

Sampling networks of ecological interactions

Pedro Jordano^{*a}

^aIntegrative Ecology Group, Estación Biológica de Doñana, Consejo
Superior de Investigaciones Científicas (EBD-CSIC), Avenida
Americo Vesputio s/n, E-41092 Sevilla, Spain

Sevilla, August 30, 2015

Summary

1. Sampling ecological interactions presents similar challenges, problems, potential biases, and constraints as sampling individuals and species in biodiversity inventories. Interactions are just pairwise relationships among individuals of two unrelated species, such as those among plants and their seed dispersers in frugivory interactions or those among plants and their pollinators. Sampling interactions is a fundamental step to build robustly estimated interaction networks, yet few analyses have attempted a formal approach to their sampling protocols.

^{*}jordano@ebd.csic.es

2. 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.
3. While the complete inventory of interactions is likely impossible, a robust characterization of its main patterns and metrics is probably realistic. We must acknowledge that a sizable 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, the restrictions imposed by the organisms life-histories. Thus, the number of observed interactions I in robustly sampled networks is typically $I \ll I_{max}$, resulting in extremely sparse interaction matrices with low connectance.
4. Reasons for forbidden links are multiple but mainly stem from spatial and temporal uncoupling of partner species encounters and from intrinsically low probabilities of interspecific encounter for many of the potential pairwise interactions. Adequately assessing the completeness of a network of ecological interactions thus needs a deep knowledge of the natural history details embedded, so that forbidden links can be “discounted” when addressing sampling effort.
5. 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. This is crucial to assess the fast-paced and devastating effects of defaunation-driven loss of key ecological

interactions and the services they provide.

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 assessment aims at sampling individuals in collections and deter-
2 mining the number of species represented. Given that, by definition, samples are
3 incomplete, these collections enumerate a lower number of the species actually
4 present. The ecological literature dealing with robust estimators of species rich-
5 ness and diversity in collections of individuals is immense, and a number of useful
6 approaches have been used to obtain such estimates (Magurran, 1988; Gotelli &
7 Colwell, 2001; Hortal, Borges & Gaspar, 2006; Colwell, 2009; Gotelli & Colwell,
8 2011). Recent effort has been also focused at defining essential biodiversity vari-
9 ables (EBV) (Pereira *et al.*, 2013) that can be sampled and measured repeatedly
10 to complement biodiversity estimates. Yet sampling species or taxa-specific EBVs
11 is just probing a single component of biodiversity; interactions among species are
12 another fundamental component, the one that supports the existence of species.

13 For example, the extinction of interactions represents a dramatic loss of biodiver-
14 sity because it entails the loss of fundamental ecological functions (Valiente-Banuet
15 *et al.*, 2014). This missed component of biodiversity loss, the extinction of ecolog-
16 ical interactions, very often accompanies, or even precedes, species disappearance.
17 Interactions among species are a key component of biodiversity and here I aim to
18 show that most problems associated to sampling interactions in natural communi-
19 ties have to do with problems associated to sampling species diversity. I consider
20 pairwise interactions among species at the habitat level, in the context of alpha
21 diversity and the estimation of local interaction richness from sampling data (Mao
22 & Colwell, 2005). In the first part I provide a succinct overview of previous work
23 addressing sampling issues for ecological interaction networks. In the second part
24 I discuss specific rationales for sampling the biodiversity of ecological interactions.

25 Interactions can be a much better indicator of the richness and diversity of
26 ecosystem functions than a simple list of taxa and their abundances and/or re-
27 lated biodiversity indicator variables (EBVs). Thus, sampling interactions should
28 be a central issue when identifying and diagnosing ecosystem services (e.g., polli-
29 nation, natural seeding by frugivores, etc.). Fortunately, all the whole battery of
30 biodiversity-related tools used by ecologists to sample biodiversity (species, *sensu*
31 *stricto*) can be extended and applied to the sampling of interactions. Analogs
32 are evident between these approaches (Colwell, Dunn & Harris, 2012). Monitor-
33 ing interactions is analogous to any biodiversity sampling [i.e., a species inventory
34 Jordano (1987); Jordano, Vázquez & Bascompte (2009)] and is subject to similar
35 methodological shortcomings, especially under-sampling (Coddington *et al.*, 2009;
36 Vazquez, Chacoff & Cagnolo, 2009; Dorado *et al.*, 2011; Rivera-Hutinel *et al.*,
37 2012). For example, when we study mutualistic networks, our goal is to make an

38 inventory of the distinct pairwise interactions that made up the network. We are
 39 interested in having a complete list of all the pairwise interactions among species
 40 (e.g., all the distinct, species-species interactions, or links, among the pollinators
 41 and flowering plants) that can exist in a given community. Sampling these in-
 42 teractions thus entails exactly the same problems, limitations, constraints, and
 43 potential biases as sampling individual organisms and species diversity. As Mao &
 44 Colwell (2005) put it, these are the workings of Preston’s demon, the moving “veil
 45 line” between detected and the undetected interactions as sample size increases
 46 (Preston, 1948).

47 Early efforts to recognize and solve sampling problems in analyses of interac-
 48 tions stem from researchers interested in food web analyses and in determining the
 49 biases of undersampled food web metrics (Martinez, 1991; Cohen *et al.*, 1993; Mar-
 50 tinez, 1993; Bersier, Banasek-Richter & Cattin, 2002; Brose, Martinez & Williams,
 51 2003; Banasek-Richter, Cattin & Bersier, 2004). In addition, the myriad of classic
 52 natural history studies documenting animal diets, host-pathogen infection records,
 53 plant herbivory records, etc., represent efforts to document interactions occurring
 54 in nature. All of them share the problem of sampling incompleteness influenc-
 55 ing the patterns and metrics reported. Yet, despite the early recognition that
 56 incomplete sampling may seriously bias the analysis of ecological networks (Jor-
 57 dano, 1987), only recent studies have explicitly acknowledged it and attempted to
 58 determine its influence (Ollerton & Cranmer, 2002; Nielsen & Bascompte, 2007;
 59 Vazquez, Chacoff & Cagnolo, 2009; Gibson *et al.*, 2011; Olesen *et al.*, 2011; Cha-
 60 coff *et al.*, 2012; Rivera-Hutinel *et al.*, 2012; Olito & Fox, 2014; Bascompte &
 61 Jordano, 2014; Vizentin-Bugoni, Maruyama & Sazima, 2014; Frund, McCann &
 62 Williams, 2015). The sampling approaches have been extended to predict patterns

of coextinctions in interaction assemblages (e.g., hosts-parasites) (Colwell, Dunn & Harris, 2012). Most empirical studies provide no estimate of sampling effort, implicitly assuming that the reported network patterns and metrics are robust. Yet recent evidences point out that number of partner species detected, number of actual links, and some aggregate statistics describing network patterns, are prone to sampling bias (Nielsen & Bascompte, 2007; Dorado *et al.*, 2011; Olesen *et al.*, 2011; Chacoff *et al.*, 2012; Rivera-Hutinel *et al.*, 2012; Olito & Fox, 2014; Frund, McCann & Williams, 2015). Most of these evidences, however, come from either theoretical, simulation, studies (Frund, McCann & Williams, 2015) or from relatively species-poor assemblages. Even for species-rich, tropical assemblages it might be erroneous to conclude that network data routinely come from insufficiently sampled datasets (Ollerton & Cranmer, 2002; Chacoff *et al.*, 2012), given the extremely sparse nature of these interaction matrices because of the prevalence of forbidden links (which, by definition, cannot be documented despite extensive sampling effort). However, most certainly, sampling limitations pervade biodiversity inventories in tropical areas (Coddington *et al.*, 2009) and we might rightly expect that frequent interactions may be over-represented and rare interactions may be missed entirely in studies of mega-diverse assemblages (Bascompte & Jordano, 2014); but, to what extent?

Sampling interactions: methods

When we sample interactions in the field we record the presence of two species that interact in some way. For example, Snow and Snow(1988) recorded an interaction whenever they saw a bird “touching” a fruit on a plant. We observe and

record feeding observations, visitation, occupancy, presence in pollen loads or in fecal samples, etc., of *individual* animals or plants and accumulate pairwise interactions, i.e., lists of species partners and the frequencies with which we observe them. Therefore, estimating the sampling completeness of pairwise interactions for a whole network, requires estimating the number (richness) of distinct pairwise interactions accumulated as sampling effort is increased, pooling the data for all partner species.

Most, if not all, 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, $\Delta = AP$, is a 2D representation of the interactions among, say, A animal species (rows) and P plant species (columns) (Jordano, 1987; Bascompte & Jordano, 2014). An interaction matrix Δ consists of an array of zeroes or ones, or an array of numeric values (including zeroes)- if the data (interaction frequencies) are quantified. 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). Given that 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), a frequently used proxy for interaction intensities w_{ji} is just how frequent are new interspecific encounters, whether or not appropriately weighted to estimate interaction effectiveness (Vazquez, Morris & Jordano, 2005).

111 We need to define two basic steps in the sampling of interactions: 1) which
 112 type of interactions we sample; and 2) which type of record we get to document
 113 the existence of an interaction. In step #1 we need to take into account whether
 114 we are sampling the whole community of interactor species (all the animals, all
 115 the plants) or just a subset of them, i.e., a sub matrix $\Delta_{m,n}$ of $m < A$ animal
 116 species and $n < P$ plant species of the adjacency matrix Δ_{AP} . Subsets can be:
 117 a) all the potential plants interacting with a subset of the animals (Fig. 1a); b)
 118 all the potential animal species interacting with a subset of the plant species (Fig.
 119 1b); c) a subset of all the potential animal species interacting with a subset of all
 120 the plant species (Fig. 1c). While some discussion has considered how to establish
 121 the limits of what represents a network (Strogatz, 2001) (in analogy to discussion
 122 on food-web limits; Cohen, 1978), it must be noted that situations a-c in Fig.
 123 1 do not represent complete interaction networks. As vividly stated by Cohen
 124 et al. (1993): “*As more comprehensive, more detailed, more explicit webs become*
 125 *available, smaller, highly aggregated, incompletely described webs may progressively*
 126 *be dropped from analyses of web structure (though such webs may remain useful for*
 127 *other purposes, such as pedagogy)*”. Subnet sampling is generalized in studies of
 128 biological networks (e.g., protein interactions, gene regulation), yet it is important
 129 to recognize that most properties of subnetworks (even random subsamples) do
 130 not represent properties of whole networks (Stumpf, Wiuf & May, 2005).

131 In step #2 above we face the problem of the type of record we take to sample
 132 interactions. This is important because it defines whether we approach the problem
 133 of filling up the interaction matrix in a “zoo-centric” way or in a “phyto-centric”
 134 way. Zoo-centric studies directly sample animal activity and document the plants
 135 ‘touched’ by the animal. For example, analysis of pollen samples recovered from the

body of pollinators, analysis of fecal samples of frugivores, radio-tracking data, etc. Phyto-centric studies take samples of focal individual plant species and document which animals ‘arrive’ or ‘touch’ the plants. Examples include focal watches of 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 or plant-herbivore interactions are “host-centric” or phyto-centric (e.g., Thébault & Fontaine, 2010; 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 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). System symmetry has been suggested to influence estimations of generalization levels in plants and animals when measured as I_A and I_P (Elberling & Olesen, 1999); thus, differences in I_A and I_P between networks may arise from different $A : P$ ratios rather than other ecological factors (Olesen & Jordano, 2002).

Interestingly enough, quite complete analyses of interaction networks can be obtained when combining both phyto-centric and zoo-centric sampling. For example, Bosch *et al.* (Bosch *et al.*, 2009) showed that the addition of pollen load data on top of focal-plant sampling of pollinators unveiled a significant number

161 of interactions, resulting in important network structural changes. Connectance
 162 increased 1.43-fold, mean plant connectivity went from 18.5 to 26.4, and mean
 163 pollinator connectivity from 2.9 to 4.1; moreover, extreme specialist pollinator
 164 species (singletons in the adjacency matrix) decreased 0.6-fold. Zoo-centric sam-
 165 pling has recently been extended with the use of DNA-barcoding, for example
 166 with plant-herbivore (Jurado-Rivera *et al.*, 2009) and plant-frugivore interactions
 167 (González-Varo, Arroyo & Jordano, 2014). For mutualistic networks we would ex-
 168 pect that zoo-centric sampling could help unveiling interactions for rare species or
 169 for relatively common species which are difficult to sample by direct observation.
 170 Future methodological work may provide significant advances showing how mixing
 171 different sampling strategies strengthens the completeness of network data. These
 172 mixed strategies may combine, for instance, focal analyses, pollen load or seed
 173 contents, camera traps, and DNA barcoding records. We might expect increased
 174 power of these mixed sampling approaches when combining different methods from
 175 both phyto- and zoo-centric perspectives (Bosch *et al.*, 2009; Bluthgen, 2010).

176 Sampling interactions: rationale

177 The number of distinct pairwise interactions that we can record in a landscape (an
 178 area of relatively homogeneous vegetation, analogous to the one we would use to
 179 monitor species diversity) is equivalent to the number of distinct classes in which
 180 we can classify the recorded encounters among individuals of two different species.
 181 Yet, individual-based plant-animal interaction networks have been only recently
 182 studied (Dupont, Trøjelsgaard & Olesen, 2011). We walk in the forest and see
 183 a blackbird *Tm* picking an ivy *Hh* fruit and ingesting it: we have a record for

184 $Tm - Hh$ interaction. We keep advancing and record again a blackbird feeding
 185 on hawthorn Cm fruits so we record a $Tm - Cm$ interaction; as we advance we
 186 encounter another ivy plant and record a blackcap swallowing a fruit so we now
 187 have a new $Sa - Hh$ interaction, and so on. At the end we have a series of
 188 classes (e.g., $Sa - Hh$, $Tm - Hh$, $Tm - Cm$, etc.), along with their observed
 189 frequencies. Bunge & Fitzpatrick (1993) review the main aspects and approaches
 190 to estimate the number of distinct classes C in a sample of observations. The
 191 sampling of interactions in nature, as the sampling of species, is a cumulative
 192 process. In our analysis, we are not re-sampling individuals, but interactions, so we
 193 made interaction-based accumulation curves. If an interaction-based curve points
 194 towards a robust sampling, it does mean that no new interactions are likely to be
 195 recorded, irrespectively of the species, as it is a whole-network sampling approach
 196 (N. Gotelli, pers. com.). We add new, distinct, interactions recorded as we increase
 197 sampling effort (Fig. 2). We can obtain an Interaction Accumulation Curve (IAC)
 198 analogous to a Species cumulating Curve (SAC): the observed number of distinct
 199 pairwise interactions in a survey or collection as a function of the accumulated
 200 number of observations or samples (Colwell, 2009).

201 Our sampling above would have resulted in a vector $n = [n_1 \dots n_C]'$ where n_i is
 202 the number of records in the i^{th} class. As stressed by Bunge & Fitzpatrick (1993),
 203 however, the i^{th} class would appear in the sample if and only if $n_i > 0$, and we
 204 don't know *a priori* which n_i are zero. So, n is not observable. Rather, what we
 205 get is a vector $c = [c_1 \dots c_n]'$ where c_j is the number of classes represented j times
 206 in our sampling: c_1 is the number of singletons, c_2 is the number of twin pairs, c_3
 207 the number of triplets, etc. The problem thus turns to be estimating the number
 208 of distinct classes C from the vector of c_j values.

209 Estimating the number of interactions with resulting robust estimates of net-
 210 work parameters is a central issue in the study of ecological interaction networks
 211 (Jordano, 1987; Bascompte & Jordano, 2014). In contrast with traditional species
 212 diversity estimates, sampling networks has the paradox that despite the poten-
 213 tially interacting species being present in the sampled assemblage (i.e., included in
 214 the A and P species lists), some of their pairwise interactions are impossible to be
 215 recorded. The reason is forbidden links. Independently of whether we sample full
 216 communities or subset communities we face a problem: some of the interactions
 217 that we can visualize in the empty adjacency matrix Δ will simply not occur. Thus,
 218 independently of the sampling effort we put, we'll never document these pairwise
 219 interactions. With a total of AP "potential" interactions, a fraction of them are
 220 impossible to record, because they are forbidden (Jordano, Bascompte & Olesen,
 221 2003; Olesen *et al.*, 2011). Forbidden links are constraints for the establishment of
 222 new links, and mainly arise from the biological attributes of the species: no link
 223 can be established between a plant and an animal mutualist differing in phenology,
 224 i.e. the seeds of a winter-ripening plant cannot be dispersed by a frugivore that
 225 is a summer stopover migrant (Jordano, 1987). Or, for instance, short-tongued
 226 pollinators cannot successfully reach the nectar in long-corolla flowers and pol-
 227 linate them efficiently (Moré *et al.*, 2012). Forbidden links are thus represented
 228 as structural zeroes in the interaction matrix, i.e., matrix cells that cannot get a
 229 non-zero value. So, we need to account for the frequency of these structural zeros
 230 in our matrix before proceeding. For example, most measurements of connectance
 231 $C = I/(AP)$ implicitly ignore the fact that by taking the full product AP in the
 232 denominator they are underestimating the actual connectance value, i.e., the frac-
 233 tion of actual interactions I relative to the *biologically possible* ones, not to the

234 total maximum $I_{max} = AP$.

235 Adjacency matrices are frequently sparse, i.e., they are densely populated with
 236 zeroes, with a fraction of them being structural (i.e., unobservable interactions)
 237 (Bascompte & Jordano, 2014). It would be thus a serious interpretation error to
 238 attribute the sparseness of adjacency matrices for bipartite networks to under-
 239 sampling. The actual typology of link types in ecological interaction networks is
 240 thus more complex than just the two categories of observed and unobserved inter-
 241 actions (Table 1). Unobserved interactions are represented by zeroes and belong
 242 to two categories. Missing interactions may actually exist but require additional
 243 sampling or a variety of methods to be observed. Forbidden links, on the other
 244 hand, arise due to biological constraints limiting interactions and remain unobserv-
 245 able in nature, irrespectively of sampling effort (Jordano, Bascompte & Olesen,
 246 2003; Olesen *et al.*, 2011). Forbidden links are non-occurrences of pairwise interac-
 247 tions that can be accounted for by biological constraints, such as spatio-temporal
 248 uncoupling, size or reward mismatching, foraging constraints (e.g., accessibility),
 249 and physiological-biochemical constraints (Jordano, 1987). We still have extremely
 250 reduced information about the frequency of forbidden links in natural communi-
 251 ties (Jordano, Bascompte & Olesen, 2003; Stang *et al.*, 2009; Vazquez, Chacoff &
 252 Cagnolo, 2009; Olesen *et al.*, 2011; Ibanez, 2012; Maruyama *et al.*, 2014; Vizentin-
 253 Bugoni, Maruyama & Sazima, 2014) (Table 1). Forbidden links FL may actually
 254 account for a relatively large fraction of unobserved interactions UL when sam-
 255 pling taxonomically-restricted subnetworks (e.g., plant-hummingbird pollination
 256 networks) (Table 1). Phenological unmatching is also prevalent in most networks,
 257 and may add up to explain ca. 25–40% of the forbidden links, especially in highly
 258 seasonal habitats, and up to 20% when estimated relative to the total number

259 of unobserved interactions (Table 2). In any case, we might expect that a frac-
 260 tion of the missing links ML would be eventually explained by further biological
 261 reasons, depending on the knowledge of natural details of the particular systems.
 262 Our goal as naturalists would be to reduce the fraction of UL which remain as
 263 missing links; to this end we might search for additional biological constraints or
 264 increase sampling effort. For instance, habitat use patterns by hummingbirds in
 265 the Arima Valley network (Table 2; Snow & Snow, 1972) impose a marked pattern
 266 of microhabitat mismatches causing up to 44.5% of the forbidden links. A myr-
 267 iad of biological causes beyond those included as FL in Table 2 may contribute
 268 explanations for UL : limits of color perception and or partial preferences, pres-
 269 ence of secondary metabolites in fruit pulp and leaves, toxins and combinations
 270 of monosaccharides in nectar, etc. However, it is surprising that just the limited
 271 set of forbidden link types considered in Table 1 explain between 24.6–77.2% of
 272 the unobserved links. Notably, the Arima Valley, Santa Virgínia, and Hato Ratón
 273 networks have $> 60\%$ of the unobserved links explained, which might be related
 274 to the fact that they are subnetworks (Arima Valley, Santa Virgínia) or relatively
 275 small networks (Hato Ratón). All this means that empirical networks may have
 276 sizable fractions of structural zeroes. Ignoring this biological fact may contribute
 277 to wrongly infer undersampling of interactions in real-world assemblages.

278 To sum up, two elements of inference are required in the analysis of unobserved
 279 interactions in ecological interaction networks: first, detailed natural history infor-
 280 mation on the participant species that allows the inference of biological constraints
 281 imposing forbidden links, so that structural zeroes can be identified in the adja-
 282 cency matrix; second, a critical analysis of sampling robustness a robust estimate
 283 of the actual fraction of missing links, M , and thus, a robust estimate of I .

284 Asymptotic diversity estimates

Let's assume a sampling of the diversity in a specific locality, over relatively homogeneous landscape where we aim at determining the number of species present for a particular group of organisms. To do that we carry out transects or plot samplings across the landscape, adequately replicated so we obtain a number of samples. Briefly, S_{obs} is the total number of species observed in a sample, or in a set of samples. S_{est} is the estimated number of species in the community represented by the sample, or by the set of samples, where *est* indicates an estimator. With abundance data, let S_k be the number of species each represented by exactly k individuals in a single sample. Thus, S_0 is the number of undetected species (species present in the community but not included in the sample), S_1 is the number of singleton species, S_2 is the number of doubleton species, etc. The total number of individuals in the sample would be:

$$n = \sum_{k=1}^{S_{obs}} S_k$$

285

286 A frequently used asymptotic, bias corrected, non-parametric estimator is S_{Chao}
 287 (Hortal, Borges & Gaspar, 2006; Chao, 2005; Colwell, 2013):

$$S_{Chao} = S_{obs} + \frac{S_1(S_1 - 1)}{2(S_2 + 1)}$$

288 Another frequently used alternative is the Chao2 estimator, S_{Chao2} (Gotelli &
 289 Colwell, 2001), which has been reported to have a limited bias for small sample
 290 sizes (Colwell & Coddington, 1994; Chao, 2005):

$$S_{Chao2} = S_{obs} + \frac{S_1^2}{2S_2}$$

291 A plot of the cumulative number of species recorded, S_n , as a function of
 292 some measure of sampling effort (say, n samples taken) yields the species accu-
 293 mulation curve (SAC) or collector's curve (Colwell & Coddington, 1994). Such a
 294 curve eventually reaches an asymptote converging with S_{est} . In an analogous way,
 295 interaction accumulation curves (IAC), analogous to SACs, can be used to as-
 296 sess the robustness of interactions sampling for plant-animal community datasets
 297 (Jordano, 1987; Jordano, Vázquez & Bascompte, 2009; Olesen *et al.*, 2011). For
 298 instance, a random accumulator function (e.g., library `vegan` in the R Package, R
 299 Development Core Team, 2010) which finds the mean IAC and its standard devia-
 300 tion from random permutations of the data, or subsampling without replacement
 301 (Gotelli & Colwell, 2001) can be used to estimate the expected number of distinct
 302 pairwise interactions included in a given sampling of records (Jordano, Vázquez
 303 & Bascompte, 2009; Olesen *et al.*, 2011). We start with a vectorized interaction
 304 matrix representing the pairwise interactions (rows) recorded during a cumulative
 305 number of censuses or sampling periods (columns) (Table 3), in a way analogous
 306 to a biodiversity sampling matrix with species as rows and sampling units (e.g.,
 307 quadrats) as columns (Jordano, Vázquez & Bascompte, 2009). In this way we
 308 effectively extend sampling theory developed for species diversity to the sampling
 309 of ecological interactions. Yet future theoretical work will be needed to formally
 310 assess the similarities and differences in the two approaches and developing biolog-
 311 ically meaningful null models of expected interaction richness with added sampling
 312 effort.

Assessing sampling effort when recording interactions

The basic method we can propose 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 a community of A animal species and P plant species. The new “species” we aim to sample are the pairwise interactions (Table 3). So, if we have in our community *Turdus merula* (Tm) and *Rosa canina* (Rc) and *Prunus mahaleb* (Pm), our problem will be to sample 2 new “species”: $Tm - Rc$ and $Tm - Pm$. In general, if we have $A = 1...i$, animal species and $P = 1...j$ plant species, 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.

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

337 interactions of a given module (e.g., plants and their hummingbird pollinators; Fig.
 338 1a) may share similar detectability values, in an analogous way to species groups
 339 receiving homogeneous detectability values in mixture models (Mao & Colwell,
 340 2005). Such sampling, in its simplest form, would result in a sample with multiple
 341 pairwise interactions detected, in which the number of interaction events recorded
 342 for each distinct interaction found in the sample is recorded (i.e., a column vector
 343 in Table 3, corresponding to, say, a sampling day). The number of interactions
 344 recorded for the i_{th} pairwise interaction (i.e., $A_i P_j$ in Table 3), Y_i could be treated
 345 as a Poisson random variable with a mean parameter λ_i , its detection rate. Mix-
 346 ture models (Mao & Colwell, 2005) include estimates for abundance-based data
 347 (their analogous in interaction sampling would be weighted data), where Y_i is
 348 a Poisson random variable with detection rate λ_i . This is combined with the
 349 incidence-based model, where Y_i is a binomial random variable (their analogous
 350 in interaction sampling would be presence/absence records of interactions) with
 351 detection odds λ_i . Let T be the number of samples in an incidence-based data set.
 352 A Poisson/binomial density can be written as (Mao & Colwell, 2005):

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

353 where [1] corresponds to a weighted network, and [2] to a qualitative network.

354 The detection rates λ_i depend on the relative abundances ϕ_i of the interactions,
 355 the probability of a pairwise interaction being detected when it is present, and the
 356 sample size (the number of interactions recorded), which, in turn, is a function
 357 of the sampling effort. Unfortunately, no specific sampling model has been de-

veloped 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. 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, Maruyama & Sazima, 2014). Yet more complex models should incorporate not only interspecific encounter probabilities, but also phenotypic matching and incidence of forbidden links.

Rarefaction analysis and diversity-accumulation analysis (Magurran, 1988; Hortal, Borges & Gaspar, 2006) come 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, Vázquez & Bascompte, 2009; Olesen *et al.*, 2011). Asymptotic estimates of interaction richness and its associated standard errors and confidence intervals can thus be obtained (Hortal, Borges & Gaspar, 2006). It should be noted that the asymptotic estimate of interaction richness implicitly ignores the fact that, due to forbidden links, a number of pairwise interactions among the I_{max} number specified in the adjacency matrix Δ cannot be recorded, irrespective of sampling effort. Therefore, the asymptotic value most likely is an overestimate of the actual maximum number of links that can be present in an assemblage. If forbidden links are taken into account, the asymptotic estimate should be lower. Yet, to the best of my knowledge, there is no theory developed to estimate this “biologically real” asymptotic value.

Not unexpectedly, most recent analyses of sampling effort in ecological network studies found evidences of undersampling (Chacoff *et al.*, 2012). This needs not to be true, especially when interaction subwebs are studied (Olesen *et al.*, 2011; Vizentin-Bugoni, Maruyama & Sazima, 2014), and once the issue of structural zeroes in the interaction matrices is effectively incorporated in the estimates.

The *real* missing links

Given that a fraction of unobserved interactions can be accounted for by forbidden links, what about the remaining missing interactions? We have already discussed that some of these could still be related to unaccounted constraints, and still others would be certainly attributable to insufficient sampling. Would this always be the case? Multispecific assemblages of distinct taxonomic relatedness, whose interactions can be represented as bipartite networks (e.g., host-parasite, plant-animal mutualisms, plant-herbivore interactions- with two distinct sets of unrelated higher taxa), are shaped by interspecific encounters among individuals of the partner species (Fig. 2). A crucial ecological aspect limiting these interactions is the probability of interspecific encounter, i.e., the probability that two individuals of the partner species actually encounter each other in nature.

Given log-normally distributed abundances of the two species groups, the expected “neutral” probabilities of interspecific encounter (*PIE*) would be simply the product of the two lognormal distributions. Thus, we might expect that for low *PIE* values, pairwise interactions would be either extremely difficult to sample, or just simply non-occurring in nature. Consider the Nava de las Correhuelas interaction web (NCH, Table 2), with $A = 36$, $P = 25$, $I = 181$, and almost

half of the unobserved interactions not accounted for by forbidden links missing
 links, $M = 53.1\%$. Given the robust sampling of this network (Jordano, Vázquez
 & Bascompte, 2009), a sizable fraction of these possible but missing links would
 be simply not occurring in nature, most likely by extremely low PIE , in fact
 asymptotically zero. Given the vectorized list of pairwise interactions for NCH, I
 computed the PIE values for each one by multiplying element wise the two species
 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 simply not occur according to this neutral expectation (Jordano, 1987; Ole-
 sen *et al.*, 2011) (neutral forbidden links, *sensu* Canard *et al.*, 2012). Which is the
 expected frequency for pairwise interactions? and, which is the expected proba-
 bility for unobserved interactions? More specifically, which is the probability of
 missing interactions, M (i.e., the unobserved ones that cannot be accounted for as
 forbidden links)?

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 lin-
 guistics and word analysis (Gale & Sampson, 1995) has been recently applied in
 ecology (Chao *et al.*, 2015), 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 $\frac{1-E(1)}{T}$. This reduces all the probabilities for interactions we have recorded, and makes room for interactions we haven't seen. If we sum over the interactions we have seen, then the sum of $P(X)$ is $1 - \frac{1-E(1)}{T}$. Because probabilities sum to one, we have the left-over probability of $P_{new} = \frac{E(1)}{T}$ of seeing something new, where new means that we sample a new pairwise interaction.

Note, however, that Good-Turing estimators, as the traditional asymptotic estimators, do not account in our case for the forbidden interactions. To account for these *FL* I re-scaled the asymptotic estimates, so that a more meaningful estimate could be obtained (Table 4). The scaling was calculated as $[Chao1 * (I + ML)] / AP$, just correcting for the *FL* frequency, given that $I + ML$ represent the total *feasible* interactions when discounting the forbidden links (Table 1). After scaling, observed I values (Table 2) are within the *Chao1* and *ACE* asymptotic estimates but below the *ACE* estimates for Hato Ratón and Zackenberg (Table 4). Thus, even after re-scaling for *FL*, it is likely that adequate characterization of most interaction networks will require intensive sampling effort.

Discussion

Recent work has inferred that most data available for interaction networks are incomplete due to undersampling, resulting in a variety of biased parameters and network patterns (Chacoff *et al.*, 2012). It is important to note, however, that in practice, many surveyed networks to date have been subnets of much larger networks. This is true for protein interaction, gene regulation, and metabolic networks, where only a subset of the molecular entities in a cell have been sampled (Stumpf, Wiuf & May, 2005). Despite recent attempts to document whole ecosystem meta-networks (Pocock, Evans & Memmott, 2012), it is likely that most ecological interaction networks will illustrate just major ecosystem compartments. Due to their high generalization, high temporal and spatial turnover, and high complexity of association patterns, adequate sampling of ecological interaction networks requires extremely large sampling effort. Undersampling of ecological networks may originate from the analysis of assemblage subsets (e.g., taxonomically or functionally defined), and/or from logistically-limited sampling effort. It is extremely hard to robustly sample the set of biotic interactions even for relatively simple, species-poor assemblages; yet, concluding that all ecological network datasets are undersampled 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).

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 unobservable interactions (i.e., for-

bidden 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. Some key components of this sampling are analogous to species sampling and traditional biodiversity inventories; however, there are important differences. 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 mutualisms 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. A re-scaling of traditional asymptotic estimates for interaction richness can be applied whenever the knowledge of natural history details about the study system is sufficient to estimate at least the main causes of forbidden links. Moreover, recent implementations of inference methods for unobserved species (Chao *et al.*, 2015) can be combined with the forbidden link approach, yet they do not account either for the existence of these ecological constraints.

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. Yet we still have a limited knowledge of the biodiversity of ecological interactions, but 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 compo-

nents of their ecological complexity that need to be restored after perturbations
to rebuild functional ecosystems.

Acknowledgements

I am indebted to Robert Colwell, Jens M. Olesen, Alfredo Valido, Jordi Bascompte, Thomas Lewinshon, John N. Thompson, Nick Gotelli, Carsten Dormann, and Paulo R. Guimaraes Jr. for useful and thoughtful comments and discussion at different stages of this manuscript. The study was supported by a Junta de Andalucía Excellence Grant (RNM-5731), as well as a Severo Ochoa Excellence Award from the Ministerio de Economía y Competitividad (SEV-2012-0262). The Agencia de Medio Ambiente, Junta de Andalucía, provided generous facilities that made possible my long-term field work in different natural parks.

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

- Banasek-Richter, C., Cattin, M. & Bersier, L. (2004) Sampling effects and the robustness of quantitative and qualitative food-web descriptors. *Journal of Theoretical Biology*, **226**, 23–32.
- Bascompte, J. & Jordano, P. (2014) *Mutualistic networks*. Monographs in Population Biology, No. 53. Princeton University Press, Princeton, NJ.
- Bersier, L., Banasek-Richter, C. & Cattin, M. (2002) Quantitative descriptors of food-web matrices. *Ecology*, **83**, 2394–2407.
- Bluthgen, N. (2010) Why network analysis is often disconnected from community ecology: A critique and an ecologist’s guide. *Basic And Applied Ecology*, **11**, 185–195.
- Bosch, J., Martín González, A.M., Rodrigo, A. & Navarro, D. (2009) Plant-pollinator networks: adding the pollinator’s perspective. *Ecology Letters*, **12**, 409–419.
- Brose, U., Martinez, N. & Williams, R. (2003) Estimating species richness: Sensitivity to sample coverage and insensitivity to spatial patterns. *Ecology*, **84**, 2364–2377.
- Bunge, J. & Fitzpatrick, M. (1993) Estimating the number of species: a review. *Journal of the American Statistical Association*, **88**, 364–373.
- 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.

- 541 Chacoff, N.P., Vazquez, D.P., Lomascolo, S.B., Stevani, E.L., Dorado, J. & Padrón,
542 B. (2012) Evaluating sampling completeness in a desert plant-pollinator network.
543 *Journal of Animal Ecology*, **81**, 190–200.
- 544 Chao, A. (2005) Species richness estimation. *Encyclopedia of Statistical Sciences*,
545 pp. 7909–7916. Oxford University Press, New York, USA.
- 546 Chao, A., Hsieh, T.C., Chazdon, R.L., Colwell, R.K. & Gotelli, N.J. (2015) Un-
547 veiling the species-rank abundance distribution by generalizing the Good-Turing
548 sample coverage theory. *Ecology*, **96**, 1189–1201.
- 549 Coddington, J.A., Agnarsson, I., Miller, J.A., Kuntner, M. & Hormiga, G. (2009)
550 Undersampling bias: the null hypothesis for singleton species in tropical arthro-
551 pod surveys. *Journal of Animal Ecology*, **78**, 573–584.
- 552 Cohen, J.E. (1978) *Food webs and niche space*. Princeton University Press, Prince-
553 ton, New Jersey, US.
- 554 Cohen, J.E., Beaver, R.A., Cousins, S.H., DeAngelis, D.L., Goldwasser, L., Heong,
555 K.L., Holt, R.D., Kohn, A.J., Lawton, J.H., Martinez, N., O'Malley, R., Page,
556 L.M., Patten, B.C., Pimm, S.L., Polis, G., Rejmanek, M., Schoener, T.W.,
557 Schenly, K., Sprules, W.G., Teal, J.M., Ulanowicz, R., Warren, P.H., Wilbur,
558 H.M. & Yodis, P. (1993) Improving food webs. *Ecology*, **74**, 252–258.
- 559 Colwell, R. & Coddington, J. (1994) Estimating terrestrial biodiversity through ex-
560 trapolation. *Philosophical Transactions Of The Royal Society Of London Series*
561 *B-Biological Sciences*, **345**, 101–118.
- 562 Colwell, R.K. (2009) Biodiversity: concepts, patterns, and measurement. *The*

- 563 *Princeton Guide to Ecology* (ed. S.A. Levin), pp. 257–263. Princeton University
564 Press, Princeton.
- 565 Colwell, R.K. (2013) EstimateS: Biodiversity Estimation. -, pp. 1–33.
- 566 Colwell, R.K., Dunn, R.R. & Harris, N.C. (2012) Coextinction and persistence of
567 dependent species in a changing world. *Annual Review of Ecology Evolution and*
568 *Systematics*, **43**, 183–203.
- 569 Dorado, J., Vazquez, D.P., Stevani, E.L. & Chacoff, N.P. (2011) Rareness and
570 specialization in plant-pollinator networks. *Ecology*, **92**, 19–25.
- 571 Dormann, C.F., Frund, J., Bluthgen, N. & Gruber, B. (2009) Indices, graphs and
572 null models: Analyzing bipartite ecological networks. *Open Ecology Journal*, **2**,
573 7–24.
- 574 Dupont, Y.L., Trøjelsgaard, K. & Olesen, J.M. (2011) Scaling down from species
575 to individuals: a flower–visitation network between individual honeybees and
576 thistle plants. *Oikos*, **120**, 170–177.
- 577 Dupont, Y.L., Trøjelsgaard, K., Hagen, M., Henriksen, M.V., Olesen, J.M., Ped-
578 ersen, N.M.E. & Kissling, W.D. (2014) Spatial structure of an individual-based
579 plant-pollinator network. *Oikos*, **123**, 1301–1310.
- 580 Eklöf, A., Jacob, U., Kopp, J., Bosch, J., Castro-Urgal, R., Chacoff, N.P.,
581 Dalsgaard, B., de Sassi, C., Galetti, M., Guimaraes, P.R., Lomáscolo, S.B.,
582 Martín González, A.M., Pizo, M.A., Rader, R., Rodrigo, A., Tylianakis, J.M.,
583 Vazquez, D.P. & Allesina, S. (2013) The dimensionality of ecological networks.
584 *Ecology Letters*, **16**, 577–583.

- 585 Elberling, H. & Olesen, J.M. (1999) The structure of a high latitude plant-flower
586 visitor system: the dominance of flies. *Ecography*, **22**, 314–323.
- 587 Frund, J., McCann, K.S. & Williams, N.M. (2015) Sampling bias is a challenge
588 for quantifying specialization and network structure: lessons from a quantitative
589 niche model. *Oikos*, pp. n/a–n/a.
- 590 Gale, W.A. & Sampson, G. (1995) Good-Turing frequency estimation without
591 tears. *Journal of Quantitative Linguistics*, **2**, 217–237.
- 592 Gibson, R.H., Knott, B., Eberlein, T. & Memmott, J. (2011) Sampling method
593 influences the structure of plant–pollinator networks. *Oikos*, **120**, 822–831.
- 594 González-Varo, J.P., Arroyo, J.M. & Jordano, P. (2014) Who dispersed the seeds?
595 The use of DNA barcoding in frugivory and seed dispersal studies. *Methods in*
596 *Ecology and Evolution*, **5**, 806–814.
- 597 Good, I.J. (1953) The population frequencies of species and the estimation of
598 population parameters. *Biometrika*, **40**, 237–264.
- 599 Gotelli, N.J. & Colwell, R.K. (2011) Estimating species richness. *Biological Di-*
600 *versity Frontiers in Measurement and Assessment* (eds. A.E. Magurran & B.J.
601 McGill), pp. 39–54. Oxford University Press, Oxford, UK.
- 602 Gotelli, N. & Colwell, R. (2001) Quantifying biodiversity: procedures and pitfalls
603 in the measurement and comparison of species richness. *Ecology Letters*, **4**,
604 379–391.
- 605 Hortal, J., Borges, P. & Gaspar, C. (2006) Evaluating the performance of species

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

- 627 Martinez, N.D. (1993) Effects of resolution on food web structure. *Oikos*, **66**,
628 403–412.
- 629 Martinez, N. (1991) Artifacts or attributes? Effects of resolution on food-web
630 patterns in Little Rock Lake food web. *Ecological Monographs*, **61**, 367–392.
- 631 Maruyama, P.K., Vizentin-Bugoni, J., Oliveira, G.M., Oliveira, P.E. & Dalsgaard,
632 B. (2014) Morphological and spatio-temporal mismatches shape a neotropical
633 savanna plant-hummingbird network. *Biotropica*, **46**, 740–747.
- 634 Moré, M., Amorim, F.W., Benitez-Vieyra, S., Medina, A.M., Sazima, M. &
635 Cocucci, A.A. (2012) Armament Imbalances: Match and Mismatch in Plant-
636 Pollinator Traits of Highly Specialized Long-Spurred Orchids. *PLoS ONE*, **7**,
637 e41878.
- 638 Nielsen, A. & Bascompte, J. (2007) Ecological networks, nestedness and sampling
639 effort. *Journal of Ecology*, **95**, 1134–1141–1141.
- 640 Olesen, J.M., Bascompte, J., Dupont, Y.L., Elberling, H. & Jordano, P. (2011)
641 Missing and forbidden links in mutualistic networks. *Proceedings Of The Royal*
642 *Society B-Biological Sciences*, **278**, 725–732.
- 643 Olesen, J. & Jordano, P. (2002) Geographic patterns in plant-pollinator mutualistic
644 networks. *Ecology*, **83**, 2416–2424.
- 645 Olito, C. & Fox, J.W. (2014) Species traits and abundances predict metrics of
646 plant-pollinator network structure, but not pairwise interactions. *Oikos*, **124**,
647 428–436.

- 648 Ollerton, J. & Cranmer, L. (2002) Latitudinal trends in plant-pollinator interac-
649 tions: are tropical plants more specialised? *Oikos*, **98**, 340–350.
- 650 Pereira, H.M., Ferrier, S., Walters, M., Geller, G.N., Jongman, R.H.G., Scholes,
651 R.J., Bruford, M.W., Brummitt, N., Butchart, S.H.M., Cardoso, A.C., Coops,
652 N., Dulloo, E., Faith, D., Freyhof, J., Gregory, R.D., Heip, C., Hoft, R., Hurtt,
653 G., Jetz, W., Karp, D.S., Mcgeoch, M., Obura, D., Onoda, Y., Pettorelli, N.,
654 Reyers, B., Sayre, R., Scharlemann, J.P.W., Stuart, S., Turak, E., Walpole, M.
655 & Wegmann, M. (2013) Essential biodiversity variables. *Science*, **339**, 277–278.
- 656 Pocock, M.J.O., Evans, D.M. & Memmott, J. (2012) The Robustness and Restora-
657 tion of a Network of Ecological Networks. *Science*, **335**, 973–977.
- 658 Preston, F. (1948) The commonness, and rarity, of species. *Ecology*, **29**, 254–283.
- 659 R Development Core Team (2010) *R: A language and environment for statis-*
660 *tical computing*. R Foundation for Statistical Computing. Vienna, Austria.
661 <http://www.R-project.org>, Vienna, Austria.
- 662 Rivera-Hutinel, A., Bustamante, R.O., Marín, V.H. & Medel, R. (2012) Effects of
663 sampling completeness on the structure of plant-pollinator networks. *Ecology*,
664 **93**, 1593–1603.
- 665 Schleuning, M., Frund, J., Klein, A.M., Abrahamczyk, S., Alarcón, R., Albrecht,
666 M., Andersson, G.K.S., Bazarian, S., Böhning-Gaese, K., Bommarco, R., Dals-
667 gaard, B., Dehling, D.M., Gotlieb, A., Hagen, M., Hickler, T., Holzschuh, A.,
668 Kaiser-Bunbury, C.N., Kreft, H., Morris, R.J., Sandel, B., Sutherland, W.J.,
669 Svenning, J.C., Tschardtke, T., Watts, S., Weiner, C.N., Werner, M., Williams,

- 670 N.M., Winqvist, C., Dormann, C.F. & Blüthgen, N. (2012) Specialization of
 671 mutualistic interaction networks decreases toward tropical latitudes. *Current*
 672 *Biology*, **22**, 1925–1931.
- 673 Snow, B. & Snow, D. (1972) Feeding niches of hummingbirds in a Trinidad valley.
 674 *Journal of Animal Ecology*, **41**, 471–485.
- 675 Snow, B. & Snow, D. (1988) *Birds and berries*. Poyser, Calton, UK.
- 676 Stang, M., Klinkhamer, P., Waser, N.M., Stang, I. & van der Meijden, E. (2009)
 677 Size-specific interaction patterns and size matching in a plant-pollinator inter-
 678 action web. *Annals Of Botany*, **103**, 1459–1469.
- 679 Strogatz, S. (2001) Exploring complex networks. *Nature*, **410**, 268–276.
- 680 Stumpf, M.P.H., Wiuf, C. & May, R.M. (2005) Subnets of scale-free networks are
 681 not scale-free: Sampling properties of networks. *Proceedings of the National*
 682 *Academy of Sciences USA*, **102**, 4221–4224.
- 683 Thébault, E. & Fontaine, C. (2010) Stability of ecological communities and the
 684 architecture of mutualistic and trophic networks. *Science*, **329**, 853–856.
- 685 Valiente-Banuet, A., Aizen, M.A., Alcántara, J.M., Arroyo, J., Cocucci, A.,
 686 Galetti, M., García, M.B., García, D., Gomez, J.M., Jordano, P., Medel, R.,
 687 Navarro, L., Obeso, J.R., Oviedo, R., Ramírez, N., Rey, P.J., Traveset, A.,
 688 Verdú, M. & Zamora, R. (2014) Beyond species loss: the extinction of ecological
 689 interactions in a changing world. *Functional Ecology*, **29**, 299–307.
- 690 Vazquez, D.P., Chacoff, N.P. & Cagnolo, L. (2009) Evaluating multiple deter-

- 691 minants of the structure of plant-animal mutualistic networks. *Ecology*, **90**,
692 2039–2046.
- 693 Vazquez, D.P., Ramos-Jiliberto, R., Urbani, P. & Valdovinos, F.S. (2015) A con-
694 ceptual framework for studying the strength of plant-animal mutualistic inter-
695 actions. *Ecology Letters*, **18**, 385–400.
- 696 Vazquez, D., Morris, W. & Jordano, P. (2005) Interaction frequency as a surrogate
697 for the total effect of animal mutualists on plants. *Ecology Letters*, **8**, 1088–1094.
- 698 Vizentin-Bugoni, J., Maruyama, P.K. & Sazima, M. (2014) Processes entangling
699 interactions in communities: forbidden links are more important than abundance
700 in a hummingbird-plant network. *Proceedings Of The Royal Society B-Biological*
701 *Sciences*, **281**, 20132397–20132397.

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).

726 **Figures**

Figure 1:

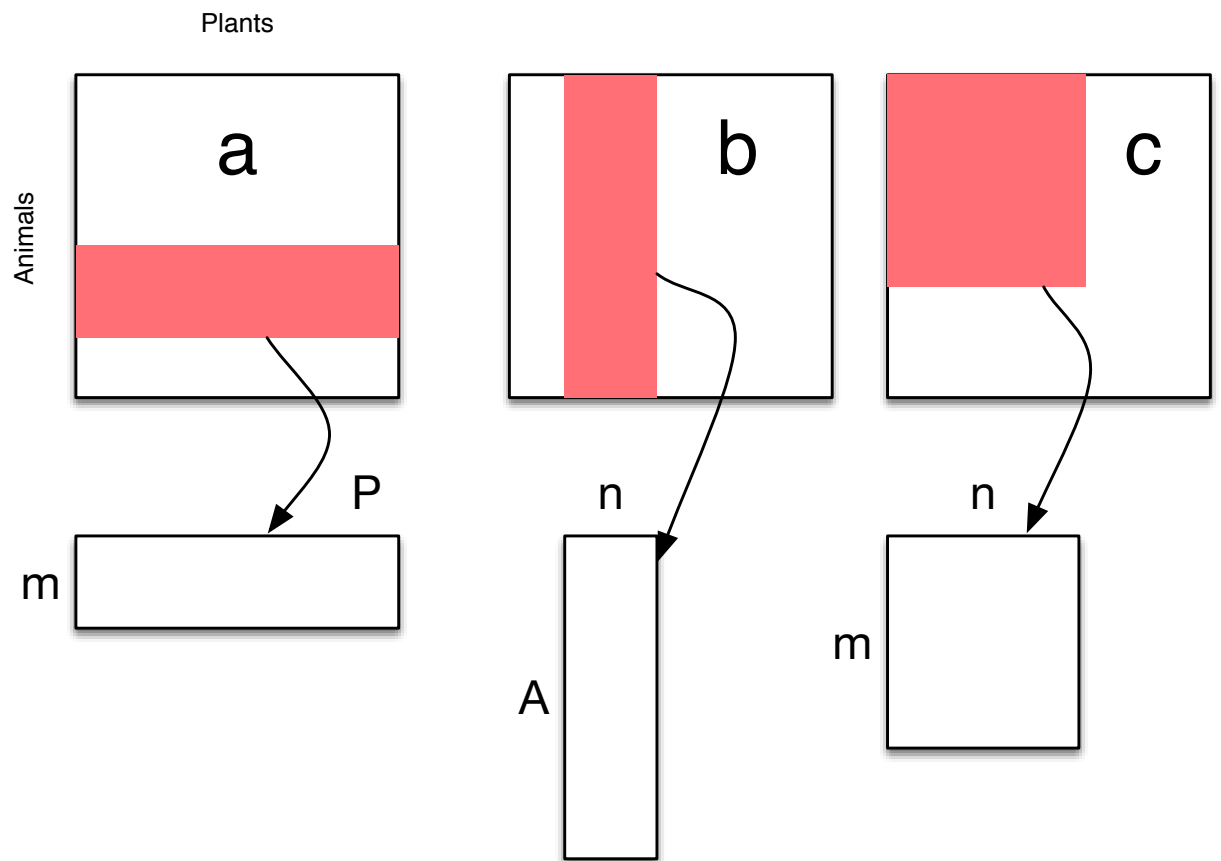
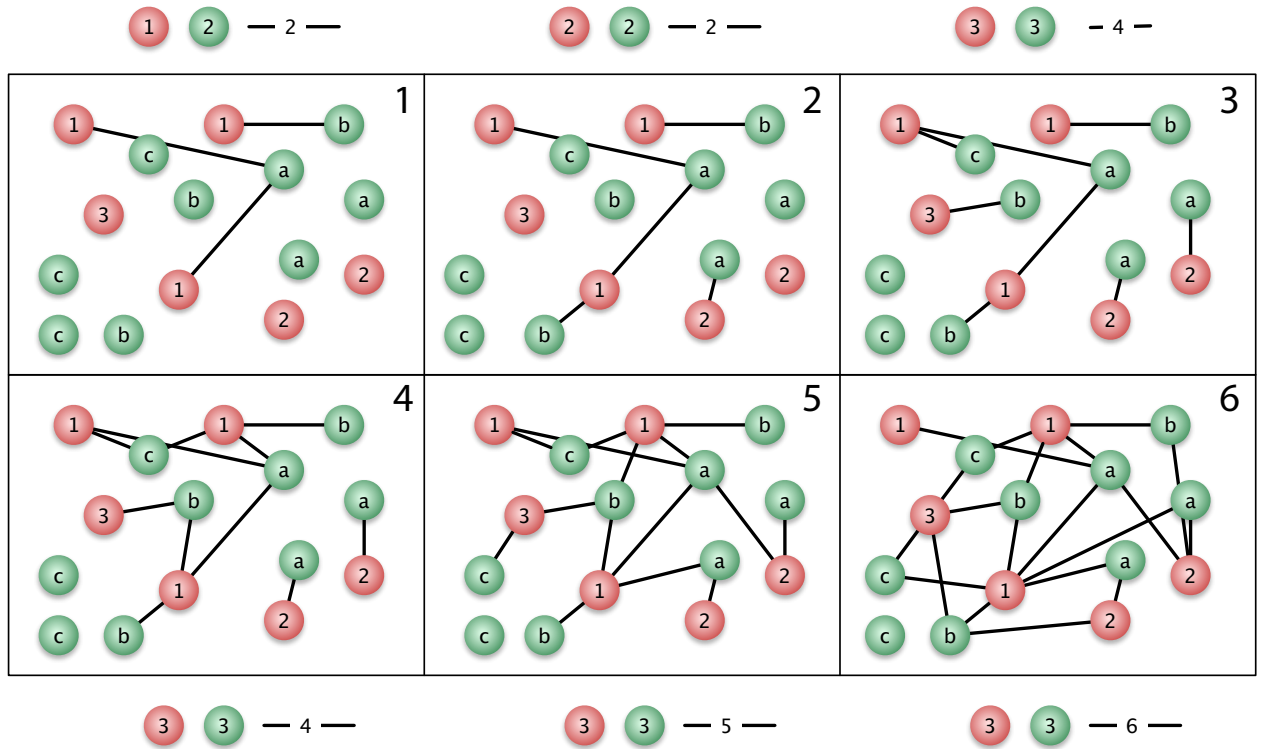


Figure 2:



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

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

755

756 **Tables**

Table 1:

Link type	Formulation	Definition
Potential links	$I_{max} = AP$	Size of network matrix, i.e. maximum number of potentially observable interactions; A and P , numbers of interacting animal and plant species, respectively.
Observed links	I	Total number of observed links in the network given a sufficient sampling effort. Number of ones in the adjacency matrix.
Unobserved links	$UL = I_{max} - I$	Number of zeroes in the adjacency matrix.
Forbidden links	FL	Number of links, which remain unobserved because of linkage constraints, irrespectively of sufficient sampling effort.
Missing links	$ML = AP - I - FL$	Number of links, which may exist in nature but need more sampling effort and/or additional sampling methods to be observed.

Table 2:

Link type	Pollination			Seed dispersal		
	Zackenber	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	$\dots(\dots)$	8 (0.0184)	30 (0.0890)	140 (0.1894)	31 (0.1834)	46 (0.0714)
FL_A	$\dots(\dots)$	5 (0.0115)	150 (0.445) ^a	$\dots(\dots)$	20 (0.1183)	61 (0.0947)
FL_O	$\dots(\dots)$	$\dots(\dots)$	38 (0.1128) ^b	$\dots(\dots)$	$\dots(\dots)$	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.

Table 3:

Interaction	Sample 1	Sample 2	Sample 3	...	Sample i
A1 - P2	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
$Scaled\ Chao$	195.4	162.7	308.4
CI	[124.5–266.3]	[148.5–176.9]	[253.6–363.1]
$Scaled\ ACE$	178.5	169.7	342.6
CI	[169.5–187.4]	[161.8–177.6]	[327.8–357.4]
% <i>unobserved</i> ^a	8.33	15.38	47.80

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