

# Evaluating sampling completeness in a desert plant–pollinator network

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## Summary

1. The study of plant–pollinator interactions in a network context is receiving increasing attention. This approach has helped to identify several emerging network patterns such as nestedness and modularity. However, most studies are based only on qualitative information, and some ecosystems, such as deserts and tropical forests, are underrepresented in these data sets.

2. We present an exhaustive analysis of the structure of a 4-year plant–pollinator network from the Monte desert in Argentina using qualitative and quantitative tools. We describe the structure of this network and evaluate sampling completeness using asymptotic species richness estimators. Our goal is to assess the extent to which the realized sampling effort allows for an accurate description of species interactions and to estimate the minimum number of additional censuses required to detect 90% of the interactions. We evaluated completeness of detection of the community-wide pollinator fauna, of the pollinator fauna associated with each plant species and of the plant–pollinator interactions. We also evaluated whether sampling completeness was influenced by plant characteristics, such as flower abundance, flower life span, number of interspecific links (degree) and selectiveness in the identity of their flower visitors, as well as sampling effort.

3. We found that this desert plant–pollinator network has a nested structure and that it exhibits modularity and high network-level generalization.

4. In spite of our high sampling effort, and although we sampled 80% of the pollinator fauna, we recorded only 55% of the interactions. Furthermore, although a 64% increase in sampling effort would suffice to detect 90% of the pollinator species, a fivefold increase in sampling effort would be necessary to detect 90% of the interactions.

5. Detection of interactions was incomplete for most plant species, particularly specialists with a long flowering season and high flower abundance, or generalists with short flowering span and scant flowers. Our results suggest that sampling of a network with the same effort for all plant species is inadequate to sample interactions.

6. Sampling the diversity of interactions is labour intensive, and most plant–pollinator networks published to date are likely to be undersampled. Our analysis allowed estimating the completeness of our sampling, the additional effort needed to detect most interactions and the plant traits that influence the detection of their interactions.

**Key-words:** Argentina, generalization, interaction web, Mendoza, modularity, Monte desert, nestedness, plant–animal mutualism, pollination, specialization, Villavicencio

## Introduction

During the last decade, studies of plant–pollinator interactions have increasingly used a community level approach, particu-

larly in the context of complex networks (Bascompte & Jordano 2007; Memmott *et al.* 2007; Aizen, Morales & Morales 2008; Petanidou *et al.* 2008). This network approach has been stimulated by recent theoretical and methodological advances, which have allowed to identify several apparently general structural properties of networks. For instance, the number of links per species (degree) is highly skewed (few species have

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many links while many species have few links; Jordano, Bascompte & Olesen 2003; Vázquez & Aizen 2003), interactions tend to be highly asymmetric and organized in a nested fashion (Bascompte *et al.* 2003; Vázquez & Aizen 2004; Bascompte, Jordano & Olesen 2006), and exhibit some degree of modularity (the existence of groups of species with high internal connectance and few connections to other species in the network; Olesen *et al.* 2006).

Network studies use a sampling procedure to identify who interacts with whom and, in quantitative studies, with what frequency or intensity. For most plant–pollinator networks, sampling centres on plants, where the identity and the number of interactions of each visitor species is recorded (e.g. Memmott 1999; Kaiser-Bunbury, Memmott & Müller 2009). An alternative approach is to centre on flower visitors, identifying pollen attached to the animal's bodies or at their nests (Bosch *et al.* 2009; Alarcón 2010). Thus, describing networks involves estimating the richness (qualitative or unweighted networks), relative abundance (quantitative or weighted networks) and composition of interactions for each species in the network. For this reason, network studies are subject to the same sampling issues of any study of diversity (Jordano 1987; Gotelli & Colwell 2001), with the additional problem that the estimated diversity of interactions is a function of the diversities of both groups of interacting species. Because limited sampling effort in network studies may affect network properties (Nielsen & Bascompte 2007; Vázquez *et al.* 2009a; Blüthgen 2010; Dorado *et al.* 2011), an accurate representation of the interactions in a community should come from a robust, comprehensive, well-designed sampling procedure (Jordano, Vázquez & Bascompte 2009).

The challenge of describing a network accurately based on plant-centered sampling lies in estimating the richness of interacting partners. How can we know if our sampling completely estimates the richness of interactions in the network? Species accumulation curves and asymptotic species richness estimators allow us to evaluate the accuracy of the sampling procedure and have been widely used in ecology to estimate the number of species present in a given area (e.g. Chazdon *et al.* 1998) and the number of interactions in food webs (Ings *et al.* 2009). Unfortunately, a substantially incomplete sampling looks very much like a substantially complete one in terms of prevalence of singletons and rare species (Colwell & Coddington 1994). Plant species attributes such as abundance, span of the flowering period and degree of specialization may influence the probability of detection of the interaction partners of a given species. For instance, rare and specialized species are expected to remain undersampled because of their low abundance, resulting in a poor estimation of the richness of their interaction partners (Dorado *et al.* 2011). Similarly, species with a short flowering stage are likely to be insufficiently sampled, again affecting detection of their interaction partners.

Here, we describe a 4-year study of a quantitative plant–pollinator network from an arid environment, the Monte desert of Mendoza, Argentina. Using these data, we describe several network properties and analyse them simultaneously in an integrative analysis. We also use species richness estima-

tors to assess the completeness of the sampling for the entire network and for each plant species in the network. Finally, we ask which combination of plant attributes explains the completeness of the sampling of interactions for each plant species. We hypothesize that as sampling effort, abundance, generalization and flowering span length increase, our detection of the interactions on each plant becomes more accurate.

## Materials and methods

### STUDY AREA

The study was conducted in the Central Monte desert biome in Mendoza, Argentina. Arid lands encompass 46% (184 million ha) of the total area of Argentina, of which 25% is Monte desert (ca. 15% of the country; Cabrera & Willink 1973), which lies entirely in Argentina (Morello 1958; Cabrera & Willink 1973). The Monte is closely related to the Pampean and Chaco biogeographic provinces, with some Antarctic and Neotropical elements and several shrub genera that are vicariant in the New World (*Aloysia*, *Capparis*, *Cercidium* and *Larrea*; Roig, Roig-Juñent & Corbalán 2009). To our knowledge, only two previous studies Jörgensen (1912) and Michelette & Camargo (2000) have considered plant–pollinator interactions at the community level in the Monte desert, and the former was the only study considered by Michener (1979) to characterize the bee fauna of the Midwestern Argentine deserts. More recent biogeographic work on pollinator fauna from deserts indicates that the Monte has a rich fauna with a high proportion of endemic genera and species of insects (Stange, Teran & Willink 1976; Roig-Juñent *et al.* 2001; Claps, Debandi & Roig-Juñent 2008). The climate is semi-arid, with 150–350 mm of mean annual rainfall occurring mostly in spring and summer (i.e. October–March; Labraga & Villalba 2009).

We worked in four 1-ha plots at Villavicencio Nature Reserve, a private protected area 30 km north of Mendoza city. The vegetation is a shrubland ca. 2 m tall, dominated by *Larrea divaricata* (Zygophyllaceae), *Condalia microphylla* (Rhamnaceae), *Prosopis flexuosa* (Fabaceae) and *Opuntia sulphurea* (Cactaceae) (Dalmaso *et al.* 1999). Most plant species flower in the spring between September and December, with a peak around mid October, although several species flower in the summer or repeatedly during the spring–summer (see Table S1, Supporting Information). Study sites were located at ca. 1270 m.a.s.l. and separated by at least 1 km (Site 1: 32°32'347"S, 68°57'210"W; Site 2: 32°31'839"S, 68°56'871"W; Site 3: 32°31'442"S, 68°56'352"W; Site 4: 32°31'405"S, 68°56'306"W).

### INTERACTION SAMPLING PROCEDURE

Plant–pollinator interactions were sampled in four consecutive flowering seasons, between September and January in 2006 and between September and December in 2007, 2008 and 2009. We sampled the whole community as comprehensively as possible. Sites 1–4 were sampled weekly in 2006 between 7 AM and 2 PM, whereas only sites 1 and 4 were sampled in the following years, twice a week. We decided to keep only sites 1 and 4 after the first year to minimize travelling time between sites and thus increase sampling effort per species without a substantial loss of species richness. For example, all plant species found in sites 2 and 3 were present either in sites 1 or 4 (Table S1, Supporting Information).

Each sampling period, hereafter 'census', consisted of 5 min of observation of a flowering branch for shrubs and trees or a whole plant or group of plants for herbaceous plants. A visit was recorded

only when the visitor touched a reproductive structure of a flower, suggesting it could be a potential pollinator. In each census, we recorded the identity of the plant species, counted the number of open flowers and recorded the species name and number of flowers visited by each flower visitor. For pollinator and plant species that could not be identified in the field, voucher specimens were collected and identified in the laboratory. We identified plant and insect specimens with the aid of specialists (see Acknowledgements); we followed Zuloaga & Morrone (1999) for the plants' nomenclature, Michener (2000) for bees, Claps, Debandi & Roig-Juñent (2008) for other insects and specialized identification keys for particular insect groups.

A total of 2048 5 min observation periods, totalling nearly 171 sampling hours, were conducted for all sites, and years, of which 511 were in 2006, 350 in 2007, 471 in 2008 and 716 in 2009. The number of sampling periods varied across species, sites, and years because species differed in their abundance, spatial distribution and duration of the flowering period (see Table S1, Supporting Information). We achieved a low sampling effort for 16 plant species; for example, nine plant species were sampled less than five times and another seven species were sampled 5–10 times (Table S1, Supporting Information). Some of these low sampled species were particularly rare, represented by few individuals in the study sites (e.g. *Hoffmanseggia eremophila*, *Petunia axillaris*, *Rosa* sp.), or flowered only during one or a few days (e.g. *Lycium gilliesianum* and *Oxybaphus ovatus*) or during one study year (e.g. *Cereus aethiops*). The maximum sampling effort was achieved for *Larrea divaricata* (286 sampling periods), followed by *Opuntia sulphurea* (185 sampling periods; Table S1, Supporting Information).

#### PLANT CHARACTERISTICS

To evaluate whether sampling completeness was explained by plant characteristics, we estimated flower abundance, flower phenological span, degree ( $k_p$ ) and selectiveness ( $d'_p$ ) for each plant species. Flower abundance was estimated weekly at each site. In 2006, we used 40  $2 \times 2$  m quadrats per site; in 2007 we used five fixed  $50 \times 2$  m transect belts; finally, in 2008–2009, we used two fixed  $50 \times 2$  m transects and four fixed  $20 \times 8$  m quadrats. Differences in the sampling methods for flower abundance responded to the needs of other related projects running simultaneously in the same sites. For comparison of flower abundance between years, we used the density of flowers in the sample quadrats or transects. At each plot, we counted the number of flowering individuals per species. In addition, for each flowering species we counted weekly the number of open flowers in 10 randomly selected individuals per site. With these data, we calculated the density of flowers of each species during each year (Table S1, Supporting Information). Flowering span was estimated for each plant species as the total number of days the species flowered each year. Specifically, we used information from the sampling carried out in transects and quadrats and from the censuses, which were usually carried out outside the transects/quadrats and thus allowed greater detection of flowering at the tails of the flowering period. Sampling effort for each plant species was calculated as the sum of the number of censuses performed on each plant over the 4 years. Quantification of degree and selectiveness is described later.

#### NETWORK STATISTICS

##### Network size, nestedness, and modularity

Network size statistics include the number of species of plants ( $P$ ), animals ( $A$ ), and the total ( $S = A + P$ ); the number of links in the

network ( $I$ ); the number of observed visits; and the size of the interaction matrix (AP).

Network structure was assessed by studying nestedness and modularity, which were calculated from the presence–absence interaction matrix (Lewinsohn *et al.* 2006). Nestedness is a measure of order of the network expressing 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, Guimarães & Lewinsohn 2007; Almeida-Neto *et al.* 2008). Nestedness values of 0 indicate non-nestedness, while 100 indicates perfect nesting. In the ordered nested matrix, species are arranged according to a decreasing degree, where specialists interact with a subset of generalists and there are minor group of generalist that interact with each other and with many specialists species. We used the NODF algorithm to estimate nestedness (Almeida-Neto, Guimarães & Lewinsohn 2007; Almeida-Neto *et al.* 2008), which overcomes some limitations of previous nestedness algorithms, correcting for matrix fill and matrix dimensions. We used a null model to assess the statistical significance of nestedness. The null model randomly assigned the  $I$  links in the original network with equal probability to all cells in the matrix, with the only constraints that the randomized matrices have the same number of plant and animals species as the original matrix and that each species has at least one interaction (i.e. the null model 1 of Vázquez & Aizen 2003). We assessed significance of nestedness by evaluating whether the observed value fell within the 95% confidence interval resulting from 1000 iterations of the null model.

Modularity ( $M$ ) is the extent to which the network is organized in subsets of interacting animals and plants, so species are more linked within than across subsets (Lewinsohn *et al.* 2006; Olesen *et al.* 2007). It ranges between 0 (random network with no modules) to 1 (maximum modularity). The presence and identity of modules were assessed using the program Netcarto, which identifies modules and classifies species according to their roles (as defined in Guimerà & Amaral 2005). The algorithm identifies groups of species that interact much more frequently with each other than with other species, based on simulated annealing (a stochastic optimization technique; Guimerà & Amaral 2005). To assess the statistical significance of observed modularity, the program calculates the value of the modularity in a random graph with the same degree (connectivity) distribution as the original network. As for nestedness, we evaluated whether observed modularity fell within the 95% confidence interval calculated from 1000 randomized matrices. The program also calculates the role of each species in the network based on its within-module degree ( $z$ ) and its among-module connectivity ( $c$ ), which defines how the species is positioned within its own module and with respect to other modules. Following Guimerà & Amaral (2005), we defined the following species roles: (R1) ultra-peripheral species, which have all their links within their module; (R2) peripheral species, with most links within their module; (R3) non-hub connector species, with many links to other modules; (R4) non-hub kinless species, whose links are homogeneously distributed among all modules; (R5) module hubs, with the vast majority of links within their module; (R6) connector hubs, with many links to most other modules; and (R7) network hubs, which have links homogeneously distributed among all modules.

##### Network- and species-level specialization

We used two measures of specialization at the network level: connectance ( $C$ ) and the network specialization index ( $H'_2$ ). Connectance is

the realized proportion of possible links,  $C = I/(AP)$ , where  $I$ ,  $A$ , and  $P$  are as explained previously (Dunne, Williams & Martinez 2002). The specialisation index ( $H'_2$ ) describes the level of specialization, or selectiveness, of an entire bipartite network (Blüthgen, Menzel & Blüthgen 2006), ranging from 0 (low selectiveness) to 1 (high selectiveness). Unlike connectance, this index has the advantage of not being affected by network size and sampling intensity, and can be applied to quantitative networks.

In addition to network-level metrics, we calculated two species-level measures of specialization: average species degree and the species specialization index. Species degree ( $k_p$  for plants and  $k_a$  for animals) is the number of species a certain species interacts with, and is a simple measure of generalization. Average degree is defined as  $k_p = I/P$  and  $k_a = I/A$  for plants and animals, respectively. The species specialization index  $d'$ , hereafter selectiveness, calculated from the quantitative interaction matrix, expresses how selective a given species is relative to available resources. This index indicates how strongly a species deviates from a random sample of the available interaction partners; it ranges from 0 (no selectiveness) to 1 (high selectiveness; Blüthgen, Menzel & Blüthgen 2006). For comparisons between plants and animals, we used the standardized average degree, calculated by dividing average degree over the number of potential interaction partners in the network ( $K_p = k_p/A$  for plants and  $K_a = k_a/P$  for animals), and the mean selectiveness index for plants ( $\bar{d}'_p$ ) and animals ( $\bar{d}'_a$ ).

All network statistics, except modularity, were calculated with the bipartite package (Dormann, Gruber & Fründ 2008) of R statistical software (R Development Core Team 2008).

#### ESTIMATING SAMPLING COMPLETENESS OF INTERACTIONS

We considered three levels of analysis to estimate sampling completeness of the interactions: the entire pollinator fauna, the plant–pollinator interactions in the whole network and the interactions for each plant species considered separately. While the first analysis, at the level of the entire pollinator fauna, is a measure of the sampling completeness of the insect community present in our study area, the second analysis, at the level of interactions in the whole network, is a measure of sampling completeness of the plant–pollinator interactions in the area; in turn, the third analysis, at the plant species level, tells us about the sampling completeness of the interactions on each plant species in the community, allowing us to estimate relative sampling completeness among species and to examine the plant characteristics that explain those differences.

To estimate the sampling completeness of the pollinator fauna, we estimated the richness of insect visitors accumulated as sampling effort increased, pooling the data for all plant species. To estimate the sampling completeness of plant–pollinator interactions in the whole network, we estimated the number (richness) of interactions accumulated as sampling effort increased, again, pooling the data for all plant species. Finally, to estimate the sampling completeness of interactions for each plant species, we estimated the number of interactions accumulated as sampling effort increased separately for each plant species.

For each of the aforementioned levels of analysis, we computed the species accumulation curve and the Chao 2 estimator of asymptotic species richness. The Chao 2 estimator, widely used in ecological studies (Chazdon *et al.* 1998; Gotelli & Colwell 2001), uses a non-parametric method to estimate species richness based on the concept that rare species carry the most information about the missing ones. This index uses the number of uniques (species that appear in only

one sample) and duplicates (species that occur in two samples) and is defined as

$$S_E = S_O + (L^2/2M), \quad \text{eqn 1}$$

where  $L$  is the number of species that occur in only one sample ('unique' species),  $M$  is the number of species that occur in exactly two samples and  $S_O$  is the observed number of species (Chao 2005).

We chose the Chao 2 estimator because it is one of the least biased estimates for small sample sizes, which is particularly important for the estimation of species richness (Colwell & Coddington 1994; Chao *et al.* 2009). In addition, for our data set it showed a good estimation stability among the estimators used; estimation stability was defined as the difference in estimation of flower visitor richness based on different sample sizes (i.e. number of 5-min observation periods; Fig. S1, Supporting Information).

We estimated the percent asymptotic richness detected in our samples, hereafter 'detection', as

$$\%S_O = 100 \frac{S_O}{S_E}, \quad \text{eqn 2}$$

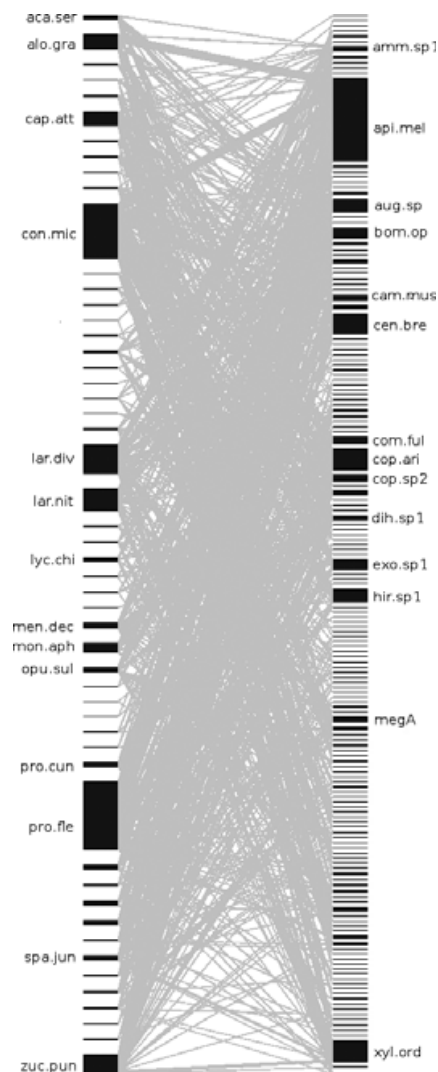
where  $S_O$  is the observed richness of species or interactions observed in our samples and  $S_E$  is the richness estimated with Chao 2. For the analysis at the plant species level, we considered only plant species where we performed more than 10 censuses and observed more than 10 visits (36 of 52 species), because asymptotic estimators of species richness are not accurate when sample sizes are extremely low. We also estimated the additional sampling needed to detect an arbitrary proportion (100%, 90% and 80%) of the estimated asymptotic species/interaction richness, using the method recently proposed by Chao *et al.* (2009). The number of uniques, duplicates and the estimation of Chao 2 were calculated with the Vegan package (Oksanen *et al.* 2010) of R statistical software (R Development Core Team 2008).

Using multiple linear regression, we explored the importance of plant characteristics and number of censuses to determine the detection of their interactions. We used stepwise regression to select among competing models using backward elimination with Akaike's Information Criterion (AIC) as the selection criterion (Burnham & Anderson 1998). We used  $\%S_O$  as the dependent variable and flower abundance (measured as the number of flowers per unit area), the number of flowering days, degree ( $k_p$ ) and selectiveness ( $d'_p$ ), and the number of censuses as explanatory variables. We started with a full model with two-way interactions (i.e. a species flowering during a long period may have greater overall censuses than a species that blooms over a short period, Gelman & Hill 2007). Explanatory variables were standardized by subtracting their mean and dividing by their standard deviation (Gelman & Hill 2007). We used the step function from the stats package in R to select the model with the lowest AIC.

## Results

### NETWORK DESCRIPTION

A total of 159 morphospecies of animals ( $A$ ) were recorded in the study sites, which visited 52 species of flowering plants ( $P$ ); thus, the total number of species in the network ( $S$ ) was 211. We recorded a total of 16895 visits and 727 interactions between different species ( $I$ ); the network size ( $AP$ ) was 8320 (Fig. 1). Among plants, 42 species (80%) were native; the introduced species included *Centaurea solstitialis*



**Fig. 1.** Graphical representation of the quantitative plant–pollinator network of Villavicencio. Black rectangles represent plant (left) and insect (right) species (Tables S1 and S2, Supporting Information for plant and insect species codes, respectively). Rectangle width is proportional to the number of interactions recorded per species. Lines represent links between species; line width is proportional to the frequency of interactions (number of visits recorded by a visitor species to a plant species). Only species codes for highly linked species are given.

(Asteraceae), *Cerastium arvense* (Caryophyllaceae), *Elaeagnus angustifolia* (Elaeagnaceae), *Spartium junceum* (Fabaceae), *Erodium cicutarium* (Geraniaceae), *Argemone subfusiformis* (Papaveraceae), *Ruta chadepensis* (Rutaceae) and *Verbascum thapsus* (Verbenaceae). The 52 plant observed species belonged to 28 different families. The most speciose families were the Asteraceae and Verbenaceae (six species) and the Fabaceae (five species). The 159 animals recorded were all insects that belonged to seven orders (Hymenoptera, Coleoptera, Diptera, Lepidoptera, Orthoptera, Hemiptera and Neuroptera), of which the hymenopterans and dipterans were the most diverse (90 and 45 species, respectively; Table 1). Among the flower visitors, only *Apis mellifera* is confirmed to be exotic to this area; in addition,

**Table 1.** Species richness, degree and selectiveness index for higher taxonomic groups of flower visitors in the plant–pollinator network of Villavicencio

Order	Family	Number of species	Degree (mean $\pm$ es)	Specialization (mean $\pm$ es)
Hymenoptera		90	4.06 $\pm$ 0.57	0.32 $\pm$ 0.02
	Andrenidae	6	2.33 $\pm$ 0.98	0.47 $\pm$ 0.08
	Apidae	20	5.85 $\pm$ 1.54	0.39 $\pm$ 0.04
	Colletidae	13	2.46 $\pm$ 0.42	0.29 $\pm$ 0.06
	Halictidae	4	13.66 $\pm$ 7.94	0.30 $\pm$ 0.09
	Megachilidae	17	3.70 $\pm$ 0.94	0.32 $\pm$ 0.03
	Formicidae	5	4.4 $\pm$ 1.91	0.29 $\pm$ 0.08
	Others <sup>a</sup>	24	3.86 $\pm$ 1.01	0.22 $\pm$ 0.03
Diptera		46	4.89 $\pm$ 0.85	0.26 $\pm$ 0.03
Lepidoptera		11	6.82 $\pm$ 1.59	0.36 $\pm$ 0.06
Coleoptera		7	2.57 $\pm$ 0.61	0.31 $\pm$ 0.07
Others <sup>b</sup>		6	2.16 $\pm$ 0.60	0.45 $\pm$ 0.01

<sup>a</sup>Vespidade, Pompilidae, Crabronidae, Eumenidae, Ichneumonidae, Sphecidae and Thynnidae.

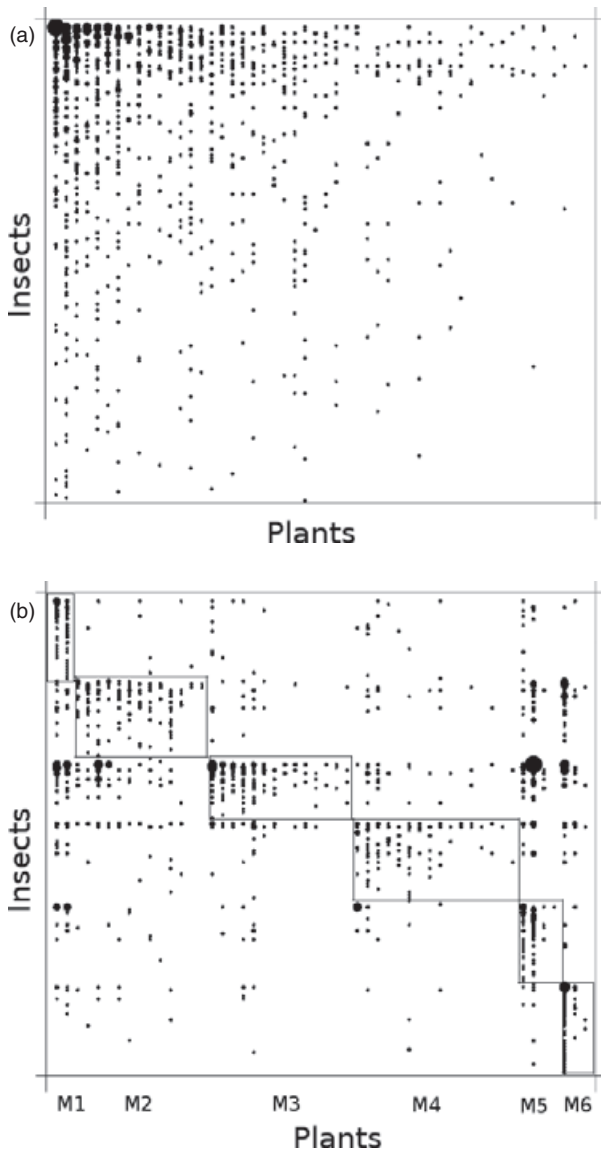
<sup>b</sup>Orthoptera, Hemiptera and Neuroptera.

*Polistes cavapyta* is also likely to be exotic. We still ignore the origin of many of the insect species recorded (Table S2, Supporting Information).

#### Nestedness and modularity

The Villavicencio plant–pollinator network is characterized by a high level of nestedness [observed  $N = 28.46$ ; 95% confidence interval of randomization (9.32, 9.78); Fig. 2a]. The core of generalists included several plant species characteristic of the Monte desert, namely *Prosopis flexuosa*, *Condalia microphylla*, *Larrea divaricata*, *L. nitida*, *Zuccagnia punctata*, *Aloysia gratissima* and *Capparis atamisquea*. Insects in this core group included some Apidae, one Halictidae and two Diptera species. The most generalized were the exotic *Apis mellifera*, followed by natives bees *Xylocopa ordinaria*, *Centris brethesi*, *Bombus opifex* and *Augochloropsis* sp., while *Hirmonera* sp. and *Copestylum aricia* were the most generalized flies.

The network exhibited a significant level of modularity [observed  $M = 0.33$ ; 95% confidence interval of randomization (0.304, 0.316)]. The modularity analysis identified six modules (Fig. 2b) containing 2–16 plant species and 20–28 insect species. Different groups of insects characterized different modules. For example, most species of dipterans were present in modules 2 and 6, while most species of megachilids were on modules 1, 4 and 5 and were completely absent from modules 2 and 6. Halictid bees were only in module 4, while andrenid bees were present in modules 4 and 1. Apid bees were absent only from module 6. Pompilid and vespid wasps were the main insect components of modules 5 and 6. Plants could not be grouped in families, as most families were represented by one or a few species. Nevertheless, three of the four species of Cactaceae were within a single module, dominated by andrenid and halictid bees. Similarly, 50% of Verbenaceae



**Fig. 2.** Plant–pollinator matrix from Villavicencio. Rows represent insect species, columns represent plant species, and circle diameter is proportional to the square root of the number of interactions between each insect and each plant. (a) Matrix arranged to maximise nestedness. (b) Matrix arranged to maximise modularity; rectangles indicate modules. Identities of species within each module are given in Tables S1 (plants) and S2 (insects), Supporting Information.

were in a module dominated by dipterans and formicids (Tables S1 and S2, Supporting Information).

Species played a variety of roles in the network. Most species (78%) had low within-module degree ( $z$ ) and among-module connectivity ( $c$ ) values, meaning that they are specialists with few links mostly within their module (peripheral and ultraperipheral roles); these species were mostly insect visitors (Table S2, Supporting Information). Generalist species were mainly plants (44% of the plant species), with either high  $z$  or high  $c$  values. Several plant species were connector species (low  $z$ , high  $c$ ) and two insect species (a nemestrinid fly and a megachilid bee) were identified as kinless, which have links to many

modules and belong to none. Five plant species and one halictid bee were hub species, with many links within their module (high  $z$ ) and many among-module links (high  $c$ ). Finally, two plant species (*Prosopis flexuosa* and *Larrea divaricata*) were defined as network hubs, that is super generalist species (species with the highest  $c$ ).

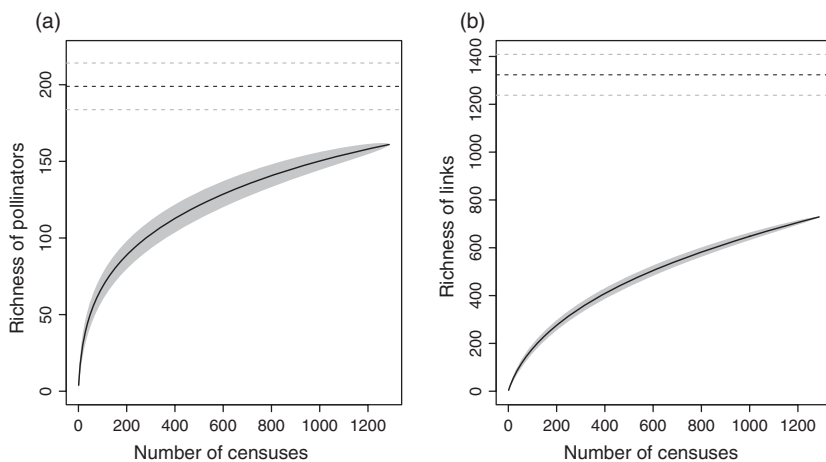
#### Network- and species-level specialization

Connectance in the Villavicencio network was low ( $C = 0.08$ ) compared with others mutualistic networks (mean  $\pm$  1 SE:  $0.29 \pm 0.18$ , Jordano 1987). The network level selectiveness index was  $H'_2 = 0.39$ , indicating high generalization at the network level (mean  $\pm$  1 SE:  $0.55 \pm 0.12$ , for plant–pollinator networks, Blüthgen *et al.* 2007). Degree for plants ranged between 1 and 54 flower visitor species (mean  $\pm$  1 SE:  $14 \pm 1.85$ ), while for visitors it ranged between 1 and 35 plant species (mean  $\pm$  1 SE:  $4.55 \pm 0.46$ ). The standardized average degree was similar for plants ( $K_p = 0.08$ ) and insects ( $K_a = 0.08$ ), suggesting no difference exists in specialisation between plants and flower visitors. In contrast, the selectiveness index  $d'$  indicates that plants ( $\bar{d}' \pm 1$  SE:  $0.39 \pm 0.02$ ) are more selective than insects ( $0.31 \pm 0.01$ ). Species-level selectiveness was substantially lower than average values reported in a review by Blüthgen *et al.* (2007) ( $\bar{d}'_p = 0.53 \pm 0.16$  for plants;  $\bar{d}'_a = 0.43 \pm 0.18$  for insects), suggesting that plants and animals in Villavicencio are unusually generalized. Halictid bees had the highest degree. Dipterans, vespids and other hymenopterans were the least selective groups, while andrenid and apid bees were the most selective (Table 1).

#### SAMPLING COMPLETENESS OF INTERACTIONS

Sampling completeness of the network varied according to the level of analysis: while we detected 80.4% of the asymptotic richness of flower visitor species, we detected only 55% of the interactions for the whole network (Fig. 3); for the plant species level of analysis, detection varied substantially among species. Seventy-five per cent of the plant species had more than 50% of their expected interaction partners detected, with an average % $S_O$  of 60.72% (SE = 20.57%; range 17–100%). Species with high % $S_O$  included some relatively highly sampled species such as *Prosopis flexuosa* (115 observation periods), *Senna aphylla* (75 periods) and *Monttea aphylla* (59 periods), and others with relatively low sampling effort such as *Pyrrhocactus pachacoensis* (14 periods) and *Buddleja mendozencis* (12 periods). On the other extreme, the species with the lowest % $S_O$  was *Trichocereus candicans* (16.97%, with 47 periods).

Using Chao *et al.*'s (2009) method, we estimated that we would need a fivefold increase in sampling effort needed to detect 100% of insect species, and a 23-fold increase to detect 100% of the interactions in the network. However, substantially lower effort is needed to sample 90% of species or interactions: we need a 64% increase in sampling effort for species and 4.7-fold increase for interactions (Table 2).



**Fig. 3.** Accumulation curves of pollinator species (a) and interaction (b) richness with increasing sampling effort. Asymptotic species/interactions richness ( $S_E$ ) (dark dashed lines) with the standard error (grey dashed lines) are shown as horizontal lines.

**Table 2.** Estimated sampling effort for incidence-based matrix

	$n$	$S_O$	$S_E$	$L$	$M$	$q_0$	Number of additional samples needed to detect $X\%$ species/interactions ( $g\%$ )			
							100%	90%	85%	80%
Insects	1288	160	198.42	42	24	0.01	7107	824	321	<sup>a</sup>
Interactions	1288	728	1323.35	334	116	0.46	16974	6144	3240	2160

$n$ , number of censuses;  $S_O$ , observed species richness;  $S_E$ , estimated asymptotic species richness, based on the Chao 2 estimator;  $L$ , the number of species/interactions represented by exactly one sample ('uniques');  $M$ , the number of species represented by exactly two samples ('duplicates');  $q_0$ , the probability that the next observed sample contains a species new to the survey (i.e. the proportion of species in the next sample that are new to the survey), estimated as  $L/T$  ( $T$  = the total number of incidences in the matrix);  $g$ , target fraction of  $S_E$  that is to be reached.

<sup>a</sup>In this case,  $g$  must be  $> 0.804$  because of the restriction  $g S_E > S_O$ .

**Table 3.** Summary statistics for the selected linear model with  $\%S_O$  as response variable and predictor variables. The estimate, standard deviation, the sums of squares and associated  $P$ -value are given for each factor

Coefficient	Estimate	SD	SumSq	$P$
Intercept	58.04	4.52	–	–
Sampling effort (no. of censuses)	11.20	6.43	87	0.565
Selectiveness ( $d$ )	-6.05	3.83	1402	<b>0.027</b>
Flower abundance (fl/ha)	1.60	4.81	637	0.127
Span of flowering (no. of days)	-10.45	7.01	2	0.924
Degree ( $k_p$ )	-16.20	6.13	9	0.850
Sampling: specialisation	14.13	6.52	232	0.351
Sampling: span of flowering	-12.56	8.89	462	0.192
Specialisation: span of flowering	-12.99	5.60	1789	<b>0.014</b>
Degree: flower abundance	6.29	3.21	1582	<b>0.020</b>
Degree: span of flowering	20.21	6.93	2186	<b>0.007</b>

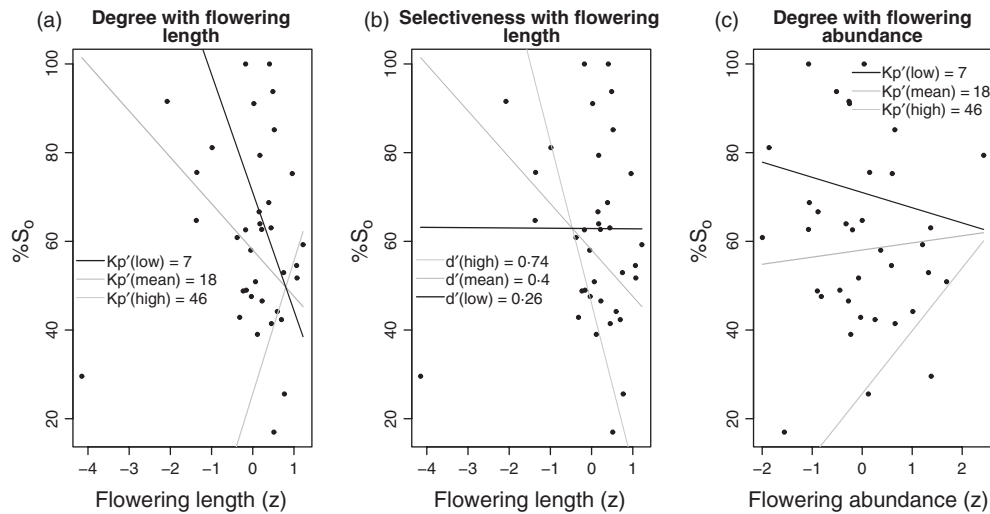
The  $P$ -values for significant effects are highlighted in bold.

Plant attributes and sampling effort explained 39.3% of the variance ( $R^2_{\text{adjusted}} = 0.393$ ,  $F = 3.265$ , d.f. = 10/25,  $P = 0.007$ ) in detection of interactions at the individual plant species level. The best-fitting model included all simple predictor variables and some interactions (Table 3). Detection increased with increasing sampling effort and flower abundance, but decreased with increasing selectiveness, length of the flowering period and degree (Table 3). In addition,

interactions meant that for plant species with low degree (i.e. specialists), detection decreases with increasing flowering length while it increases for plants with high degree (i.e. generalists) (Fig. 4a). Similarly, for plant species with high selectiveness, detection increases with increasing length of flowering period, while it decreases with decreasing selectiveness (Fig. 4b); finally, for plants with low degree, detection decreases with increasing flower abundance while it increases with increasing degree (Fig. 4c). Thus, plant species with either low degree, high selectivity and long flowering period or generalists species with low flower abundance and short flowering span had the lowest detection of the estimated number of interactions.

## Discussion

Our study adds important information for the study of plant–animal mutualistic interactions by contributing highly detailed, quantitative data of a species-rich network from a mid-latitude desert environment, an underrepresented type of biome in network studies. This network is characterized by a relatively large size, low connectance, a nested and modular structure, and a high generalization level of both plants and insects. Our analysis of sampling completeness revealed that, despite a large sampling effort, our network was undersampled, as we detected  $< 60\%$  of the potential interactions, in spite of having detected a relatively high proportion of the



**Fig. 4.** Relationship between the detection of the interacting partners ( $\%S_0$ ) and flowering length (a), for different degree values ( $k_p$ ) and (b) different selectiveness values ( $d'$ ). (c) Relationship between ( $\%S_0$ ) and flowering abundance for the degree ( $k_p$ ). Lines represent different levels of degree (a, c) and selectiveness (b). Flowering length and abundance are standardised by subtracting the mean and dividing by their standard deviation.

visitor species in the community (80%). Increasing the detection of interactions would require a substantial increase in sampling effort (e.g. a fivefold increase to achieve 90% detection of interactions). Although for some plant species interactions were relatively well sampled, most plant species were undersampled. The best-sampled species were those with large sampling effort, specialized plants with short flowering span and generalized plants with long flowering span or with abundant flowers.

The high generalization found for species in our study sheds light on the debate on the degree of specialization of the interactions between plants and pollinators in desert ecosystems. Desert organisms are hypothesized to cope with the high seasonality, variability and unpredictability of these arid ecosystems by generalizing on a variety of resources, which allows them to meet their food (animals) or reproductive (plants) requirements (Noy-Meir 1973; Moldenke 1975; Chesson *et al.* 2004). In contrast, Michener (1979) suggested that the high degree of synchronization of flowering phenologies and the high degree of overlap of activity periods of bees should lead to specialization, which would allow food resource partitioning among species, reducing interspecific competition. Our results are more consistent with the former conjecture.

Our network exhibited a significantly nested structure, which agrees with results from previous studies on mutualistic networks (Bascompte *et al.* 2003; Dupont, Hansen & Olesen 2003; Alarcón, Waser & Ollerton 2008). Our network also exhibited a significantly modular structure, another common property of plant–pollinator networks, particularly large ones (Olesen *et al.* 2007). The coexistence of a nested and a modular structure has been reported for other pollination networks (Olesen *et al.* 2007; Valdovinos *et al.* 2009), especially in networks with low connectance, in which high nestedness usually means high modularity (Fortuna *et al.* 2010). Nestedness is likely to result from the simultaneous

influence of species' relative abundances, the constraints imposed by complementarity in species phenotypes, phenologies, spatial distributions and phylogenies, and sampling artefacts (Vázquez *et al.* 2009a; Vázquez, Chacoff & Cagnolo 2009b). In turn, the existence of modules in a network may reflect habitat heterogeneity, divergent selection regimes and phylogenetic clustering of closely related species (Lewinsohn *et al.* 2006; Olesen *et al.* 2007), leading to nonrandom patterns of interaction. However, we still need a better understanding of the combined implications of these two patterns for community stability and functioning of the interactions.

The composition of modules might reflect some phylogenetic signal (Olesen *et al.* 2007). Some modules found in our network were defined by groups of insect species of the same family that tended to interact together, which might reflect some phylogenetic influence on modularity. For example, the andrenids and halictids were grouped together in a single module and interacted mainly with Cactaceae, while several dipteran families were grouped together with Formicidae and interacted mostly with Verbenaceae. In contrast, some highly connected species did not belong to particular modules; for example, *Larrea divaricata* and *Prosopis flexuosa* were identified as network hubs, which means that they provide floral resources to a high diversity of flower visiting insects and they can be of particular importance for the maintenance of the structure and functioning of this mutualistic network.

The evaluation of sampling completeness allowed us to estimate the proportion of the entire pollinator fauna and the plant–pollinator interactions that were undetected in our study. There are several possible explanations for the relatively low detection of species and interactions in our study. One is that sampling effort was indeed insufficient; this seems to be true for some specialist plants, as indicated by our analysis of sampling completeness. Another explanation is that we could be overestimating the proportion of, for example,

undetected interactions, because our measure of detection, the Chao 2 estimator, uses information on infrequent species (uniques and duplicates) to estimate the number of undetected species. Completeness is possible only when all species are observed in more than two censuses, which implies mathematically the absence of rare species (Chao *et al.* 2009; Colwell & Coddington 1994). Thus, all species or interactions that are seen only once or twice, something very common in a plant–pollinator network such as the one reported here, increase the expected richness. Hence, the community of interacting organisms and the recorded interactions appear as undersampled. In communities, the abundance of species is heterogeneous, with a few abundant and many rare species; unfortunately, the estimation of expected richness does not take this fact into account, assuming that all species that were detected once or twice should increase their abundance with more sampling. Although increasing sampling should increase the probability of detection of all species/interactions, some extremely rare species or opportunistic interactions might never, or only rarely, be detected a second time. As suggested by Chazdon *et al.* (1998), this implies that the Chao 2 estimator might overestimate the expected richness of insects, and this can be particularly important for many interactions that were observed only a few times. We found that the community of insects was relatively well sampled (88%), with most insect species observed many times, compared with a lower detection of interactions (55%); this difference could be partly attributed to the many rare interactions, or to the opportunistic visits made by highly generalized insects, rather than to true undersampling of interactions. Recently, Stang *et al.* (2009) found that generalist insect species visit each plant species less often than specialist insect species. If general, this result means that it increases the expected richness of interactions for plant species, as most generalist insect species will be unique and duplicate interactions. A third possible explanation is that the presence and abundance of plant flowers and insects vary substantially between years (e.g. Herrera 1988), as observed in other systems (e.g. Petanidou *et al.* 2008). Such year-to-year compositional turnover is likely to affect our asymptotic estimates of species richness (Colwell, Rahbek & Gotelli 2004), because plants or animals that were present in only 1 year, for example, will necessarily increase the number of uniques and duplicates in our total sample. We still need to disentangle true ecological factors, such as species abundance and dynamics, from sampling biases (Vázquez *et al.* 2009a) to improve our ability to detect interactions in mutualistic networks.

Plant species attributes such as abundance, span of flowering period and degree of specialization influenced the detection probability of the interaction partners of plant species. We have shown that for specialized plants (i.e. those with high selectiveness or low degree) with long flowering span, we detected fewer of their estimated total interactions. Our results also indicate that a short flowering season and low flower abundance also lead to low detection of interactions in generalized plants. Thus, our overall ability to detect interactions could be enhanced by increasing the sampling effort on

specialized plants with long flowering periods and on generalized plants that are rare or have a short flowering span. Thus, the appropriate sampling procedure to detect interactions in a plant–pollinator network should be one that considers plant specialisation, abundance and floral phenological span.

The ongoing debate on the effect of sampling effort on the structure and metrics of mutualistic networks (Nielsen & Bascompte 2007; Blüthgen *et al.* 2008; Dorado *et al.* 2011) and food webs (Cohen *et al.* 1993; Goldwasser & Roughgarden 1997; Martinez *et al.* 1999) is indicative of the importance of the topic for the field of ecological research. One of the major current challenges is to understand sampling accuracy and how to improve it. We believe our analysis of a desert plant–pollinator network is a significant step in that direction.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Fig. S1.** The difference between estimated richness for each number of census and estimated richness for all censuses is plotted against the number of censuses.

**Fig. S2.** Number of observed species for each plant and number of expected species to be found based on Chao 2 estimators. See Table S1 for plant species codes.

**Table S1.** Information on plant species in the Villavicencio network: species code used in figures, species origin (native or exotic), life form

(small tree, shrub, vine, herb), number of observation periods conducted, cumulative flower abundance over the whole flowering season (flowers/ha), cumulative flowering span, degree, specialization index, total interaction frequency, module membership, role in the network, sites where the species was found, estimated total richness using  $S_{\text{Chao 2}}$ ,  $S_{\text{ACE}}$  and  $S_{\text{Bootstrap}}$ .

**Table S2.** Information on insect species in the Villavicencio network: species code used in figures, species origin (native or exotic), degree of sociality, typical nesting habitat (only for hymenopterans), preferred type of floral resource, degree, specialization index, interaction frequency, module membership and role in the network.

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