

Rareness and specialization in plant–pollinator networks

JIMENA DORADO,^{1,3} DIEGO P. VÁZQUEZ,^{1,2} ERICA L. STEVANI,¹ AND NATACHA P. CHACOFF¹

¹*Instituto Argentino de Investigaciones de las Zonas Áridas, CCT CONICET Mendoza, CC 507, 5500 Mendoza, Argentina*

²*Instituto de Ciencias Básicas, Universidad Nacional de Cuyo, Centro Universitario, M5502JMA Mendoza, Argentina*

Abstract. Most rare species appear to be specialists in plant–pollinator networks. This observation could result either from real ecological processes or from sampling artifacts. Several methods have been proposed to overcome these artifacts, but they have the limitation of being based on visitation data, causing interactions involving rare visitor species to remain undersampled. We propose the analysis of food composition in bee trap nests to assess the reliability of network specialization estimates. We compared data from a plant–pollinator network in the Monte Desert of Villavicencio Nature Reserve, Argentina, sampled by visit observation, and data from trap nests sampled at the same time and location. Our study shows that trap nest sampling was good for estimating rare species degree. The rare species in the networks appear to be more specialized than they really are, and the bias in the estimation of the species degree increases with the rareness. The low species degree of these rare species in the visitation networks results from insufficient sampling of the rare interactions, which could have important consequences for network structure.

Key words: Monte Desert, Villavicencio Nature Reserve, Argentina; plant–pollinator networks; rare species; sampling artifact; solitary bees; specialists; species degree; trap nest.

INTRODUCTION

Plant–pollinator networks depict the interspecific interactions between plants and pollinators in a community (Memmott 1999, Bascompte and Jordano 2007). These networks are usually built with observations of pollinator visits to flowers (visitation networks). A common observation in these studies is that most rare species appear to be specialists (Bascompte et al. 2003, Jordano et al. 2003, Vázquez and Aizen 2003, 2004). In other words, the number of interspecific links of a species (hereafter species degree) and its observed visitation frequency are usually positively correlated. This correlation could result either from real ecological mechanisms or from sampling artifacts (Vázquez et al. 2009a). True ecological mechanisms include two distinct types of processes: interaction neutrality and trait matching. Interaction neutrality refers to the effect of abundance on the probability of interspecific encounters, so that abundant species tend to encounter other species more frequently than rare species. Trait matching refers to the correspondence between phenotypic traits of interacting individuals, so that only individuals with certain traits can actually interact. A sampling artifact is likely because rare species of pollinators and their interactions have a low probability of being observed at flowers, and thus may appear as extreme specialists when they are, in fact, generalists.

Low detection probability of interspecific links of a species could distort network patterns and thus the understanding of the studied community. Three methods have been proposed to overcome the potential biases in specialization estimates resulting from sampling artifacts. The first one consists of increasing sampling effort, either by investing more time in visual surveys per unit time (Vázquez and Aizen 2006, Nielsen and Bascompte 2007) or by increasing the time span of the study (Petanidou et al. 2008). The second approach is to apply statistical methods to attempt to remove sampling artifacts (Blüthgen et al. 2006, Dormann et al. 2009). A third approach consists of studying the pollen carried by pollinators (Bosch et al. 2009, Alarcón 2010), which provides cumulative information on the identity of plants visited in successive visits, thus increasing the detection probability of interactions for rare pollinator species. Their virtues notwithstanding, the three previous methods have the limitation of being based on visitation data, which makes interactions involving rare visitor species to remain undersampled.

We propose the analysis of food composition in bee trap nests as a tool to assess the reliability of specialization estimates from plant–pollinator visitation networks. Trap nests are a simple, albeit powerful tool to study feeding habits of bees, providing substantial information on their feeding habits because they contain large quantities of pollen (Krombein 1967, Michener 2000). By identifying the pollen from the nests, we can find out the identity of plants visited by each bee species when it built the nest. If flowers of some plant species were visited by bees only for adult feeding or nectar collection, those species should be represented in small

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³ E-mail: jdorado@mendoza-conicet.gov.ar

quantities in the nests (Williams and Tepedino 2003, Rust et al. 2004). Although trap nests are used by a relatively small group of pollinator species, those that use them occupy many trap nests, which provides a tool for obtaining copious information on the specialization of rare species.

We hypothesize that degree estimates from visitation data for rare species are highly biased, and that trap nests provide more accurate information on species degree than visitation observations, both because each bee species occupies many traps in spite of being rare in visitation observations and because each bee individual collects pollen from many visits. We evaluate four predictions of our hypothesis by comparing data from visitation observations and trap nests. First, the identities of plant species recorded in visitation observations should be a subset of those observed in trap nests. Second, the species degree estimated using visitation observations and trap nest data should not be correlated, which would indicate that the two methods are not equivalent for estimating species degree. Third, the positive relationship between species degree and observation frequency recorded for visitation data should disappear when using trap nest data instead. Fourth, degree estimates from the visits and the trap nests should converge as observation frequency increases. We end with a simulation to evaluate the consequences of the improved interaction information on the network structure.

METHODS

Plant–pollinator visitation sampling

We used data from the plant–pollinator network described by Vázquez et al. (2009b). The visit observations were carried out during 2006 and 2007 in four plots in the Monte Desert of Villavicencio Nature Reserve, Mendoza, Argentina. Visit observations consisted of 5-min observation of a flowering branch for shrubs and trees or a whole plant or group of plants for herbs. During each observation period, we recorded each flower visitor contacting floral reproductive parts. We conducted 894 flower visitor observation periods (nearly 75 h of observation), of which 556 involved plant species included in this analysis. For flower visitors and plants that could not be identified in the field, voucher specimens were collected and identified in the laboratory. The resulting network included 41 plant species, 97 visitor species, and 6301 interactions. Of the 97 floral visitor species, 50 were singletons.

The identity of some insect specimens from the visits data was reassessed after publication of the Vázquez et al. (2009b) study. As a result, two specimens formerly identified as one of our study species (*Megachile* sp. A) were reidentified as a different species and thus were not included in our analyses. We also eliminated some observations for which individual visitors could not be collected and visual identification was dubious. As

results did not differ significantly after making these changes, we report only results for the corrected data.

Trap nest sampling

We carried out the trap nest sampling at the same time and on the same four 1-ha plots where the visitation network was described, so that the same interactions could be recorded in both the trap nests and the visitation observations. We placed trap nests in 30 points per plot, spaced at least 20 m from each other. Each point had nine trap nests consisting of a wood piece (2 × 15 cm) with a longitudinal 5- or 8-mm hole; wood pieces of different hole sizes were interspersed. Trap nests were checked weekly; occupied traps were taken to the laboratory and replaced by empty ones. Each trap nest constitutes one bee nest (Fig. 1a). Once in the laboratory, nests were opened to record the number of cells (Fig. 1b). One cell of each nest was extracted for pollen identification. Taxonomic identification of pollen was conducted by comparison with a reference collection, prepared including all plant species flowering at each study plot. The rest of the cells in each nest were kept in the laboratory at ambient conditions until adult emergence. After emergence, the number of adults, parasites and cleptoparasites, and the species identity were recorded. Whenever possible, emerged adults were released in the field at the original nest collection site. Parasites and cleptoparasites that emerged from the trap nests were not included in the analysis because the pollen in the nests does not represent any flower visit from the adult.

Statistical analysis

To evaluate if pollen from the trap nests gives good information for inferring the species degree, we compared the identity of plant species recorded by visitation observation and trap nest data. If the plant identities of the visitation observation data were a subset of those found in the trap nests, we would conclude that the pollen from the trap nests is good. Alternatively, if the plant identities from trap nests were a subset of those from the visitation observation, we would conclude that the pollen from the trap nests gives incomplete information for inferring the species degree.

To evaluate if visitation and trap nest data are equivalent for estimating the species degree, we evaluated Spearman rank correlations between them; lack of correlation would indicate that the two methods are not equivalent for estimating species degree. To evaluate if degree estimates from visitation data result from a sampling artifact, we compared Spearman rank correlations between the species degree and the visit observation frequency estimated from visitation observations and trap nest data; a positive correlation for visitation data but not for trap nest data would mean that a sampling artifact was important in generating observed degree. To evaluate if degree estimates from visits and trap nests become similar as observation frequency in visitation data increases, we evaluated the

Spearman rank correlation between the standardized difference between degree estimates calculated with visitation and trap data (hereafter visit–trap degree difference) and the observation frequency for each bee species; a positive correlation would indicate that for visitation data the sampling bias of degree estimates increases with rareness. Standardization in the visit–trap degree difference was done by dividing it by the total number of interspecific interactions observed for the bee species. Correlation analyses were done using the `cor.test` function in the base package of R statistical software (*available online*).⁴

Simulation of improved interaction information

We conducted a simulation to evaluate how network structure would change if improved interaction information was available for all species. To this end, we first assigned a probability of error in estimation of degree for each species in the network. For trap-nesting species, this probability was estimated as the visit–trap degree difference, assuming that trap nests reveal the “true” interaction information. We used the relationship between the visit–trap degree difference and observation frequency to estimate this probability for the remaining visitor species that did not nest in our traps, truncating the relationship and assigning zero probability to species with negative values in the ordinates. For species with probability values greater than zero, we assigned a new number of links between k and 9, where k is the original degree of a species and 9 is the maximum degree observed for the species that used trap nests ($k < 9$ for species receiving new links). The new number of links was assigned using the binomial probability distribution of each species’ error probability (with parameters n and p), where n is the number of trials ($n = 9$) and p is the error probability for each species. Next we added links to the original interaction matrix by assigning new links to plants with probability proportional to the product of a plant’s degree and its abundance in the field. We compared connectance, mean degree, and nestedness, three widely used matrix indices, between the original and the modified matrices. Connectance is the proportion of realized interspecific links, defined as $C = L/(I \times J)$, where L is the number of nonzero entries in the binary interaction network and I and J are 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). Nestedness was calculated with the NODF metric (Almeida-Neto et al. 2008), which corrects biases resulting from matrix fill and matrix dimensions. Values of 0 indicate non-

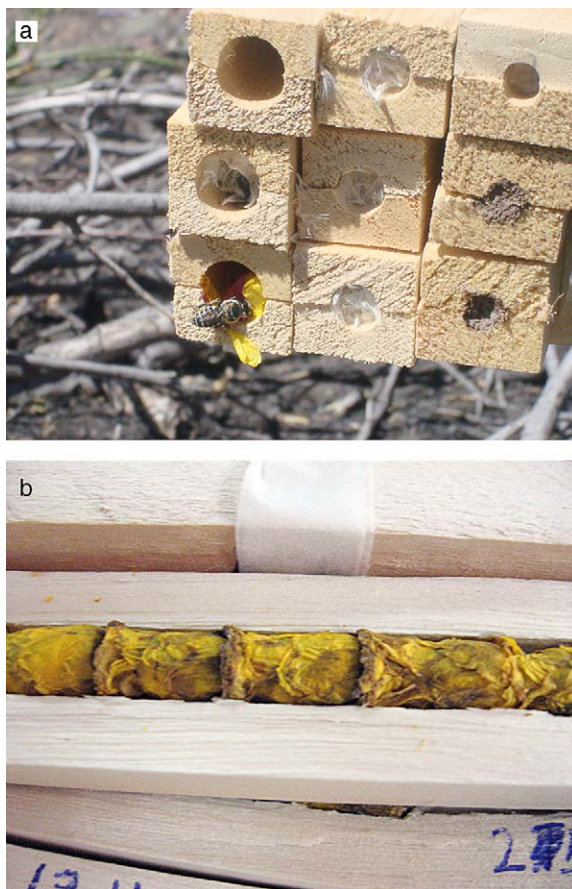


FIG. 1. Trap nests consist of wood pieces with a longitudinal hole where bee species nest. One occupied trap nest constitutes one bee nest. (a) *Megachile* sp.: a female building the nest with *Larrea divaricata* petals. The holes covered with mud belong to *Megachile* spp., and the holes covered with fruit fibers belong to *Anthidium* spp. Photo credit: Natacha P. Chacoff. (b) Open *Megachile* sp.: a nest. Each cell contains stored pollen to feed one larva. Photo credit: E. L. Stevani.

nestedness, those of 100 perfect nesting. It is implemented in the “nestednodf” function of the bipartite package (Dormann et al. 2008) of R statistical software (see footnote 4). The bipartite package was also used to calculate connectance and mean degree.

RESULTS

A total of 460 trap nests were occupied by seven solitary bee species, 121 of which were analyzed for pollen content (25 in 2006 and 96 in 2007). The five more abundant species in the traps were also observed in the visits: two unnamed *Megachile* species (hereafter called *Megachile* sp. A and *Megachile* sp. C), *Megachile ctenophora* Holmberg, *Anthidium vigintipunctatum* Friese, and *Trichothurgus laticeps* Friese. The two remaining species, *Anthidium rubripes* Friese and *Anthidium decaspilum* Mours, were observed in only a handful of traps and absent from visitation data. *Coelioxys inconspicua* Holmberg, an abundant cleptoparasite of *Megachile*, and another five parasite species that

⁴ <http://www.r-project.org/>

TABLE 1. Species degree recorded using trap nests and visit observation frequency from a plant–pollinator network in the Villavicencio Nature Reserve, Mendoza, Argentina.

| Species | <i>Megachile</i> sp. A | | <i>Megachile</i> sp. C | | <i>Megachile ctenophora</i> Holmberg | | <i>Anthidium vigintipunctatum</i> Friese | |
|--|------------------------|-----------------|------------------------|----------------|--------------------------------------|----------------|--|-----------------|
| | Visits (19) | Trap nests (48) | Visits (7) | Trap nests (3) | Visits (1) | Trap nests (8) | Visits (1) | Trap nests (37) |
| <i>Larrea divaricata</i> auct. non Cav. (170) | 15 | 48 | 5 | 3 | – | 7 | 1 | 20 |
| <i>Prosopis flexuosa</i> L. C. (60) | – | 4 | – | 1 | – | 6 | – | 30 |
| <i>Opuntia sulphurea</i> G. Don in Loudon (64) | – | 2 | 2 | 1 | – | 3 | – | – |
| <i>Thymophylla pentachaeta</i> (DC.) Small (DC.) Strother (12) | – | 4 | – | – | – | 5 | – | 4 |
| <i>Lecanophora heterophylla</i> (Cav.) Krapov (22) | – | 1 | – | 1 | 1 | 2 | – | 1 |
| <i>Zuccagnia punctata</i> Cav. (59) | – | 3 | – | – | – | 1 | – | 3 |
| <i>Menodora decemfida</i> (Gillies ex Hook. & Arn.) A. Gray (56) | – | – | – | – | – | 4 | – | 4 |
| <i>Senna aphylla</i> (Cav.) H. S. Irwin & Barney (28) | 2 | 1 | – | – | – | – | – | – |
| <i>Lycium</i> spp. (22) | – | – | – | – | – | – | – | 3 |
| <i>Buddleja mendozensis</i> Benth. (7) | – | – | – | – | – | – | – | 2 |
| <i>Larrea nitida</i> Cav. (29) | 1 | – | – | – | – | – | – | – |
| <i>Helenium donianum</i> (Hook. & Arn.) Seckt (27) | 1 | – | – | – | – | – | – | – |

Notes: Plants are in rows, and bee species are in columns. Each trap nest constitutes one bee nest. Visits refers to the number of individuals of a particular bee species recorded in visit observations; “trap nests” is the total number of trap nests found for each bee species. Values in parentheses following plant species names in column 1 report the number of observation periods carried out on a particular plant species. Values within the cells give the number of interactions between a plant and a bee species recorded in visits and trap nests; cells with dashes indicate absence of that interaction. Notice that the sum of the interactions recorded in nests of a given bee species may be greater than its total number of nests, because a single trap nest can have more than one plant species.

emerged from the trap nests were also unobserved in the visits. Thus, we restrict our analysis to the five most abundant bee species, for which we recorded 33 visits to flowers. The number of visits and trap nests recorded for each bee species, as well as the number of observation periods for plants associated with these bee species, are in Table 1 (see Plate 1).

For all study species, the species degree estimated from trap nests was greater than in visitation data (Table 1). For all but one bee species the identities of plant species recorded in visits was a subset of those recorded in trap nests (Table 1); the exception was *Megachile* sp. A, which was recorded visiting some plant species not represented in trap pollen. Thus, while bee species appear as extreme specialists in visitation data, they appear more generalized in the trap nest data. For example, *M. ctenophora* and *A. vigintipunctatum* were observed only once in visits and thus their species degree is one in the visitation network, but it becomes much greater (7 and 8) in trap nests.

There was no relationship between degree estimated from visitation and trap nest data ($r = -0.28$, $N = 5$, $P = 0.64$). This shows that one estimate cannot be predicted by the other.

There was a positive correlation between species degree and visit observation frequency when using visitation data to estimate degree ($r = 0.91$, $N = 5$, $P = 0.028$; Fig. 2a), which is consistent with previous studies of plant–pollinator networks, in which rare species have a low species degree (Bascompte et al. 2003, Jordano et al. 2003, Vázquez and Aizen 2003, 2004). This correlation disappears when degree is estimated from trap nest data ($r = -0.5$, $N = 5$, $P = 0.391$; Fig. 2b).

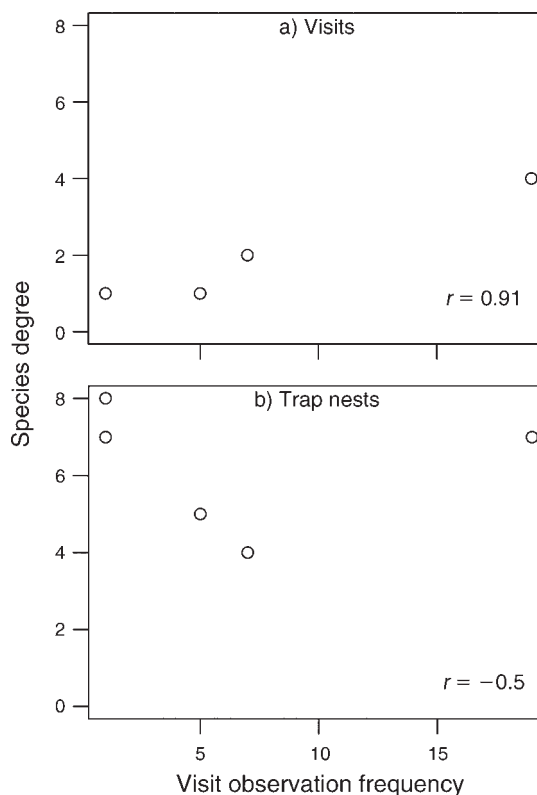


FIG. 2. Correlation between the species degree and visit observation frequency. (a) Bee species degree (number of interspecific interactions) estimated from visit observations (Vázquez et al. 2009b). Notice that there are four points instead of five, because there are two species overlapping that were observed once. (b) Bee species degree estimated from trap nests. Each trap nest constitutes one bee nest.

TABLE 1. Extended.

| <i>Trichothurgus laticeps</i> Friese | | <i>Anthidium rubripes</i> Friese | | <i>Anthidium decapilum</i> Moure | |
|---|-----------------------|---|----------------------|---|----------------------|
| Visits (5) | Trap nests (22) | Visits (0) | Trap nests (2) | Visits (0) | Trap nests (1) |
| – | 1 | – | 2 | – | 1 |
| – | 1 | – | 1 | – | – |
| 5 | 22 | – | – | – | – |
| – | 3 | – | 1 | – | – |
| – | 1 | – | – | – | – |
| – | – | – | – | – | – |
| – | – | – | – | – | – |
| – | – | – | – | – | – |
| – | – | – | – | – | – |
| – | – | – | – | – | – |
| – | – | – | – | – | – |
| – | – | – | – | – | – |
| – | – | – | – | – | – |

There was a negative relationship between the visit–trap degree difference and visit observation frequency ($r = -0.97$, $N = 5$, $P = 0.0048$; Fig. 3). This negative relationship shows that estimates of degree for visitation and trap nest data become closer as observation frequency increases.

The simulation showed that improved interaction information would result in connectance increased by 60% (from 0.081 to 0.134), mean degree increased by 60% (from 2.33 to 3.85), and nestedness increased by 50% (from 21 to 42).

DISCUSSION

Our study shows that trap nest sampling provided substantially better estimates of species degree than visitation observations. Trap nests also allowed the detection of bee species unrecorded in visit observations. Thus, our results clearly show that the rare species in visitation networks appear more specialized than they really are, and the bias in the estimation of the species degree increases with the rareness of the species. The low species degree of these rare species in the visitation networks results from insufficient sampling of the rare interactions, which could have important consequences in our perception of network structure.

Trap nests provided useful information for estimating species degree of most study bee species, as interspecific interactions recorded from visits were a subset of those observed in trap nests. The exception was *Megachile* sp. A (Table 1), which visited two plant species unrecorded in trap nests, *Larrea nitida* and *Helenium donianum*. One plausible explanation is that this bee species uses these plants only for nectar; however, we believe this is not the most likely explanation for the absence of those plant species in the trap nests. Exclusive use of *L. nitida* for nectar is unlikely, given that *L. divaricata*, a morphologically similar species, is used mostly as a pollen source. Alternatively, *L. nitida* could have been present

but unnoticed in trap nests, as its pollen grains are undistinguishable from those of *Larrea divaricata*. In our analysis, we assumed all *Larrea* pollen grains observed in trap nests were of *L. divaricata*, because it flowers more widely and abundantly during the nest building season, whereas *L. nitida* flowers briefly at the beginning of the season; however, it should be born in mind that this assumption could be incorrect. In turn, it is also unlikely that *H. donianum* was used only for nectar because its pollen appears in nests from other sites not included in this analysis; alternatively, it was probably either unused in our study sites for provisioning the nests or undetected in the pollen samples. Thus the visit observation data complement the trap nest data, although the latter method is substantially more sensitive than the former.

Our results demonstrate that our study species are more generalized than they appear from the visitation data, indicating that the large number of rare specialists in plant–pollinator networks results from a sampling artifact, as conjectured by previous studies (Vázquez et al. 2009a). This conclusion comes from the observations that the degrees estimated with both methods are uncorrelated, indicating they are not equivalent, and that the positive relationship between species degree and visit observation frequency observed for visitation data (Fig. 2a) disappears when we use degree estimated from trap nests instead (Fig. 2b). The latter results agree with those of Bosch et al. (2009), where the number of extreme specialists of the network decreased by 0.6-fold when including the carried pollen information, suggesting that ecological specialization is often overestimated in plant–pollinator networks.

It is possible that the observed sample bias results from the low sampling size achieved for some rare plant species used as pollen sources by the bees, as sampling

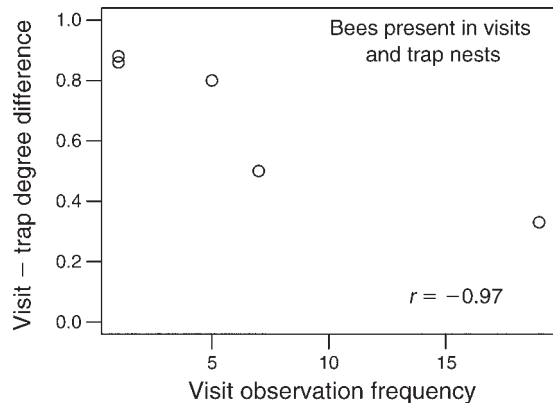


FIG. 3. Visit – trap degree difference vs. visit observation frequency for rare bee species that used trap nests. The visit – trap degree difference is the difference between the species degree (number of interspecific interactions) estimated from visits and from trap nests divided by the total number of interactions the bee species had (i.e., standardized).

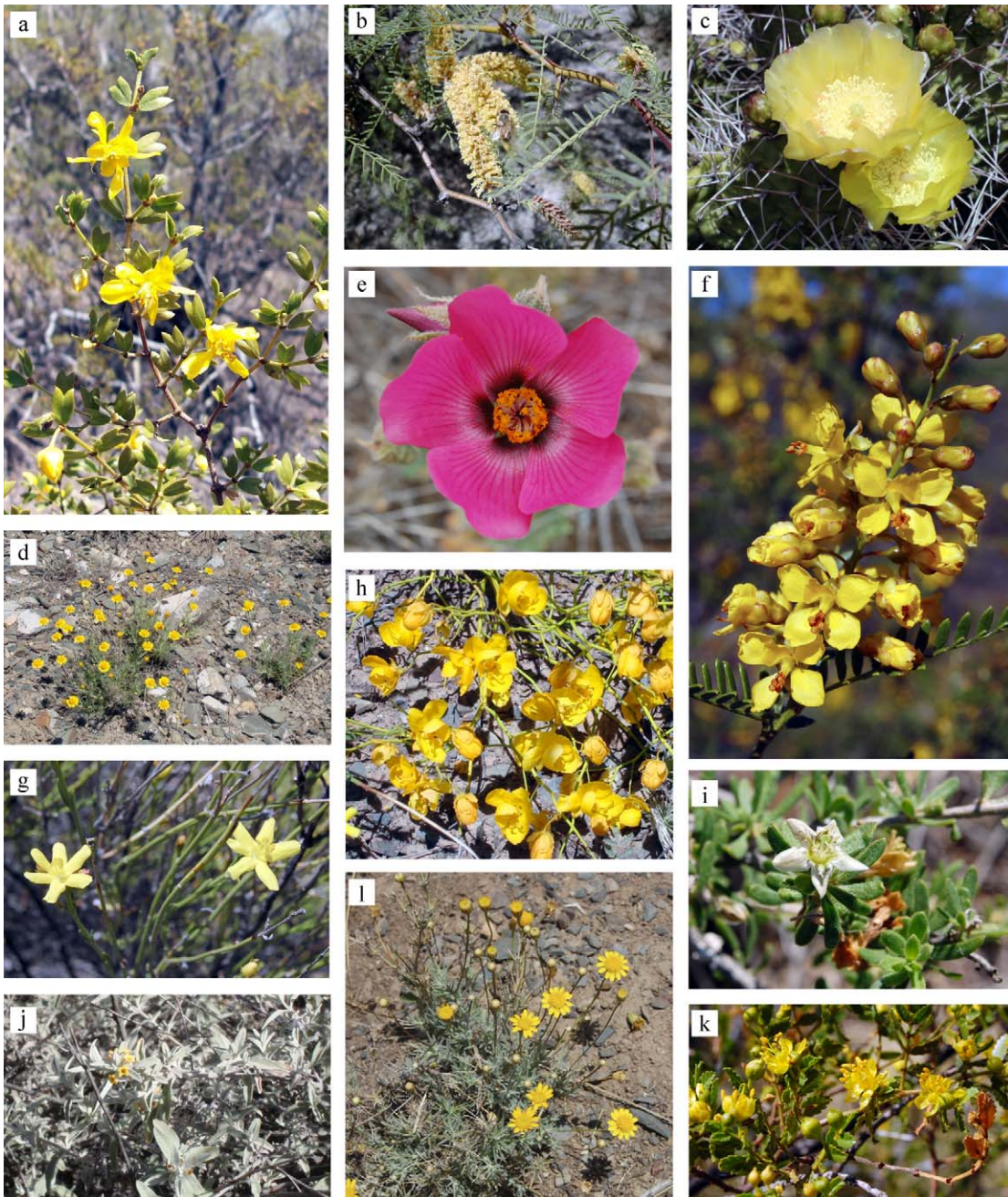


PLATE 1. Plant species used by rare bees that nest in wood holes in the Monte Desert of Villavicencio, Mendoza, Argentina (listed in Table 1). (a) *Larrea divaricata* auct. non Cav., (b) *Prosopis flexuosa* L. C., (c) *Opuntia sulphurea* G. Don in Loudon, (d) *Thymophylla pentachaeta* (DC.) Small (DC.) Strother, (e) *Lecanophora heterophylla* (Cav.) Krapov, (f) *Zuccagnia punctata* Cav., (g) *Menodora decemfida* (Gillies ex Hook. & Arn.) A. Gray, (h) *Senna aphylla* (Cav.) H. S. Irwin & Barney, (i) *Lycium* sp., (j) *Buddleja mendozensis* Benth., (k) *Larrea nitida* Cav., (l) *Helenium donianum* (Hook. & Arn.) Sect. Photo credits: (a, b, c, d, h, and j) N. P. Chacoff; (e, f, g, i, k, and l) D. P. Vázquez.

intensity in our study (and most other plant–pollinator network studies) was positively correlated with plant abundance. However, we believe this is an unlikely explanation, because bees used both abundant and rare plants and the bias occurred in both plant groups (see Table 1). Notice for example that for *L. divaricata*, the

most abundant plant, the visit observations detected only one-half of the interactions recorded in trap nests. Consequently, we think the bias is given by the difficulty of sampling the interactions involving rare pollinator species in the visit observations and not by the distribution of the sampling effort among the plants.

Our study also demonstrates that sampling artifacts become more important with increasing rarity of species, as the visit–trap degree difference increases with decreasing visit observation frequency (Fig. 3). A greater value of the visit–trap degree difference means that the estimation from visits is biased toward specialization because of lack of information compared to the estimation from trap nests. Although we worked with a small number of bees that nest in wood holes, the same could happen for other rare species in the network. Thus, as others have cautioned before (Blüthgen et al. 2006, 2008, Petanidou et al. 2008), defining specialization as species degree could be highly misleading.

The simulation using improved data showed that the type of sampling artifact considered here may exert strong influences on overall network structure, as connectance, mean degree, and nestedness increased by >50% when assuming the same frequency-dependent sampling artifact operated for all species in the network. This assumption is surely questionable, as the different insect groups of the network have different life histories and morphological, behavioral, and physiological constraints from the observed bees. This caveat notwithstanding, the previous assumption allowed us to conduct this simulation as an exercise to provide a rough estimate of the magnitude and the direction of the changes in network parameters resulting from sampling biases. It is noteworthy that the changes in the network parameters in our simulation have the same trend found by Bosch et al. (2009) when comparing networks constructed with visitation and pollen transport data.

In summary, our results indicate that a sampling artifact is likely to pervade the conclusions we make about the structure of plant–animal mutualistic networks. This conclusion underscores the need to apply methods to remove these artifacts. Increased natural history information such as that provided by trap nests is an invaluable tool to achieve this goal.

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