

Sampling networks of ecological interactions

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Summary

1. Sampling ecological interactions presents similar challenges, problems, potential biases, and constraints as sampling individuals and species in biodiversity inventories. Robust estimates of the actual number of interactions (links) within diversified ecological networks require adequate sampling effort that needs to be explicitly gauged. Yet we still lack a sampling theory explicitly focusing on ecological interactions.
2. While the complete inventory of interactions is likely impossible, a robust characterization of its main patterns and metrics is probably realistic. We

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must acknowledge that a sizeable fraction of the maximum number of interactions I_{max} among, say, A animal species and P plant species (i.e., $I_{max} = AP$) is impossible to record due to forbidden links, i.e., life-history restrictions. Thus, the number of observed interactions I in robustly sampled networks is typically $I \ll I_{max}$, resulting in sparse interaction matrices with low connectance.

3. Here I provide a review and outline a conceptual framework for interaction sampling by building an explicit analogue to individuals and species sampling, thus extending diversity-monitoring approaches to the characterization of complex networks of ecological interactions. Contrary to species inventories, a sizable fraction of non-observed pairwise interactions cannot be sampled, due to biological constraints that forbid their occurrence.
4. Reasons for forbidden links are multiple but mainly stem from spatial and temporal uncoupling, size mismatches, and intrinsically low probabilities of interspecific encounter for most potential interactions of partner species. Adequately assessing the completeness of a network of ecological interactions thus needs knowledge of the natural history details embedded, so that forbidden links can be accounted for when addressing sampling effort.
5. Recent implementations of inference methods for unobserved species or for individual-based data can be combined with the assessment of forbidden links. This can help in estimating their relative importance, simply by the difference between the asymptotic estimate of interaction richness *in a robustly-sampled* assemblage and the maximum richness I_{max} of interactions. This is crucial to assess the rapid and devastating effects of defauna-

driven loss of key ecological interactions and the services they provide and the analogous losses related to interaction gains due to invasive species and biotic homogenization.

Keywords

complex networks, food webs, frugivory, mutualism, plant-animal interactions, pollination, seed dispersal

Introduction

Biodiversity sampling is a labour-intensive activity, and sampling is often not sufficient to detect all or even most of the species present in an assemblage. Gotelli & Colwell (2011).

1 Biodiversity species assessment aims at sampling individuals in collections and
2 determining the number of species represented. Given that, by definition, samples
3 are incomplete, these collections do not enumerate the species actually present.
4 The ecological literature dealing with robust estimators of species richness and di-
5 versity in collections of individuals is immense, and a number of useful approaches
6 have been used to obtain such estimates (Magurran, 1988; Gotelli & Colwell, 2001;
7 Colwell *et al.*, 2004; Hortal *et al.*, 2006; Colwell, 2009; Gotelli & Colwell, 2011; Chao
8 *et al.*, 2014). Recent effort has been also focused at defining essential biodiversity
9 variables (EBV) (Pereira *et al.*, 2013) that can be sampled and measured repeat-
10 edly to complement biodiversity estimates. Yet sampling species or taxa-specific

EBVs is just probing a single component of biodiversity; interactions among species are another fundamental component, one that supports the existence, but in some cases also the extinction, of species. For example, the extinction of interactions represents a dramatic loss of biodiversity because it entails the loss of fundamental ecological functions (Valiente-Banuet *et al.*, 2014). This missed component of biodiversity loss, the extinction of ecological interactions, very often accompanies, or even precedes, species disappearance. Interactions among species are a key component of biodiversity and here I aim to show that most problems associated with sampling interactions in natural communities relate to, and are even worse than, problems associated with sampling species diversity. I consider pairwise interactions among species at the habitat level, in the context of alpha diversity and the estimation of local interaction richness from sampling data (Chao *et al.*, 2014). In the first part I provide a succinct overview of previous work addressing sampling issues for ecological interaction networks. In the second part, I discuss specific rationales for sampling the biodiversity of ecological interactions. Finally, I provide a short overview of asymptotic diversity estimates (Gotelli & Colwell, 2001), and a discussion of its application to interaction sampling. Most of the examples come from the analysis of plant-animal interaction networks, yet are applicable to other types of interspecific interactions.

Interactions can be a much better indicator of the richness and diversity of ecosystem functions than a simple list of taxa and their abundances and/or related biodiversity indicator variables (EBVs) (Memmott *et al.*, 2006; Valiente-Banuet *et al.*, 2014). Thus, sampling interactions should be a central issue when identifying and diagnosing ecosystem services (e.g., pollination, seeding by frugivores, etc.). Fortunately, the whole battery of biodiversity-related tools used

by ecologists to sample biodiversity (species, *sensu stricto*) can be extended and applied to the sampling of interactions (see Table 2 in Colwell *et al.*, 2004). Monitoring interactions is a type of biodiversity sampling and is subject to similar methodological shortcomings, especially under-sampling (Jordano, 1987; Jordano *et al.*, 2009; Coddington *et al.*, 2009; Vázquez *et al.*, 2009; Dorado *et al.*, 2011; Rivera-Hutinel *et al.*, 2012). For example, when we study mutualistic networks, our goal is to make an inventory of the distinct pairwise interactions that made up the network. We are interested in having a complete list of all the pairwise interactions among species (e.g., all the distinct, species-species interactions, or links, among the pollinators and flowering plants) that do actually exist in a given community. Sampling these interactions thus entails exactly the same problems, limitations, constraints, and potential biases as sampling individual organisms and species diversity. As Mao & Colwell (2005) put it, these are the workings of Preston’s demon, the moving “veil line” (Preston, 1948) between the detected and the undetected interactions as sample size increases.

Early efforts to recognize and solve sampling problems in analyses of interactions stem from research on food webs and to determine how undersampling biases food web metrics (Martinez, 1991; Cohen *et al.*, 1993; Wells & O’Hara, 2012, among others). In addition, the myriad of classic natural history studies documenting animal diets, host-pathogen infection records, plant herbivory records, etc., represent efforts to document interactions occurring in nature. All of them share the problem of sampling incompleteness influencing the patterns and metrics reported. Yet, despite the early recognition that incomplete sampling may seriously bias the analysis of ecological networks (Jordano, 1987), only recent studies have explicitly acknowledged it and attempted to determine its influence (Ollerton

61 & Cranmer, 2002; Nielsen & Bascompte, 2007; Vázquez *et al.*, 2009; Gibson *et al.*,
 62 2011; Olesen *et al.*, 2011; Chacoff *et al.*, 2012; Rivera-Hutinel *et al.*, 2012; Olito &
 63 Fox, 2014; Bascompte & Jordano, 2014; Vizentin-Bugoni *et al.*, 2014, 2016; Frund
 64 *et al.*, 2015). The sampling approaches have been extended to predict patterns of
 65 coextinctions in interaction assemblages (e.g., hosts-parasites) (Colwell *et al.*, 2012).
 66 Most empirical studies provide no indication of sampling effort, implicitly assuming
 67 that the reported network patterns and metrics are robust. Yet recent evidences
 68 point out that number of partner species detected, number of actual links, and
 69 some aggregate statistics describing network patterns, are prone to sampling bias
 70 (Nielsen & Bascompte, 2007; Dorado *et al.*, 2011; Olesen *et al.*, 2011; Chacoff *et al.*,
 71 2012; Rivera-Hutinel *et al.*, 2012; Olito & Fox, 2014; Frund *et al.*, 2015). Most of
 72 these evidences, however, come either from simulation studies (Frund *et al.*, 2015)
 73 or from relatively species-poor assemblages. Most certainly, sampling limitations
 74 pervade biodiversity inventories in tropical areas (Coddington *et al.*, 2009) and
 75 we might rightly expect that frequent interactions may be over-represented and
 76 rare interactions may be missed entirely in studies of mega-diverse assemblages
 77 (Bascompte & Jordano, 2014); but, to what extent?

78 Sampling interactions: methods

79 When we sample interactions in the field we record the presence of two species
 80 that interact in some way. For example, Snow and Snow(1988) recorded an inter-
 81 action whenever they saw a bird “touching” a fruit on a plant. We observe and
 82 record feeding observations, visitation, occupancy, presence in pollen loads or in
 83 fecal samples, etc., of *individual* animals or plants and accumulate pairwise inter-

actions, i.e., lists of species partners and the frequencies with which we observe them. We assume that the matrix (species numbers) is predefined (i.e., all species interacting are well documented). Therefore, estimating the sampling completeness of pairwise interactions for a whole network, requires some gauging of how the number (richness) of distinct pairwise interactions accumulates as sampling effort is increased) and/or estimating the uncertainty around the missed links (Wells & O’Hara, 2012).

Most types of ecological interactions can be illustrated with bipartite graphs, with two or more distinct groups of interacting partners (Bascompte & Jordano, 2014); for illustration purposes I’ll focus more specifically on plant-animal interactions. Sampling interactions requires filling the cells of an interaction matrix with data. The matrix, $\Delta = AP$ (the adjacency matrix for the graph representation of the network), is a 2D inventory of the interactions among, say, A animal species (rows) and P plant species (columns) (Jordano, 1987; Bascompte & Jordano, 2014). The matrix entries illustrate the values of the pairwise interactions visualized in the Δ matrix, and can be 0 or 1, for presence-absence of a given pairwise interaction, or take a quantitative weight w_{ji} to represent the interaction intensity or unidirectional effect of species j on species i (Bascompte & Jordano, 2014; Vazquez *et al.*, 2015). The outcomes of most ecological interactions are dependent on frequency of encounters (e.g., visit rate of pollinators, number of records of ant defenders, frequency of seeds in fecal samples). Thus, a frequently used proxy for interaction intensities w_{ji} is just how frequent new interspecific encounters are, whether or not appropriately weighted to estimate interaction effectiveness (Vazquez *et al.*, 2005).

We need to define two basic steps in the sampling of interactions: 1) which

109 type of interactions we sample; and 2) which type of record we get to document
 110 the existence of an interaction. In step #1 we need to take into account whether
 111 we are sampling the whole community of interactor species (all the animals, all
 112 the plants) or just a subset of them, i.e., a sub matrix $\Delta_{m,n}$ of $m < A$ animal
 113 species and $n < P$ plant species of the adjacency matrix Δ_{AP} (i.e., the matrix
 114 representation of interactions among the partner species). Subsets can be: a) all
 115 the potential plants interacting with a subset of the animals (Fig. 1a); b) all the
 116 potential animal species interacting with a subset of the plant species (Fig. 1b);
 117 c) a subset of all the potential animal species interacting with a subset of all the
 118 plant species (Fig. 1c). While some discussion has considered how to establish
 119 the limits of what represents a network (Strogatz, 2001) (in analogy to discussion
 120 on food-web limits; Cohen, 1978), it must be noted that situations a-c in Fig. 1
 121 do not represent complete interaction networks. Subnet sampling is generalized
 122 in studies of biological networks (e.g., protein interactions, gene regulation), yet
 123 it is important to recognize that most properties of subnetworks (even random
 124 subsamples) do not represent properties of whole networks (Stumpf *et al.*, 2005).

125 In step #2 above we face the problem of the type of record we take to sample
 126 interactions. This is important because it defines whether we approach the problem
 127 of filling up the interaction matrix in a “zoo-centric” way or in a “phyto-centric”
 128 way. Zoo-centric studies directly sample animal activity and document the plants
 129 ‘touched’ by the animal. For example, analysis of pollen samples recovered from the
 130 body of pollinators, analysis of fecal samples of frugivores, radio-tracking data, etc.
 131 Phyto-centric studies take samples of focal individual plant species and document
 132 which animals ‘arrive’ or ‘touch’ the plants. Examples include focal watches of
 133 fruiting or flowering plants to record visitation by animals, raising insect herbivores

from seed samples, identifying herbivory marks in samples of leaves, etc.

Most recent analyses of plant-animal interaction networks are phyto-centric; just 3.5% of available plant-pollinator ($N=58$) or 36.6% plant-frugivore ($N=22$) interaction datasets are zoo-centric (see Schleuning *et al.*, 2012). Moreover, most available datasets on host-parasite (parasitoid) or plant-herbivore interactions are “host-centric” or phyto-centric (e.g., Thébault & Fontaine, 2010; Morris *et al.*, 2013; Eklöf *et al.*, 2013). This may be related to a variety of causes, like preferred methodologies by researchers working with a particular group or system, logistic limitations, or inherent taxonomic focus of the research questions. A likely result of phyto-centric sampling would be adjacency matrices with large $A : P$ ratios. In contrast, zoo-centric samplings might be prone to detect plants from outside the habitat, complicating the definition of network boundaries. In any case we don’t have a clear view of the potential biases that taxa-focused sampling may generate in observed network patterns, for example by generating consistently asymmetric interaction matrices (Dormann *et al.*, 2009).

Reasonably complete analyses of interaction networks can be obtained when combining both phyto-centric and zoo-centric sampling. For example, Bosch *et al.* (2009) showed that the addition of pollen load data on top of focal-plant sampling of pollinators unveiled a significant number of interactions, resulting in important network structural changes. Olesen *et al.* (2011) identified pollen loads on sampled insects and added the new links to an observation-based visitation matrix, with an extra 5% of links representing the estimated number of missing links in the pollination network. The overlap between observational and pollen-load recorded links was only 33%, underscoring the value of combining methodological approaches. Zoo-centric sampling has recently been extended with the use

159 of DNA-barcoding, for example with plant-herbivore (Jurado-Rivera *et al.*, 2009),
 160 host-parasitoid (Wirta *et al.*, 2014), and plant-frugivore interactions (González-
 161 Varo *et al.*, 2014). For mutualistic networks we would expect that zoo-centric
 162 sampling could help unveiling interactions of the animals with rare plant species
 163 or for relatively common plants species which are difficult to sample by direct ob-
 164 servation. Future methodological work may provide significant advances showing
 165 how mixing different sampling strategies strengthens the completeness of network
 166 data. These mixed strategies may combine, for instance, timed watches at focal
 167 plants, spot censuses along walked transects, pollen load or seed contents analy-
 168 ses, monitoring with camera traps, and DNA barcoding records. However, there
 169 are no tested protocols and/or sampling designs for ecological interaction studies
 170 to suggest an optimum combination of approaches. Ideally, pilot studies would
 171 provide adequate information for each specific study setting.

172 Sampling interactions: rationale

173 The number of distinct pairwise interactions that we can record in a landscape (an
 174 area of relatively homogeneous vegetation) is equivalent to the number of distinct
 175 classes in which we can classify the recorded encounters among *individuals* of
 176 two different species. Yet, individual-based interaction networks have been only
 177 recently studied (Dupont *et al.*, 2011; Wells & O'Hara, 2012). The most usual
 178 approach has been to pool individual-based interaction data into species-based
 179 summaries, an approach that ignores the fact that only a fraction of individuals
 180 may actually interact given a per capita interaction effect (Wells & O'Hara, 2012).
 181 Wells & O'Hara (2012) illustrate the pros and cons of the approach. We walk in

the forest and see a blackbird Tm picking an ivy Hh fruit and ingesting it: we have a record for $Tm - Hh$ interaction. We keep advancing and record again a blackbird feeding on hawthorn Cm fruits so we record a $Tm - Cm$ interaction; as we advance we encounter another ivy plant and record a blackcap swallowing a fruit so we now have a new $Sa - Hh$ interaction, and so on. At the end we have a series of classes (e.g., $Sa - Hh$, $Tm - Hh$, $Tm - Cm$, etc.), along with their observed frequencies.

We get a vector $c = [c_1 \dots c_n]'$ where c_j is the number of classes represented j times in our sampling: c_1 is the number of singletons (interactions recorded once), c_2 is the number of twin pairs (interactions with just two records), c_3 the number of triplets, etc. The problem thus turns to be estimating the number of distinct classes C from the vector of c_j values and the frequency of unobserved interactions (see “The real missing links” below).

More specifically, we usually obtain a type of reference sample (Chao *et al.*, 2014) for interactions: a series of repeated samples (e.g., observation days, 1h watches, etc.) with quantitative information, i.e., recording the number of instances of each interaction type on each day. This replicated abundance data, can be treated in three ways: 1) Abundance data within replicates: the counts of interactions, separately for each day; 2) Pooled abundance data: the counts of interactions, summed over all days (the most usual approach); and 3) Replicated incidence data: the number of days on which we recorded each interaction. Assuming a reasonable number of replicates, replicated incidence data is considered to be the most robust statistically, as it takes account of heterogeneity among days (Colwell *et al.*, 2004, 2012; Chao *et al.*, 2014). Thus, both presence-absence and weighted information on interactions can be accommodated for this purpose.

207 The species assemblage

208 When we consider an observed and recorded sample of interactions on a particular
 209 assemblage of A_{obs} and P_{obs} species (or a set of replicated samples) as a reference
 210 sample (Chao *et al.*, 2014) we may have three sources of undersampling error.
 211 These sources are ignored if we treat the reference sample as a true representation
 212 of the interactions in a well-defined assemblage: 1) some animal species are actually
 213 present but not observed (zero abundance or incidence in the interactions in the
 214 reference sample), A_0 ; 2) some plant species are actually present but not observed
 215 (zero abundance or incidence in the interactions in the reference sample), P_0 ; 3)
 216 some unobserved links (the zeroes in the adjacency matrix, UL) may actually
 217 occur but not recorded. Thus a first problem is determining if A_{obs} and P_{obs} truly
 218 represent the actual species richness interacting in the assemblage. To this end
 219 we might use the replicated reference samples to estimate the true number of
 220 interacting animal A_{est} and plant P_{est} species as in traditional diversity estimation
 221 analysis (Chao *et al.*, 2014). If there are no uniques (species seen on only one day),
 222 then A_0 and P_0 will be zero (based on the Chao2 formula), and we have A_{obs} and
 223 P_{obs} as robust estimates of the actual species richness of the assemblage. If A_0
 224 and P_0 are not zero they estimate the minimum number of undetected animal and
 225 plant species that can be expected with a sufficiently large number of replicates,
 226 taken from the same assemblage/locality by the same methods in the same time
 227 period. We can use extrapolation methods (Colwell *et al.*, 2012) to estimate how
 228 many additional replicate surveys it would take to reach a specified proportion g
 229 of A_{est} and P_{est} .

The interactions

We are then faced with assessing the sampling of interactions I . Table 1 summarizes the main components and targets for estimation of interaction richness. In contrast with traditional species diversity estimates, sampling networks has the paradox that despite the potentially interacting species being present in the sampled assemblage (i.e., included in the A_{obs} and P_{obs} species lists), some of their pairwise interactions are impossible to record. The reason is forbidden links. Independently of whether we sample full communities or subset communities we face a problem: some of the interactions that we can visualize in the empty adjacency matrix Δ will simply not occur. With a total of $A_{obs}P_{obs}$ “potential” interactions (eventually augmented to $A_{est}P_{est}$ in case we have undetected species), a fraction of them are impossible to record, because they are forbidden (Jordano *et al.*, 2003; Olesen *et al.*, 2011).

Our goal is to estimate the true number of non-null AP interactions, including interactions that actually occur but have not been observed (I_0) from the replicated incidence frequencies of interaction types: $I_{est} = I_{obs} + I_0$. Note that I_0 estimates the minimum number of undetected plant-animal interactions that can be expected with a sufficiently large number of replicates, taken from the same assemblage/locality by the same methods in the same time period. Therefore we have two types of non-observed links: UL^* and UL , corresponding to the real assemblage species richness and to the observed assemblage species richness, respectively (Table 1).

Forbidden links are non-occurrences of pairwise interactions that can be accounted for by biological constraints, such as spatio-temporal uncoupling (Jor-

dano, 1987), size or reward mismatching, foraging constraints (e.g., accessibility) (Moré *et al.*, 2012), and physiological-biochemical constraints (Jordano, 1987). We still have very little information about the frequency of forbidden links in natural communities (Jordano *et al.*, 2003; Stang *et al.*, 2009; Vázquez *et al.*, 2009; Olesen *et al.*, 2011; Ibanez, 2012; Maruyama *et al.*, 2014; Vizentin-Bugoni *et al.*, 2014) (Table 1). Forbidden links are thus represented as structural zeroes in the interaction matrix, i.e., matrix cells that cannot get a non-zero value. Therefore, we need to account for the frequency of these structural zeros in our matrix before proceeding.

Our main problem then turns to estimate the number of true missed links, i.e., those that can't be accounted for by biological constraints and that might suggest undersampling. Thus, the sampling of interactions in nature, as the sampling of species, is a cumulative process. In our analysis, we are not re-sampling individuals, but interactions, so we built interaction-based accumulation curves. If an interaction-based curve suggests a robust sampling, it does mean that no new interactions are likely to be recorded, irrespectively of the species, as it is a whole-network sampling approach. We add new, distinct, interactions recorded as we increase sampling effort (Fig. 2). We can obtain an Interaction Accumulation Curve (*IAC*) analogous to a Species Curve (*SAC*) (see Supporting Information in the online data availability repository): the observed number of distinct pairwise interactions in a survey or collection as a function of the accumulated number of observations or samples (Colwell, 2009).

Empirical data on Forbidden Links

Adjacency matrices are frequently sparse, i.e., they are densely populated with zeroes, with a fraction of them being structural (unobservable interactions) (Bascompte & Jordano, 2014). Thus, it would be a serious interpretation error to attribute the sparseness of adjacency matrices for bipartite networks to just the result of undersampling. The actual typology of link types in ecological interaction networks is thus more complex than just the two categories of observed and unobserved interactions (Table 1). Unobserved interactions are represented by zeroes and belong to two categories. Missing interactions may actually exist but require additional sampling or a variety of methods to be observed. Forbidden links, on the other hand, arise due to biological constraints limiting interactions and remain unobservable in nature, irrespectively of sampling effort (Table 1). Forbidden links FL may actually account for a relatively large fraction of unobserved interactions UL when sampling taxonomically-restricted subnetworks (e.g., plant-hummingbird pollination networks) (Table 1). Phenological uncoupling is also prevalent in most networks, and may add up to explain ca. 25-40% of the forbidden links, especially in highly seasonal habitats, and up to 20% when estimated relative to the total number of unobserved interactions (Table 2). In any case, we might expect that a fraction of the missing links ML would be eventually explained by further biological reasons, depending on the knowledge of natural details of the particular systems. Our goal as naturalists would be to reduce the fraction of UL which remain as missing links; to this end we might search for additional biological constraints or increase sampling effort. For instance, habitat use patterns by hummingbirds in the Arima Valley network (Table 2; Snow & Snow, 1972) impose a

300 marked pattern of microhabitat mismatches causing up to 44.5% of the forbidden
 301 links. A myriad of biological causes beyond those included as *FL* in Table 1 may
 302 contribute explanations for *UL*: limits of color perception, presence of secondary
 303 metabolites in fruit pulp and leaves, toxins and combinations of monosaccharides
 304 in nectar, etc. For example, aside from *FL*, some pairwise interactions may sim-
 305 ply have an asymptotically-zero probability of interspecific encounter between the
 306 partner species, if they are very rare. However, it is surprising that just the limited
 307 set of forbidden link types considered in Table 1 explain between 24.6-77.2% of
 308 the unobserved links. Notably, the Arima Valley, Santa Virgínia, and Hato Ratón
 309 networks have $> 60\%$ of the unobserved links explained, which might be related
 310 to the fact that they are subnetworks (Arima Valley, Santa Virgínia) or relatively
 311 small networks (Hato Ratón). All this means that empirical networks may have
 312 sizable fractions of structural zeroes. Ignoring this biological fact may contribute
 313 to wrongly inferring undersampling of interactions in real-world assemblages.

314 To sum up, two elements of inference are required in the analysis of unobserved
 315 interactions in ecological interaction networks: first, detailed natural history infor-
 316 mation on the participant species that allows the inference of biological constraints
 317 imposing forbidden links, so that structural zeroes can be identified in the adja-
 318 cency matrix. Second, a critical analysis of sampling robustness and a robust
 319 estimate of the actual fraction of missing links, M , resulting in a robust estimate
 320 of I . In the next sections we explore these elements of inference, using *IACs* as
 321 analogs to *SACs* to assess the robustness of interaction sampling.

Assessing sampling effort when recording interactions: asymptotic diversity estimates

A plot of the cumulative number of species recorded, S_n , as a function of some measure of sampling effort (say, n samples taken) yields the species accumulation curve (SAC) or collector's curve (Colwell & Coddington, 1994). Similarly, interaction accumulation curves (IAC), analogous to SACs (Gotelli & Colwell, 2001; Hortal *et al.*, 2006; Chao, 2005; Colwell, 2013), can be used to assess the robustness of interactions sampling for plant-animal community datasets (Jordano, 1987; Jordano *et al.*, 2009; Olesen *et al.*, 2011).

The basic method to estimate sampling effort and explicitly show the analogues with rarefaction analysis in biodiversity research is to vectorize the interaction matrix AP so that we get a vector of all the potential pairwise interactions (I_{max} , Table 1) that can occur in the observed assemblage with A_{obs} animal species and P_{obs} plant species. The new “species” we aim to sample are the pairwise interactions (Table 3), as previously discussed. In general, if we have $A = 1...i$, animal species and $P = 1...j$ plant species (assuming a complete list of species in the assemblage), we'll have a vector of “new” species to sample: $A_1P_1, A_1P_2, ...A_2P_1, A_2P_2, ...A_iP_j$. We can represent the successive samples where we can potentially get records of these interactions in a matrix with the vectorized interaction matrix and columns representing the successive samples we take (Table 3). This is simply a vectorized version of the interaction matrix. This is analogous to a biodiversity sampling matrix with species as rows and sampling units (e.g., quadrats) as columns (Jordano *et al.*, 2009). The package *EstimateS* (Colwell, 2013) includes a complete set of functions for estimating the mean IAC and its unconditional standard deviation.

tion from random permutations of the data, or subsampling without replacement (Gotelli & Colwell, 2001); it further reports asymptotic estimators for the expected number of distinct pairwise interactions included in a given reference sample of interaction records (see also the `specaccum` function in library `vegan` of the R Package)(R Development Core Team, 2010; Jordano *et al.*, 2009; Olesen *et al.*, 2011). In particular, we may take advantage of replicated incidence data, as it takes account of heterogeneity among samples (days, censuses, etc.; R.K Colwell, pers. comm.) (see also Colwell *et al.*, 2004, 2012; Chao *et al.*, 2014). Future theoretical work will be needed to formally assess the similarities and differences between the species vs. interactions sampling approaches and developing biologically meaningful null models of expected interaction richness with added sampling effort.

Diversity-accumulation analysis (Magurran, 1988; Hortal *et al.*, 2006) comes up immediately with this type of dataset. This procedure plots the accumulation curve for the expected number of distinct pairwise interactions recorded with increasing sampling effort (Jordano *et al.*, 2009; Olesen *et al.*, 2011). Asymptotic estimates of interaction richness and its associated standard errors and confidence intervals can thus be obtained (Hortal *et al.*, 2006) (see Table 4 and Supplementary Online Material). The characteristic feature of interaction datasets is that, due to forbidden links, a number of pairwise interactions among the I_{max} number specified in the Δ adjacency matrix cannot be recorded, irrespective of sampling effort.

We may expect undersampling specially in moderate to large sized networks with multiple modules (i.e., species subsets requiring different sampling strategies) (Jordano, 1987; Olesen *et al.*, 2011; Chacoff *et al.*, 2012); adequate sampling may

be feasible when interaction subwebs are studied (Olesen *et al.*, 2011; Vizentin-Bugoni *et al.*, 2014), typically with more homogeneous subsets of species (e.g., bumblebee-pollinated flowers).

For example, mixture models incorporating detectabilities have been proposed to effectively account for rare species (Mao & Colwell, 2005). In an analogous line, mixture models could be extended to samples of pairwise interactions, also with specific detectability values. These detection rate/odds could be variable among groups of interactions, depending on their specific detectability. For example, detectability of flower-pollinator interactions involving bumblebees could have a higher detectability than flower-pollinator pairwise interactions involving, say, nitidulid beetles. These more homogeneous groupings of pairwise interactions within a network define modules (Bascompte & Jordano, 2014), so we might expect that interactions of a given module (e.g., plants and their hummingbird pollinators; Fig. 1a) may share similar detectability values, in an analogous way to species groups receiving homogeneous detectability values in mixture models (Mao & Colwell, 2005). In its simplest form, this would result in a sample with multiple pairwise interactions detected, in which the number of interaction events recorded for each distinct interaction found in the sample is recorded (i.e., a column vector in Table 3, corresponding to, say, a sampling day). The number of interactions recorded for the i_{th} pairwise interaction (i.e., $A_i P_j$ in Table 3), Y_i could be treated as a Poisson random variable with a mean parameter λ_i , its detection rate. Mixture models (Mao & Colwell, 2005) include estimates for abundance-based data (their analogs in interaction sampling would be weighted data), where Y_i is a Poisson random variable with detection rate λ_i . This is combined with the incidence-based model, where Y_i is a binomial random variable (their analogous in interaction sampling

would be presence/absence records of interactions) with detection odds λ_i . Let T be the number of samples in an incidence-based data set. A Poisson/binomial density can be written as (Mao & Colwell, 2005):

$$g(y; \lambda) = \begin{cases} \frac{\lambda^y}{y!e^\lambda} & [1] \\ \binom{T}{y} \frac{\lambda^y}{(1+\lambda)^T} & [2] \end{cases}$$

where [1] corresponds to a weighted network, and [2] to a qualitative network. The detection rates λ_i depend on the relative abundances ϕ_i of the interactions, the probability of a pairwise interaction being detected when it is present, and the sample size (the number of interactions recorded), which, in turn, is a function of the sampling effort. Unfortunately, no specific sampling model has been developed along these lines for species interactions and their characteristic features. For example, a complication factor might be that interaction abundances, ϕ_i , in real assemblages are a function of the abundances of interacting species that determine interspecific encounter rates; yet they also depend on biological factors that ultimately determine if the interaction occurs when the partner species are present. For example, λ_i should be set to zero for all FL . In its simplest form, ϕ_i could be estimated from just the product of partner species abundances, an approach recently used as a null model to assess the role of biological constraints in generating forbidden links and explaining interaction patterns (Vizentin-Bugoni *et al.*, 2014). Yet more complex models (e.g., Wells & O'hara 2012) should incorporate not only interspecific encounter probabilities, but also interaction detectabilities, phenotypic matching and incidence of forbidden links. Mixture models are certainly complex and for most situations of evaluating sampling effort better alternatives

417 include the simpler incidence-based rarefaction and extrapolation (Colwell *et al.*,
418 2012; Chao *et al.*, 2014).

419 The *real* missing links

420 Given that a fraction of unobserved interactions can be accounted for by forbidden
421 links, what about the remaining missing interactions? We have already discussed
422 that some of these could still be related to unaccounted constraints, and still others
423 would be certainly attributable to insufficient sampling. Would this always be the
424 case? A crucial ecological aspect limiting interactions within multispecific assem-
425 blages of distinct taxonomic relatedness (Fig. 2) is the probability of interspecific
426 encounter, i.e., the probability that two individuals of the partner species actually
427 encounter each other in nature.

428 Given log-normally distributed abundances of the two species groups, the ex-
429 pected probabilities of interspecific encounter (*PIE*) would be simply the product
430 of the two lognormal distributions. Thus, we might expect that for very low *PIE*
431 values, pairwise interactions would be either extremely difficult to sample, or sim-
432 ply do not occur in nature. Consider the Nava de las Correhuelas interaction
433 web (NCH, Table 2, 4), with $A = 36$, $P = 25$, $I = 181$, and almost half of the
434 unobserved interactions not accounted for by forbidden links, thus $M = 53.1\%$
435 (Jordano *et al.*, 2009). A sizable fraction of these possible but missing links would
436 be simply not occurring in nature, most likely due to extremely low *PIE*, in fact
437 asymptotically zero. Given the vectorized list of pairwise interactions for NCH, I
438 computed the *PIE* values for each one by multiplying element-wise the two species
439 abundance distributions. The $PIE_{max} = 0.0597$, being a neutral estimate, based

on the assumption that interactions occur in proportion to the species-specific local abundances. With $PIE_{median} < 1.4 \cdot 10^{-4}$ we may safely expect (note the quantile estimate $Q_{75\%} = 3.27 \cdot 10^{-4}$) that a sizable fraction of these missing interactions may not occur according to this neutral expectation (Jordano, 1987; Olesen *et al.*, 2011) (neutral forbidden links, *sensu* Canard *et al.*, 2012).

When we consider the vectorized interaction matrix, enumerating all pairwise interactions for the AP combinations, the expected probabilities of finding a given interaction can be estimated with a Good-Turing approximation (Good, 1953). The technique, developed by Alan Turing and I.J. Good with applications to linguistics and word analysis (Gale & Sampson, 1995) has been recently extended in novel ways for ecological analyses (Chao *et al.*, 2015). In our present context it estimates the probability of recording an interaction of a hitherto unseen pair of partners, given a set of past records of interactions between other species pairs. Let a sample of N interactions so that n_r distinct pairwise interactions have exactly r records. All Good-Turing estimators obtain the underlying frequencies of events as:

$$P(X) = \frac{(N_X + 1)}{T} \left(1 - \frac{E(1)}{T}\right) \quad (1)$$

where X is the pairwise interaction, N_X is the number of times interaction X is recorded, T is the sample size (number of distinct interactions recorded) and $E(1)$ is an estimate of how many different interactions were recorded exactly once. Strictly speaking Equation (1) gives the probability that the next interaction type recorded will be X , after sampling a given assemblage of interacting species. In other words, we scale down the maximum-likelihood estimator $\frac{n}{T}$ by a factor of

462 $\frac{1-E(1)}{T}$. This reduces all the probabilities for interactions we have recorded, and
 463 makes room for interactions we haven't seen. If we sum over the interactions we
 464 have seen, then the sum of $P(X)$ is $1 - \frac{1-E(1)}{T}$. Because probabilities sum to one,
 465 we have the left-over probability of $P_{new} = \frac{E(1)}{T}$ of seeing something new, where
 466 new means that we sample a new pairwise interaction.

467 Discussion

468 Recent work has inferred that most data available for interaction networks are
 469 incomplete due to undersampling, resulting in a variety of biased parameters and
 470 network patterns (Chacoff *et al.*, 2012). It is important to note, however, that in
 471 practice, most surveyed networks to date have been subnets of much larger net-
 472 works. This is also true for protein interaction, gene regulation, and metabolic
 473 networks, where only a subset of the molecular entities in a cell have been sam-
 474 pled (Stumpf *et al.*, 2005). Despite recent attempts to document whole ecosystem
 475 meta-networks (Pocock *et al.*, 2012), it is likely that most ecological interaction
 476 networks will illustrate just major ecosystem compartments. Due to their high
 477 generalization, high temporal and spatial turnover, and high complexity of associ-
 478 ation patterns, adequate sampling of ecological interaction networks is challenging
 479 and requires extremely large sampling effort. Undersampling of ecological net-
 480 works may originate from the analysis of assemblage subsets (e.g., taxonomically
 481 or functionally defined), and/or from logistically-limited sampling effort. It is ex-
 482 tremely hard to robustly sample the set of biotic interactions even for relatively
 483 simple, species-poor assemblages; thus, we need to assess how robust is the char-
 484 acterization of the adjacency matrix Δ . Concluding that an ecological network

dataset is undersampled just by its sparseness would be unrealistic. The reason stems from a biological fact: a sizeable fraction of the maximum, potential links that can be recorded among two distinct sets of species is simply unobservable, irrespective of sampling effort (Jordano, 1987). In addition, sampling effort needs to be explicitly gauged because of its potential influence on parameter estimates for the network.

Missing links are a characteristic feature of all plant-animal interaction networks, and likely pervade other ecological interactions. Important natural history details explain a fraction of them, resulting in unrealizable interactions (i.e., forbidden interactions) that define structural zeroes in the interaction matrices and contribute to their extreme sparseness. Sampling interactions is a way to monitor biodiversity beyond the simple enumeration of component species and to develop efficient and robust inventories of functional interactions. Yet no sampling theory for interactions is available. Focusing just on the realized interactions or treating missing interactions as the expected unique result of sampling bias would miss important components to understand how all sorts of interactions coevolve within complex webs of interdependence among species.

Contrary to species inventories, a sizable fraction of non-observed pairwise interactions cannot be sampled, due to biological constraints that forbid their occurrence. Moreover, recent implementations of inference methods for unobserved species (Chao *et al.*, 2015) or for individual-based data (Wells & O'Hara, 2012) can be combined with the forbidden link approach. They do not account either for the existence of these ecological constraints, but can help in estimating their relative importance, simply by the difference between the asymptotic estimate of interaction richness *in a robustly-sampled* assemblage and the maximum richness

I_{max} of interactions.

Ecological interactions provide the wireframe supporting the lives of species, and they also embed crucial ecosystem functions which are fundamental for supporting the Earth system. We still have a limited knowledge of the biodiversity of ecological interactions, and they are being lost (extinct) at a very fast pace, frequently preceding species extinctions (Valiente-Banuet *et al.*, 2014). We urgently need robust techniques to assess the completeness of ecological interactions networks because this knowledge will allow the identification of the minimal components of their ecological complexity that need to be restored to rebuild functional ecosystems after perturbations.

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Data accessibility

This review does not use new raw data, but includes some re-analyses of previously published material. All the original data supporting the paper, R code, supplementary figures, and summaries of analytical protocols is available at the author's GitHub repository (https://github.com/pedroj/MS_Network-Sampling), with DOI: 10.5281/zenodo.29437.

References

- Bascompte, J. & Jordano, P. (2014) *Mutualistic networks*. Monographs in Population Biology, No. 53, Princeton University Press, Princeton, NJ.
- Bosch, J., Martín González, A.M., Rodrigo, A. & Navarro, D. (2009) Plant-pollinator networks: adding the pollinator's perspective. *Ecology Letters* **12**, 409–419.
- Canard, E., Mouquet, N., Marescot, L., Gaston, K.J., Gravel, D. & Mouillot, D. (2012) Emergence of structural patterns in neutral trophic networks. *PLoS ONE* **7**, e38295.
- Chacoff, N.P., Vazquez, D.P., Lomascolo, S.B., Stevani, E.L., Dorado, J. & Padrón, B. (2012) Evaluating sampling completeness in a desert plant-pollinator network. *Journal of Animal Ecology* **81**, 190–200.

- 553 Chao, A. (2005) Species richness estimation. *Encyclopedia of Statistical Sciences*,
554 pp. 7909–7916, Oxford University Press, New York, USA.
- 555 Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K. & Elli-
556 son, A.M. (2014) Rarefaction and extrapolation with Hill numbers: a framework
557 for sampling and estimation in species diversity studies. *Ecological Monographs*
558 **84**, 45–67.
- 559 Chao, A., Hsieh, T.C., Chazdon, R.L., Colwell, R.K. & Gotelli, N.J. (2015) Un-
560 veiling the species-rank abundance distribution by generalizing the Good-Turing
561 sample coverage theory. *Ecology* **96**, 1189–1201.
- 562 Coddington, J.A., Agnarsson, I., Miller, J.A., Kuntner, M. & Hormiga, G. (2009)
563 Undersampling bias: the null hypothesis for singleton species in tropical arthro-
564 pod surveys. *Journal of Animal Ecology* **78**, 573–584.
- 565 Cohen, J.E. (1978) *Food webs and niche space*. Princeton University Press, Prince-
566 ton, New Jersey, US.
- 567 Cohen, J.E., Beaver, R.A., Cousins, S.H., DeAngelis, D.L., Goldwasser, L., Heong,
568 K.L., Holt, R.D., Kohn, A.J., Lawton, J.H., Martinez, N., O'Malley, R., Page,
569 L.M., Patten, B.C., Pimm, S.L., Polis, G., Rejmanek, M., Schoener, T.W.,
570 Schoenly, K., Sprules, W.G., Teal, J.M., Ulanowicz, R., Warren, P.H., Wilbur,
571 H.M. & Yodzis, P. (1993) Improving Food Webs. *Ecology* **74**, 252–258.
- 572 Colwell, R. & Coddington, J. (1994) Estimating terrestrial biodiversity through ex-
573 trapolation. *Philosophical Transactions Of The Royal Society Of London Series*
574 *B-Biological Sciences* **345**, 101–118.

- 575 Colwell, R.K. (2009) Biodiversity: concepts, patterns, and measurement. *The*
 576 *Princeton Guide to Ecology* (ed. S.A. Levin), pp. 257–263, Princeton Univer-
 577 sity Press, Princeton.
- 578 Colwell, R.K. (2013) EstimateS: Biodiversity Estimation. - pp. 1–33.
- 579 Colwell, R.K., Dunn, R.R. & Harris, N.C. (2012) Coextinction and persistence of
 580 dependent species in a changing world. *Annual Review of Ecology Evolution and*
 581 *Systematics* **43**, 183–203.
- 582 Colwell, R.K., Mao, C.X. & Chang, J. (2004) Interpolating, extrapolating, and
 583 comparing incidence-based species accumulation curves. *Ecology* **85**, 2717–2727.
- 584 Dorado, J., Vazquez, D.P., Stevani, E.L. & Chacoff, N.P. (2011) Rareness and
 585 specialization in plant-pollinator networks. *Ecology* **92**, 19–25.
- 586 Dormann, C.F., Frund, J., Bluthgen, N. & Gruber, B. (2009) Indices, graphs and
 587 null models: Analyzing bipartite ecological networks. *Open Ecology Journal* **2**,
 588 7–24.
- 589 Dupont, Y.L., Trøjelsgaard, K., Hagen, M., Henriksen, M.V., Olesen, J.M., Ped-
 590 ersen, N.M.E. & Kissling, W.D. (2014) Spatial structure of an individual-based
 591 plant-pollinator network. *Oikos* **123**, 1301–1310.
- 592 Dupont, Y.L., Trøjelsgaard, K. & Olesen, J.M. (2011) Scaling down from species
 593 to individuals: a flower–visitation network between individual honeybees and
 594 thistle plants. *Oikos* **120**, 170–177.
- 595 Eklöf, A., Jacob, U., Kopp, J., Bosch, J., Castro-Urgal, R., Chacoff, N.P.,
 596 Dalsgaard, B., de Sassi, C., Galetti, M., Guimaraes, P.R., Lomáscolo, S.B.,

- 597 Martín González, A.M., Pizo, M.A., Rader, R., Rodrigo, A., Tylianakis, J.M.,
598 Vazquez, D.P. & Allesina, S. (2013) The dimensionality of ecological networks.
599 *Ecology Letters* **16**, 577–583.
- 600 Frund, J., McCann, K.S. & Williams, N.M. (2015) Sampling bias is a challenge
601 for quantifying specialization and network structure: lessons from a quantitative
602 niche model. *Oikos* pp. n/a–n/a.
- 603 Gale, W.A. & Sampson, G. (1995) Good-Turing frequency estimation without
604 tears. *Journal of Quantitative Linguistics* **2**, 217–237.
- 605 Gibson, R.H., Knott, B., Eberlein, T. & Memmott, J. (2011) Sampling method
606 influences the structure of plant–pollinator networks. *Oikos* **120**, 822–831.
- 607 González-Varo, J.P., Arroyo, J.M. & Jordano, P. (2014) Who dispersed the seeds?
608 The use of DNA barcoding in frugivory and seed dispersal studies. *Methods in*
609 *Ecology and Evolution* **5**, 806–814.
- 610 Good, I.J. (1953) The population frequencies of species and the estimation of
611 population parameters. *Biometrika* **40**, 237–264.
- 612 Gotelli, N. & Colwell, R. (2001) Quantifying biodiversity: procedures and pitfalls
613 in the measurement and comparison of species richness. *Ecology Letters* **4**, 379–
614 391.
- 615 Gotelli, N.J. & Colwell, R.K. (2011) Estimating species richness. *Biological Di-*
616 *versity Frontiers in Measurement and Assessment* (eds. A.E. Magurran & B.J.
617 McGill), pp. 39–54, Oxford University Press, Oxford, UK.

- 618 Hortal, J., Borges, P. & Gaspar, C. (2006) Evaluating the performance of species
619 richness estimators: sensitivity to sample grain size. *Journal of Animal Ecology*
620 **75**, 274–287.
- 621 Ibanez, S. (2012) Optimizing size thresholds in a plant–pollinator interaction web:
622 towards a mechanistic understanding of ecological networks. *Oecologia* **170**, 233–
623 242.
- 624 Jordano, P. (1987) Patterns of mutualistic interactions in pollination and seed dis-
625 persal: connectance, dependence asymmetries, and coevolution. *The American*
626 *Naturalist* **129**, 657–677.
- 627 Jordano, P., Bascompte, J. & Olesen, J. (2003) Invariant properties in coevolu-
628 tionary networks of plant-animal interactions. *Ecology Letters* **6**, 69–81.
- 629 Jordano, P., Vázquez, D. & Bascompte, J. (2009) Redes complejas de interac-
630 ciones planta—animal. *Ecología y evolución de interacciones planta-animal* (eds.
631 R. Medel, R. Dirzo & R. Zamora), pp. 17–41, Editorial Universitaria, Santiago,
632 Chile.
- 633 Jurado-Rivera, J.A., Vogler, A.P., Reid, C.A.M., Petitpierre, E. & Gomez-Zurita,
634 J. (2009) DNA barcoding insect-host plant associations. *Proceedings Of The*
635 *Royal Society B-Biological Sciences* **276**, 639–648.
- 636 Magurran, A. (1988) *Ecological diversity and its measurement*. Princeton Univer-
637 sity Press, Princeton, US.
- 638 Mao, C. & Colwell, R.K. (2005) Estimation of species richness: mixture models,
639 the role of rare species, and inferential challenges. *Ecology* **86**, 1143–1153.

- 640 Martinez, N. (1991) Artifacts or attributes? Effects of resolution on food-web
641 patterns in Little Rock Lake food web. *Ecological Monographs* **61**, 367–392.
- 642 Maruyama, P.K., Vizentin-Bugoni, J., Oliveira, G.M., Oliveira, P.E. & Dalsgaard,
643 B. (2014) Morphological and spatio-temporal mismatches shape a neotropical
644 savanna plant-hummingbird network. *Biotropica* **46**, 740–747.
- 645 Memmott, J., Alonso, D., Berlow, E., Dobson, A., Dunne, J.A., Solé, R.V. &
646 Weitz, J.S. (2006) Biodiversity loss and ecological network structure. *Ecological*
647 *Networks Linking Structure to Dynamics in Food Webs* (eds. M. Pascual & J.A.
648 Dunne), pp. 325–347, Oxford University Press, Oxford, UK.
- 649 Moré, M., Amorim, F.W., Benitez-Vieyra, S., Medina, A.M., Sazima, M. &
650 Cocucci, A.A. (2012) Armament Imbalances: Match and Mismatch in Plant-
651 Pollinator Traits of Highly Specialized Long-Spurred Orchids. *PLoS ONE* **7**,
652 e41878.
- 653 Morris, R.J., Gripenberg, S., Lewis, O.T. & Roslin, T. (2013) Antagonistic inter-
654 action networks are structured independently of latitude and host guild. *Ecology*
655 *Letters* **17**, 340–349.
- 656 Nielsen, A. & Bascompte, J. (2007) Ecological networks, nestedness and sampling
657 effort. *Journal of Ecology* **95**, 1134–1141–1141.
- 658 Olesen, J.M., Bascompte, J., Dupont, Y.L., Elberling, H. & Jordano, P. (2011)
659 Missing and forbidden links in mutualistic networks. *Proceedings Of The Royal*
660 *Society B-Biological Sciences* **278**, 725–732.
- 661 Olito, C. & Fox, J.W. (2014) Species traits and abundances predict metrics of

- 662 plant-pollinator network structure, but not pairwise interactions. *Oikos* **124**,
663 428–436.
- 664 Ollerton, J. & Cranmer, L. (2002) Latitudinal trends in plant-pollinator interac-
665 tions: are tropical plants more specialised? *Oikos* **98**, 340–350.
- 666 Pereira, H.M., Ferrier, S., Walters, M., Geller, G.N., Jongman, R.H.G., Scholes,
667 R.J., Bruford, M.W., Brummitt, N., Butchart, S.H.M., Cardoso, A.C., Coops,
668 N., Dulloo, E., Faith, D., Freyhof, J., Gregory, R.D., Heip, C., Hoft, R., Hurtt,
669 G., Jetz, W., Karp, D.S., Mcgeoch, M., Obura, D., Onoda, Y., Pettorelli, N.,
670 Reyers, B., Sayre, R., Scharlemann, J.P.W., Stuart, S., Turak, E., Walpole, M.
671 & Wegmann, M. (2013) Essential biodiversity variables. *Science* **339**, 277–278.
- 672 Pocock, M.J.O., Evans, D.M. & Memmott, J. (2012) The Robustness and Restora-
673 tion of a Network of Ecological Networks. *Science* **335**, 973–977.
- 674 Preston, F. (1948) The commonness, and rarity, of species. *Ecology* **29**, 254–283.
- 675 R Development Core Team (2010) *R: A language and environment for statis-*
676 *tical computing*. R Foundation for Statistical Computing. Vienna, Austria.
677 <http://www.R-project.org>, Vienna, Austria.
- 678 Rivera-Hutinel, A., Bustamante, R.O., Marín, V.H. & Medel, R. (2012) Effects
679 of sampling completeness on the structure of plant-pollinator networks. *Ecology*
680 **93**, 1593–1603.
- 681 Schleuning, M., Frund, J., Klein, A.M., Abrahamczyk, S., Alarcón, R., Albrecht,
682 M., Andersson, G.K.S., Bazarian, S., Böhning-Gaese, K., Bommarco, R., Dals-
683 gaard, B., Dehling, D.M., Gotlieb, A., Hagen, M., Hickler, T., Holzschuh, A.,

- 684 Kaiser-Bunbury, C.N., Kreft, H., Morris, R.J., Sandel, B., Sutherland, W.J.,
685 Svenning, J.C., Tschardtke, T., Watts, S., Weiner, C.N., Werner, M., Williams,
686 N.M., Winqvist, C., Dormann, C.F. & Blüthgen, N. (2012) Specialization of
687 mutualistic interaction networks decreases toward tropical latitudes. *Current*
688 *Biology* **22**, 1925–1931.
- 689 Snow, B. & Snow, D. (1972) Feeding niches of hummingbirds in a Trinidad valley.
690 *Journal of Animal Ecology* **41**, 471–485.
- 691 Snow, B. & Snow, D. (1988) *Birds and berries*. Poyser, Calton, UK.
- 692 Stang, M., Klinkhamer, P., Waser, N.M., Stang, I. & van der Meijden, E. (2009)
693 Size-specific interaction patterns and size matching in a plant-pollinator inter-
694 action web. *Annals Of Botany* **103**, 1459–1469.
- 695 Strogatz, S. (2001) Exploring complex networks. *Nature* **410**, 268–276.
- 696 Stumpf, M.P.H., Wiuf, C. & May, R.M. (2005) Subnets of scale-free networks
697 are not scale-free: Sampling properties of networks. *Proceedings of the National*
698 *Academy of Sciences USA* **102**, 4221–4224.
- 699 Thébault, E. & Fontaine, C. (2010) Stability of ecological communities and the
700 architecture of mutualistic and trophic networks. *Science* **329**, 853–856.
- 701 Valiente-Banuet, A., Aizen, M.A., Alcántara, J.M., Arroyo, J., Cocucci, A.,
702 Galetti, M., García, M.B., García, D., Gomez, J.M., Jordano, P., Medel, R.,
703 Navarro, L., Obeso, J.R., Oviedo, R., Ramírez, N., Rey, P.J., Traveset, A.,
704 Verdú, M. & Zamora, R. (2014) Beyond species loss: the extinction of ecological
705 interactions in a changing world. *Functional Ecology* **29**, 299–307.

- 706 Vazquez, D., Morris, W. & Jordano, P. (2005) Interaction frequency as a surrogate
707 for the total effect of animal mutualists on plants. *Ecology Letters* **8**, 1088–1094.
- 708 Vázquez, D.P., Chacoff, N.P. & Cagnolo, L. (2009) Evaluating multiple determi-
709 nants of the structure of plant-animal mutualistic networks. *Ecology* **90**, 2039–
710 2046.
- 711 Vazquez, D.P., Ramos-Jiliberto, R., Urbani, P. & Valdovinos, F.S. (2015) A con-
712 ceptual framework for studying the strength of plant-animal mutualistic inter-
713 actions. *Ecology Letters* **18**, 385–400.
- 714 Vizentin-Bugoni, J., Maruyama, P.K., Debastiani, V.J., Duarte, L.d.S., Dalsgaard,
715 B. & Sazima, M. (2016) Influences of sampling effort on detected patterns and
716 structuring processes of a neotropical plant–hummingbird network. *Journal of*
717 *Animal Ecology* **85**, 262–272.
- 718 Vizentin-Bugoni, J., Maruyama, P.K. & Sazima, M. (2014) Processes entangling
719 interactions in communities: forbidden links are more important than abundance
720 in a hummingbird-plant network. *Proceedings Of The Royal Society B-Biological*
721 *Sciences* **281**, 20132397–20132397.
- 722 Wells, K. & O’Hara, R.B. (2012) Species interactions: estimating per-individual
723 interaction strength and covariates before simplifying data into per-species eco-
724 logical networks. *Methods in Ecology and Evolution* **4**, 1–8.
- 725 Wirta, H.K., Hebert, P.D.N., Kaartinen, R., Prosser, S.W., Várkonyi, G. & Roslin,
726 T. (2014) Complementary molecular information changes our perception of food
727 web structure. *Proceedings of the National Academy of Sciences USA* **111**, 1885–
728 1890.

Figure captions

Figure 1. Sampling ecological interaction networks (e.g., plant-animal interactions) usually focus on different types of subsampling the full network, yielding submatrices $\Delta[m, n]$ of the full interaction matrix Δ with A and P animal and plant species. a) all the potential plants interacting with a subset of the animals (e.g., studying just the hummingbird-pollinated flower species in a community); b) all the potential animal species interacting with a subset of the plant species (e.g., studying the frugivore species feeding on figs *Ficus* in a community); and c) sampling a subset of all the potential animal species interacting with a subset of all the plant species (e.g., studying the plant-frugivore interactions of the rainforest understory).

Figure 2. Sampling species interactions in natural communities. Suppose an assemblage with $A = 3$ animal species (red, species 1–3 with three, two, and 1 individuals, respectively) and $P = 3$ plant species (green, species a-c with three individuals each) (colored balls), sampled with increasing effort in steps 1 to 6 (panels). In Step 1 we record animal species 1 and plant species 1 and 2 with a total of three interactions (black lines) represented as two distinct interactions: $1 - a$ and $1 - b$. As we advance our sampling (panels 1 to 6, illustrating e.g., additional sampling days) we record new distinct interactions. Note that we actually sample and record interactions among individuals, yet we pool the data across species to get a species by species interaction matrix. Few network analyses have been carried out on individual data (Dupont *et al.*, 2014). Above and below each panel are the cumulative number of distinct species and interactions sampled, so

753 that panel 6 illustrates the final network.

754

755 **Figures**

Figure 1:

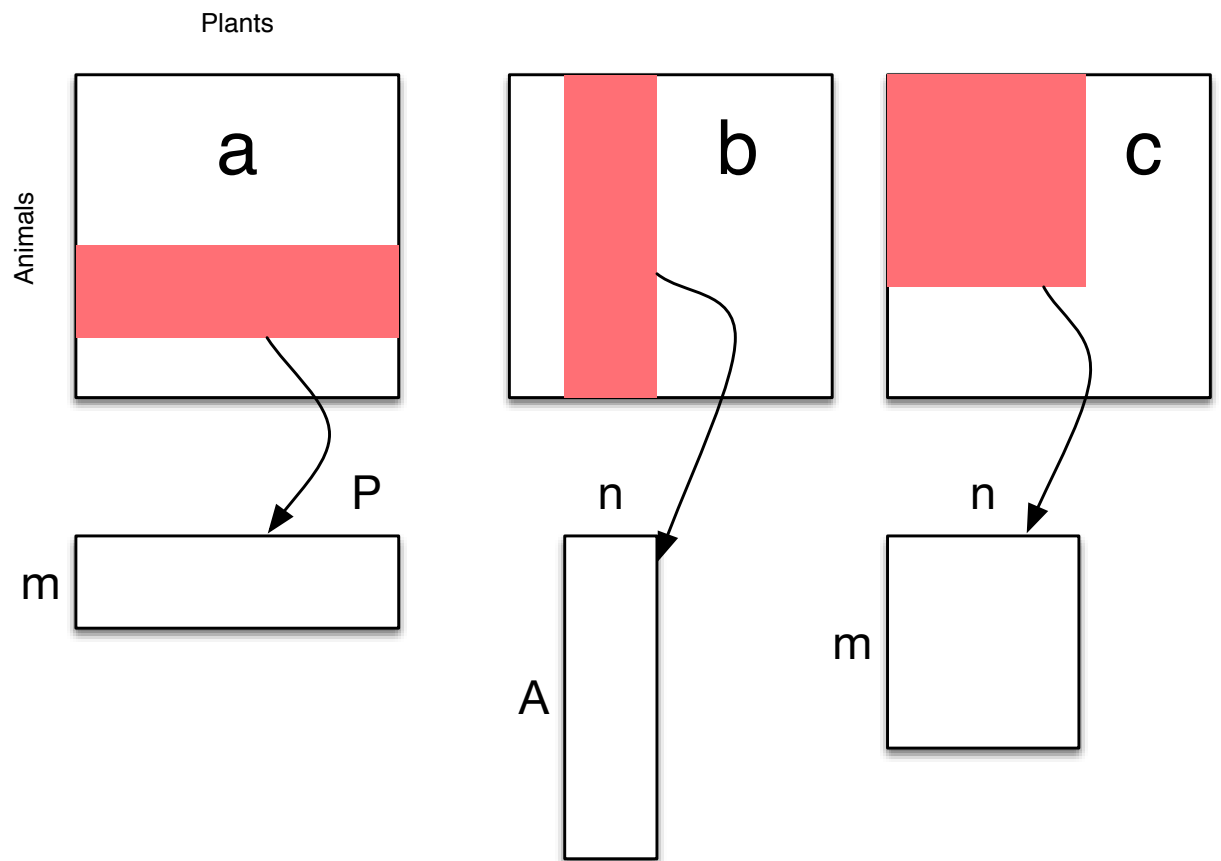
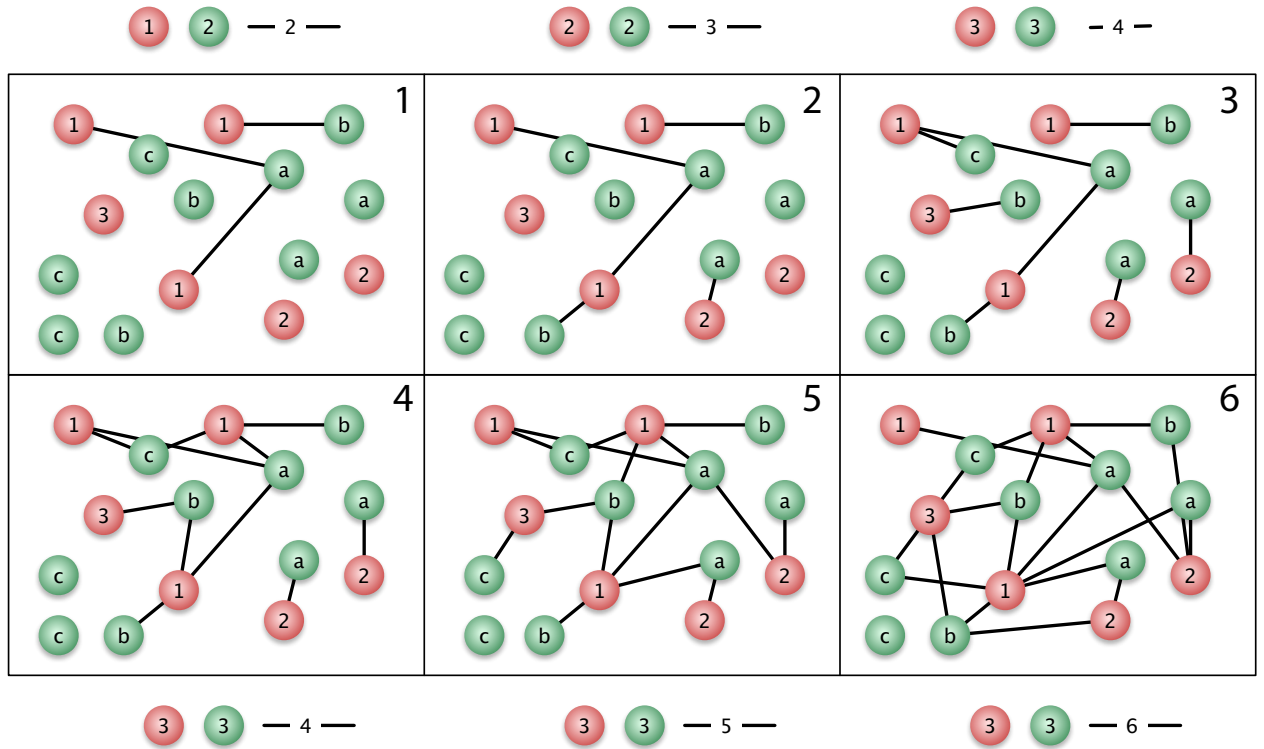


Figure 2:



Jordano - Figure 1

Table captions

Table 1. A taxonomy of link types for ecological interactions (Olesen *et al.* 2011).

A , number of animal species; P , number of plant species; I , number of observed links; $C = 100I/(AP)$, connectance; FL , number of forbidden links; and ML , number of missing links. As natural scientists, our ultimate goal is to eliminate ML from the equation $FL = AP - I - ML$, which probably is not feasible given logistic sampling limitations. When we, during our study, estimate ML to be negligible, we cease observing and estimate I and FL .

Table 2. Frequencies of different type of forbidden links in natural plant-animal interaction assemblages. AP , maximum potential links, I_{max} ; I , number of observed links; UL , number of unobserved links; FL , number of forbidden links; FL_P , phenology; FL_S , size restrictions; FL_A , accessibility; FL_O , other types of restrictions; ML , unknown causes (missing links). Relative frequencies (in parentheses) calculated over $I_{max} = AP$ for I , ML , and FL ; for all forbidden links types, calculated over FL . References, from left to right: Olesen *et al.* 2008; Olesen & Myrthue unpubl.; Snow & Snow 1972 and Jordano *et al.* 2006; Vizentin-Bugoni *et al.* 2014; Jordano *et al.* 2009; Olesen *et al.* 2011.

Table 3. A vectorized interaction matrix.

Table 4. Sampling statistics for three plant-animal interaction networks (Olesen *et al.* 2011). Symbols as in Table 1; N , number of records; $Chao1$ and ACE are asymptotic estimators for the number of distinct pairwise interactions I (Hortal

780 *et al.* 2006), and their standard errors; C , sample coverage for rare interactions
781 (Chao & Jost 2012). Scaled asymptotic estimators and their confidence intervals
782 (CI) were calculated by weighting *Chao1* and *ACE* with the observed frequencies
783 of forbidden links.

784

785 Tables

Table 1:

Link type	Formulation	Definition
Potential links	$I_{max} = A_{obs}P_{obs}$	Size of observed network matrix, i.e. maximum number of potentially observable interactions; A_{obs} and P_{obs} , numbers of interacting animal and plant species, respectively. These might be below the real numbers of animal and plant species, A_{est} and P_{est} .
Observed links	I_{obs}	Total number of observed links in the network given a sufficient sampling effort. Number of ones in the adjacency matrix.
True links	I_{est}	Total number of links in the network given a sufficient sampling effort; expected for the augmented $A_{est}P_{est}$ matrix.
Unobserved links	$UL = I_{max} - I_{obs}$	Number of zeroes in the adjacency matrix.
True unobserved links	$UL* = I_{max} - I_{obs}$	Number of zeroes in the augmented adjacency matrix that, eventually, includes unobserved species.
Forbidden links	FL	Number of links, which remain unobserved because of linkage constraints, irrespectively of sufficient sampling effort.
Observed Missing links	$ML = A_{obs}P_{obs} - I_{obs} - FL$	Number of links, which may exist in nature but need more sampling effort and/or additional sampling methods to be observed.
True Missing links	$ML* = A_{est}P_{est} - I_{est} - FL$	Number of links, which may exist in nature but need more sampling effort and/or additional sampling methods to be observed. Augments ML for the $A_{est}P_{est}$ matrix.

Table 2:

Link type	Pollination			Seed dispersal		
	Zackenberg	Grundvad	Arima Valley	Sta. Virginia	Hato Ratón	Nava Correhuelas
I_{max}	1891	646	522	423	272	825
I	268 (0.1417)	212 (0.3282)	185 (0.3544)	86 (0.1042)	151 (0.4719)	181 (0.2194)
UL	1507 (0.7969)	434 (0.6718)	337 (0.6456)	337 (0.4085)	169 (0.5281)	644 (0.7806)
FL	530 (0.3517)	107 (0.2465)	218 (0.6469)	260 (0.7715)	118 (0.6982)	302 (0.4689)
FL_P	530 (1.0000)	94 (0.2166)	0 (0.0000)	120 (0.1624)	67 (0.3964)	195 (0.3028)
FL_S	... (...)	8 (0.0184)	30 (0.0890)	140 (0.1894)	31 (0.1834)	46 (0.0714)
FL_A	... (...)	5 (0.0115)	150 (0.445) ^a	... (...)	20 (0.1183)	61 (0.0947)
FL_O	... (...)	... (...)	38 (0.1128) ^b	... (...)	... (...)	363 (0.5637)
ML	977 (0.6483)	327 (0.7535)	119 (0.3531)	77 (0.1042)	51 (0.3018)	342 (0.5311)

^a, Lack of accessibility due to habitat uncoupling, i.e., canopy-foraging species vs. understory species.

^b, Colour restrictions, and reward per flower too small relative to the size of the bird.

Dots indicate no data available for the FL type.

Table 3:

Interaction	Sample 1	Sample 2	Sample 3	...	Sample i
A1 - P1	12	2	0	...	6
A1 - P2	0	0	0	...	1
...
A5 - P3	5	0	1	...	18
A5 - P4	1	0	1	...	3
...
A _i - P _i	1	0	1	...	2

Table 4:

	Hato Ratón	Nava Correhuelas	Zackenberg
A	17	33	65
P	16	25	31
I_{max}	272	825	1891
N	3340	8378	1245
I	151	181	268
C	0.917	0.886	0.707
$Chao1$	263.1 ± 70.9	231.4 ± 14.2	509.6 ± 54.7
ACE	240.3 ± 8.9	241.3 ± 7.9	566.1 ± 14.8
% <i>unobserved</i> ^a	8.33	15.38	47.80

^a, estimated with library Jade (R Core Development Team 2010, Chao *et al.* 2015)