photo credits, respectively: N. P. Chacoff, D. P. Vázquez, D. P. Vázquez, Benigno Padrón.

Of course, the above results for the Villavicencio network are by no means a general evaluation of the relative contribution of neutrality and forbidden links to the structure of mutualistic networks. Only future studies applying our (or a similar) approach to multiple data sets will allow such general evaluation. Unfortunately, most data sets available to date do not include the sort of detailed information needed for this comparison. Clearly, further progress in the understanding of the determinants of network patterns requires spatiotemporally explicit data sets with detailed natural history information that may allow deriving sensible rules of phenotypic complementarity.

We believe this goal will be facilitated if research efforts are focused on a sample of representative study systems with contrasting ecological conditions throughout the world.

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APPENDIX

Phylogenetic analyses (*Ecological Archives* E090-141-A1).

SUPPLEMENT

R functions used for the analysis (*Ecological Archives* E090-141-S1).