# 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. Interactions are just pairwise relationships among individuals of two different 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.

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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, i.e., life-history restrictions. Thus, the number of observed interactions $I$ in robustly sampled networks is typically $I << I_{max}$, resulting in extremely sparse interaction matrices with low connectance.

4. Reasons for forbidden links are multiple but mainly stem from spatial and temporal uncoupling, 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 "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. Contrary to species inventories, a sizable fraction of non-observed pairwise interactions cannot be sampled, due to biological constraints that forbid their occurrence.

6. 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 defaunation-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

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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).
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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-

versity in collections of individuals is immense, and a number of useful approaches have been used to obtain such estimates (Magurran, 1988; Gotelli & Colwell, 2001; Colwell, Mao & Chang, 2004; Hortal, Borges & Gaspar, 2006; Colwell, 2009; Gotelli & Colwell, 2011; Chao *et al.*, 2014). Recent effort has been also focused at defining essential biodiversity variables (EBV) (Pereira *et al.*, 2013) that can be sampled and measured repeatedly 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 we 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. We 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 we provide a succinct overview of previous work addressing sampling issues for ecological interaction networks. In the second part, we discuss specific rationales for sampling the biodiversity of ecological interactions. Finally, we 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.

30 Interactions can be a much better indicator of the richness and diversity of
31 ecosystem functions than a simple list of taxa and their abundances and/or related
32 biodiversity indicator variables (EBVs) (Memmott *et al.*, 2006; Valiente-Banuet
33 *et al.*, 2014). Thus, sampling interactions should be a central issue when identifying
34 and diagnosing ecosystem services (e.g., pollination, natural seeding by frugivores,
35 etc.). Fortunately, the whole battery of biodiversity-related tools used by ecologists
36 to sample biodiversity (species, *sensu stricto*) can be extended and applied to the
37 sampling of interactions. Analogs are evident between these approaches (see Table
38 2 in Colwell, Mao & Chang, 2004). Monitoring interactions is a type of biodiversity
39 sampling and is subject to similar methodological shortcomings, especially under-
40 sampling (Jordano, 1987; Jordano, Vázquez & Bascompte, 2009; Coddington *et al.*,
41 2009; Vázquez, Chacoff & Cagnolo, 2009; Dorado *et al.*, 2011; Rivera-Hutinel *et al.*,
42 2012). For example, when we study mutualistic networks, our goal is to make an
43 inventory of the distinct pairwise interactions that made up the network. We are
44 interested in having a complete list of all the pairwise interactions among species
45 (e.g., all the distinct, species-species interactions, or links, among the pollinators
46 and flowering plants) that do actually exist in a given community. Sampling these
47 interactions thus entails exactly the same problems, limitations, constraints, and
48 potential biases as sampling individual organisms and species diversity. As Mao
49 & Colwell (2005) put it, these are the workings of Preston's demon, the moving
50 "veil line" (Preston, 1948) between the detected and the undetected interactions
51 as sample size increases.

52 Early efforts to recognize and solve sampling problems in analyses of interac-
53 tions stem from research on food webs and to determine how undersampling biases
54 food web metrics (Martinez, 1991; Cohen *et al.*, 1993; Martinez, 1993; Bersier,

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55  Banasek-Richter & Cattin, 2002; Brose, Martinez & Williams, 2003; Banasek-

56  Richter, Cattin & Bersier, 2004; Wells & O'Hara, 2012). In addition, the myriad

57  of classic natural history studies documenting animal diets, host-pathogen infection

58  records, plant herbivory records, etc., represent efforts to document interactions

59  occurring in nature. All of them share the problem of sampling incompleteness in-

60  fluencing the patterns and metrics reported. Yet, despite the early recognition that

61  incomplete sampling may seriously bias the analysis of ecological networks (Jor-

62  dano, 1987), only recent studies have explicitly acknowledged it and attempted to

63  determine its influence (Ollerton & Cranmer, 2002; Nielsen & Bascompte, 2007;

64  Vázquez, Chacoff & Cagnolo, 2009; Gibson *et al.*, 2011; Olesen *et al.*, 2011; Chacoff

65  *et al.*, 2012; Rivera-Hutinel *et al.*, 2012; Olito & Fox, 2014; Bascompte & Jordano,

66  2014; Vizentin-Bugoni, Maruyama & Sazima, 2014; Frund, McCann & Williams,

67  2015). The sampling approaches have been extended to predict patterns of coex-

68  tintions in interaction assemblages (e.g., hosts-parasites) (Colwell, Dunn & Harris,

69  2012). Most empirical studies provide no indication of sampling effort, implicitly

70  assuming that the reported network patterns and metrics are robust. Yet recent ev-

71  idences point out that number of partner species detected, number of actual links,

72  and some aggregate statistics describing network patterns, are prone to sampling

73  bias (Nielsen & Bascompte, 2007; Dorado *et al.*, 2011; Olesen *et al.*, 2011; Chacoff

74  *et al.*, 2012; Rivera-Hutinel *et al.*, 2012; Olito & Fox, 2014; Frund, McCann &

75  Williams, 2015). Most of these evidences, however, come either from simulation

76  studies (Frund, McCann & Williams, 2015) or from relatively species-poor assem-

77  blages. Most certainly, sampling limitations pervade biodiversity inventories in

78  tropical areas (Coddington *et al.*, 2009) and we might rightly expect that frequent

79  interactions may be over-represented and rare interactions may be missed entirely

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80 in studies of mega-diverse assemblages (Bascompte & Jordano, 2014); but, to what

81 extent?

# Sampling interactions: methods

83 When we sample interactions in the field we record the presence of two species

84 that interact in some way. For example, Snow and Snow(1988) recorded an inter-

85 action whenever they saw a bird "touching" a fruit on a plant. We observe and

86 record feeding observations, visitation, occupancy, presence in pollen loads or in

87 fecal samples, etc., of *individual* animals or plants and accumulate pairwise inter-

88 actions, i.e., lists of species partners and the frequencies with which we observe

89 them. Therefore, estimating the sampling completeness of pairwise interactions for

90 a whole network, requires some gauging of how the number (richness) of distinct

91 pairwise interactions accumulates as sampling effort is increased) and/or estimat-

92 ing the uncertainty around the missed links (Wells & O'Hara, 2012).

93 Most types of ecological interactions can be illustrated with bipartite graphs,

94 with two or more distinct groups of interacting partners (Bascompte & Jordano,

95 2014); for illustration purposes I'll focus more specifically on plant-animal inter-

96 actions. Sampling interactions requires filling the cells of an interaction matrix

97 with data. The matrix, $\Delta = AP$ (the adjacency matrix for the graph representa-

98 tion of the network), is a 2D inventory of the interactions among, say, $A$ animal

99 species (rows) and $P$ plant species (columns) (Jordano, 1987; Bascompte & Jor-

100 dano, 2014). The matrix entries illustrate the values of the pairwise interactions

101 visualized in the $\Delta$ matrix, and can be 0 or 1, for presence-absence of a given

102 pairwise interaction, or take a quantitative weight $w_{ji}$ to represent the interaction

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103 intensity or unidirectional effect of species $j$ on species $i$ (Bascompte & Jordano,

104 2014; Vazquez *et al.*, 2015). The outcomes of most ecological interactions are

105 dependent on frequency of encounters (e.g., visit rate of pollinators, number of

106 records of ant defenders, frequency of seeds in fecal samples). Thus, a frequently

107 used proxy for interaction intensities $w_{ji}$ is just how frequent new interspecific

108 encounters are, whether or not appropriately weighted to estimate interaction ef-

109 fectiveness (Vazquez, Morris & Jordano, 2005).

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

111 type of interactions we sample; and 2) which type of record we get to document

112 the existence of an interaction. In step #1 we need to take into account whether

113 we are sampling the whole community of interactor species (all the animals, all

114 the plants) or just a subset of them, i.e., a sub matrix $\Delta_{m,n}$ of $m < A$ animal

115 species and $n < P$ plant species of the adjacency matrix $\Delta_{AP}$ (i.e., the matrix

116 representation of interactions among the partner species). Subsets can be: a) all

117 the potential plants interacting with a subset of the animals (Fig. 1a); b) all the

118 potential animal species interacting with a subset of the plant species (Fig. 1b);

119 c) a subset of all the potential animal species interacting with a subset of all the

120 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

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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

136 body of pollinators, analysis of fecal samples of frugivores, radio-tracking data, etc.

137 Phyto-centric studies take samples of focal individual plant species and document

138 which animals 'arrive' or 'touch' the plants. Examples include focal watches of

139 fruiting or flowering plants to record visitation by animals, raising insect herbivores

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

141 Most recent analyses of plant-animal interaction networks are phyto-centric;

142 just 3.5% of available plant-pollinator ($N = 58$) or 36.6% plant-frugivore ($N = 22$)

143 interaction datasets are zoo-centric (see Schleuning *et al.*, 2012). Moreover, most

144 available datasets on host-parasite (parasitoid) or plant-herbivore interactions are

145 "host-centric" or phyto-centric (e.g., Thébault & Fontaine, 2010; Morris *et al.*,

146 2013; Eklöf *et al.*, 2013). This may be related to a variety of causes, like preferred

147 methodologies by researchers working with a particular group or system, logistic

148 limitations, or inherent taxonomic focus of the research questions. A likely result

149 of phyto-centric sampling would be adjacency matrices with large $A : P$ ratios.

150 In any case we don't have a clear view of the potential biases that taxa-focused

151 sampling may generate in observed network patterns, for example by generating

152 consistently asymmetric interaction matrices (Dormann *et al.*, 2009). System sym-

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metry 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).

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. Connectance increased 1.43-fold, mean plant connectivity went from 18.5 to 26.4, and mean pollinator connectivity from 2.9 to 4.1; moreover, extreme specialist pollinator species (singletons in the adjacency matrix) decreased 0.6-fold. 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 of DNA-barcoding, for example with plant-herbivore (Jurado-Rivera *et al.*, 2009), host-parasiotid (Wirta *et al.*, 2014), and plant-frugivore interactions (González-Varo, Arroyo & Jordano, 2014). For mutualistic networks we would expect that zoo-centric sampling could help unveiling interactions of the animals with rare plant species or for relatively common plants species which are difficult to sample by direct observation. Future methodological work may provide significant advances showing how mixing different sampling strategies strengthens the completeness of network data. These mixed strategies may combine, for instance, timed watches at focal plants, spot

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178 censuses along walked transects, pollen load or seed contents analyses, monitoring

179 with camera traps, and DNA barcoding records. We might expect increased power

180 of these mixed sampling approaches when combining different methods from both

181 phyto- and zoo-centric perspectives (Bosch *et al.*, 2009; Blüthgen, 2010). Note also

182 that the different methods could be applied in different combinations to the two

183 distinct sets of species. However, there are no tested protocols and/or sampling

184 designs for ecological interaction studies to suggest an optimum combination of

185 approaches. Ideally, pilot studies would provide adequate information for each

186 specific study setting.

# Sampling interactions: rationale

188 The number of distinct pairwise interactions that we can record in a landscape

189 (an area of relatively homogeneous vegetation, analogous to the one we would

190 use to monitor species diversity) is equivalent to the number of distinct classes in

191 which we can classify the recorded encounters among *individuals* of two different

192 species. Yet, individual-based interaction networks have been only recently studied

193 (Dupont, Trøjelsgaard & Olesen, 2011; Wells & O'Hara, 2012). The most usual

194 approach has been to pool indiviudal-based interaction data into species-based

195 summaries, an approach that ignores the fact that only a fraction of individuals

196 may actually interact given a per capita interaction effect (Wells & O'Hara, 2012).

197 Wells & O'Hara (2012) illustrate the pros and cons of the approach. We walk in

198 the forest and see a blackbird $Tm$ picking an ivy $Hh$ fruit and ingesting it: we

199 have a record for $Tm - Hh$ interaction. We keep advancing and record again a

200 blackbird feeding on hawthorn $Cm$ fruits so we record a $Tm - Cm$ interaction;

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201 as we advance we encounter another ivy plant and record a blackcap swallowing a

202 fruit so we now have a new $Sa - Hh$ interaction, and so on. At the end we have

203 a series of classes (e.g., $Sa - Hh$, $Tm - Hh$, $Tm - Cm$, etc.), along with their

204 observed frequencies. Bunge & Fitzpatrick (1993) provide an early review of the

205 main aspects and approaches to estimate the number of distinct classes $C$ in a

206 sample of observations.

207 Our sampling above would have resulted in a vector $n = [n_1...n_C]'$ where $n_i$ is

208 the number of records in the $i^{th}$ class. As stressed by Bunge & Fitzpatrick (1993),

209 however, the $i^{th}$ class would appear in the sample if and only if $n_i > 0$, and we

210 don't know *a priori* which $n_i$ are zero. So, $n$ is not observable. Rather, what we

211 get is a vector $c = [c_1...c_n]'$ where $c_j$ is the number of classes represented $j$ times

212 in our sampling: $c_1$ is the number of singletons (interactions recorded once), $c_2$

213 is the number of twin pairs (interactions with just two records), $c_3$ the number

214 of triplets, etc. The problem thus turns to be estimating the number of distinct

215 classes $C$ from the vector of $c_j$ values and the frequency of unobserved interactions

216 (see "The real missing links" below).

217 More specifically, we usually obtain a type of reference sample (Chao *et al.*,

218 2014) for interactions: a series of replicated samples (e.g., observation days, 1h

219 watches, etc.) with quantitative information, i.e., recording the number of in-

220 stances of each interaction type on each day. This replicated abundance data,

221 can be treated in three ways: 1) Abundance data within replicates: the counts

222 of interactions, separately for each day; 2) Pooled abundance data: the counts of

223 interactions, summed over all days (the most usual approach); and 3) Replicated

224 incidence data: the number of days on which we recorded each interaction. Assum-

225 ing a reasonable number of replicates, replicated incidence data is considered the

226 most robust statistically, as it takes account of heterogeneity among days (Colwell,

227 Mao & Chang, 2004; Colwell, Dunn & Harris, 2012; Chao *et al.*, 2014). Thus, both

228 presence-absence and weighted information on interactions can be accommodated

229 for this purpose.

## The species assemblage

231 When we consider an observed and recorded sample of interactions on a particular

232 assemblage of $A_{obs}$ and $P_{obs}$ species (or a set of replicated samples) as a reference

233 sample (Chao *et al.*, 2014) we may have three sources of undersampling error that

234 are ignored by treating a reference sample as a true representation of the inter-

235 actions in well-defined assemblage: 1) some animal species are actually present

236 but not observed (zero abundance or incidence in the interactions in the reference

237 sample), $A_0$; 2) some plant species are actually present but not observed (zero

238 abundance or incidence in the interactions in the reference sample), $P_0$; 3) some

239 unobserved links (the zeroes in the adjacency matrix, $UL$) may actually occur but

240 not recorded. Thus a first problem is determining if $A_{obs}$ and $P_{obs}$ truly represent

241 the actual species richness interacting in the assemblage. To this end we might use

242 the replicated reference samples to estimate the true number of interacting animal

243 $A_{est}$ and plant $P_{est}$ species as in traditional diversity estimation analysis (Chao

244 *et al.*, 2014). If there are no uniques (species seen on only one day), then $A_0$ and

245 $P_0$ will be zero, and we have $A_{obs}$ and $P_{obs}$ as robust estimates of the actual species

246 richness of the assemblage. If $A_0$ and $P_0$ are not zero they estimate the minimum

247 number of undetected animal and plant species that can be expected with a suf-

248 ficiently large number of replicates, taken from the same assemblage/locality by

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the same methods in the same time period. We can use extrapolation methods (Colwell, Dunn & Harris, 2012) to estimate how many additional replicate surveys it would take to reach a specified proportion $g$ 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 $\Delta$ 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, Bascompte & Olesen, 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

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272 assemblage species richness and to the observed assemblage species richness, re-

273 spectively (Table 1).

274 Forbidden links are non-occurrences of pairwise interactions that can be ac-

275 counted for by biological constraints, such as spatio-temporal uncoupling (Jor-

276 dano, 1987), size or reward mismatching, foraging constraints (e.g., accessibility)

277 (Moré *et al.*, 2012), and physiological-biochemical constraints (Jordano, 1987). We

278 still have very little information about the frequency of forbidden links in natural

279 communities (Jordano, Bascompte & Olesen, 2003; Stang *et al.*, 2009; Vázquez,

280 Chacoff & Cagnolo, 2009; Olesen *et al.*, 2011; Ibanez, 2012; Maruyama *et al.*, 2014;

281 Vizentin-Bugoni, Maruyama & Sazima, 2014) (Table 1). Forbidden links are thus

282 represented as structural zeroes in the interaction matrix, i.e., matrix cells that

283 cannot get a non-zero value.

284 We might expect different types of $FL$ to occupy different parts of the $\Delta$ ma-

285 trix, with missing cells due to phenological uncoupling, $FL_P$, largely distributed

286 in the lower-right half $\Delta$ matrix and actually missed links $ML$ distributed in its

287 central part (Olesen *et al.*, 2010). Yet, most of these aspects remain understud-

288 ied. Therefore, we need to account for the frequency of these structural zeros in

289 our matrix before proceeding. For example, most measurements of connectance

290 $C = I/(AP)$ implicitly ignore the fact that by taking the full product $AP$ in the

291 denominator they are underestimating the actual connectance value, i.e., the frac-

292 tion of actual interactions $I$ relative to the *biologically possible* ones, not to the

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

294 Our main problem then turns to estimate the number of true missed links,

295 i.e., those that can't be accounted for by biological constraints and that might

296 suggest undersampling. Thus, the sampling of interactions in nature, as the sam-

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pling of species, is a cumulative process. In our analysis, we are not re-sampling individuals, but interactions, so we made 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 (N. Gotelli, pers. com.). 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 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 sam-

pling 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 marked pattern of microhabitat mismatches causing up to 44.5% of the forbidden links. A myriad of biological causes beyond those included as $FL$ in Table 2 may contribute explanations for $UL$: limits of color perception and or partial preferences, presence of secondary metabolites in fruit pulp and leaves, toxins and combinations of monosaccharides in nectar, etc. For example, aside from $FL$, some pairwise interactions may simply have an asymptotically-zero probability of interspecific encounter between the partner species, if they are very rare. However, it is surprising that just the limited set of forbidden link types considered in Table 1 explain between 24.6-77.2% of the unobserved links. Notably, the Arima Valley, Santa Virgínia, and Hato Ratón networks have $> 60\%$ of the unobserved links explained, which might be related to the fact that they are subnetworks (Arima Valley, Santa Virgínia) or relatively small networks (Hato Ratón). All this means that empirical networks may have sizable fractions of structural zeroes. Ignoring this biological fact may contribute to wrongly inferring undersampling of interactions in real-world assemblages.

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345     To sum up, two elements of inference are required in the analysis of unobserved

346 interactions in ecological interaction networks: first, detailed natural history infor-

347 mation on the participant species that allows the inference of biological constraints

348 imposing forbidden links, so that structural zeroes can be identified in the adja-

349 cency matrix. Second, a critical analysis of sampling robustness and a robust

350 estimate of the actual fraction of missing links, $M$, resulting in a robust estimate

351 of $I$. In the next sections we explore these elements of inference, using $IACs$ as

352 analogs to $SACs$ to assess the robustness of interaction sampling.

# Asymptotic diversity estimates

354 Let's assume a sampling of the diversity in a specific locality, over relatively ho-

355 mogeneous landscape where we aim at determining the number of species present

356 for a particular group of organisms. To do that we carry out transects or plot

357 samplings across the landscape or use any other type of direct or indirect record-

358 ing method, adequately replicated so we obtain a number of samples. Briefly, $S_{obs}$

359 is the total number of species observed in a sample, or in a set of samples. $S_{est}$

360 is the estimated number of species in the community represented by the sample,

361 or by the set of samples, where $est$ indicates an estimator. With abundance data,

362 let $S_k$ be the number of species each represented by exactly $k$ individuals in a sin-

363 gle sample. Thus, $S_0$ is the number of undetected species (species present in the

364 community but not included in the sample), $S_1$ is the number of singleton species

365 (represented by just one individual), $S_2$ is the number of doubleton species (species

366 with two individuals), etc. The total number of individuals in the sample would be:

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

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369 A frequently used asymptotic, bias corrected, non-parametric estimator is $S_{Chao1}$
370 (Hortal, Borges & Gaspar, 2006; Chao, 2005; Colwell, 2013):

$$S_{Chao1} = S_{obs} + \frac{S_1(S_1 - 1)}{2(S_2 + 1)}$$

371 Another frequently used alternative is the Chao2 estimator, $S_{Chao2}$ (Gotelli &
372 Colwell, 2001), which has been reported to have a limited bias for small sample
373 sizes (Colwell & Coddington, 1994; Chao, 2005). Instead of using counts it uses
374 incidence frequencies ($Q_k$) among samples (number of species present in just one
375 sample, in two samples, etc.):

$$S_{Chao2} = S_{obs} + \frac{Q_1(Q_1 - 1)}{2(Q_2 + 1)}$$

376 A plot of the cumulative number of species recorded, $S_n$, as a function of some
377 measure of sampling effort (say, $n$ samples taken) yields the species accumulation
378 curve (SAC) or collector's curve (Colwell & Coddington, 1994). Similarly, inter-
379 action accumulation curves (IAC), analogous to SACs, can be used to assess the
380 robustness of interactions sampling for plant-animal community datasets (Jordano,
381 1987; Jordano, Vázquez & Bascompte, 2009; Olesen *et al.*, 2011), as discussed in
382 the next section.

# 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 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). 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 (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, Vázquez & Bascompte, 2009). The package *EstimateS* (Colwell, 2013) includes a complete set of functions for estimating the mean IAC and its unconditional standard deviation 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, Vázquez & Bascompte,

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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, Mao & Chang, 2004; Colwell, Dunn & Harris, 2012; Chao *et al.*, 2014).

In this way we effectively extend sampling theory developed for species diversity to the sampling of ecological interactions. Yet future theoretical work will be needed to formally assess the similarities and differences in the two approaches and developing biologically meaningful null models of expected interaction richness with added sampling effort.

Diversity-accumulation analysis (Magurran, 1988; Hortal, Borges & Gaspar, 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, 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) (see Supplementary Online Material). It should be noted that the asymptotic estimate of interaction richness explicitly ignores the fact that, due to forbidden links, a number of pairwise interactions among the $I_{max}$ number specified in the adjacency matrix $\Delta$ 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, Maruyama & Sazima, 2014), typically with more homogeneous subsets of species

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<sup>432</sup> (e.g., bumblebee-pollinated flowers). In any case the sparseness of the $\Delta$ matrix

<sup>433</sup> is by no means an indication of undersampling whenever the issue of structural

<sup>434</sup> zeroes in the interaction matrices is effectively incorporated in the estimates.

<sup>435</sup> For example, mixture models incorporating detectabilities have been proposed

<sup>436</sup> to effectively account for rare species (Mao & Colwell, 2005). In an analogous line,

<sup>437</sup> mixture models could be extended to samples of pairwise interactions, also with

<sup>438</sup> specific detectability values. These detection rate/odds could be variable among

<sup>439</sup> groups of interactions, depending on their specific detectability. For example,

<sup>440</sup> detectability of flower-pollinator interactions involving bumblebees could have a

<sup>441</sup> higher detectability than flower-pollinator pairwise interactions involving, say, ni-

<sup>442</sup> tidulid beetles. These more homogeneous groupings of pairwise interactions within

<sup>443</sup> a network define modules (Bascompte & Jordano, 2014), so we might expect that

<sup>444</sup> interactions of a given module (e.g., plants and their hummingbird pollinators; Fig.

<sup>445</sup> 1a) may share similar detectability values, in an analogous way to species groups

<sup>446</sup> receiving homogeneous detectability values in mixture models (Mao & Colwell,

<sup>447</sup> 2005). In its simplest form, this would result in a sample with multiple pairwise

<sup>448</sup> interactions detected, in which the number of interaction events recorded for each

<sup>449</sup> distinct interaction found in the sample is recorded (i.e., a column vector in Table

<sup>450</sup> 3, corresponding to, say, a sampling day). The number of interactions recorded for

<sup>451</sup> the $i_{th}$ pairwise interaction (i.e., $A_i P_j$ in Table 3), $Y_i$ could be treated as a Poisson

<sup>452</sup> random variable with a mean parameter $\lambda_i$, its detection rate. Mixture models

<sup>453</sup> (Mao & Colwell, 2005) include estimates for abundance-based data (their analogs

<sup>454</sup> in interaction sampling would be weighted data), where $Y_i$ is a Poisson random

<sup>455</sup> variable with detection rate $\lambda_i$. This is combined with the incidence-based model,

<sup>456</sup> where $Y_i$ is a binomial random variable (their analogous in interaction sampling

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would be presence/absence records of interactions) with detection odds $\lambda_i$. 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 $\lambda_i$ depend on the relative abundances $\phi_i$ 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, $\phi_i$, 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, $\lambda_i$ should be set to zero for all $FL$. It its simplest form, $\phi_i$ 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 (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 sam-

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478 pling effort better alternatives include the simpler incidence-based rarefaction and

479 extrapolation (Colwell, Dunn & Harris, 2012; Chao *et al.*, 2014).

# The *real* missing links

481 Given that a fraction of unobserved interactions can be accounted for by for-

482 bidden links, what about the remaining missing interactions? We have already

483 discussed that some of these could still be related to unaccounted constraints, and

484 still others would be certainly attributable to insufficient sampling. Would this

485 always be the case? Multispecific assemblages of distinct taxonomic relatedness,

486 whose interactions can be represented as bipartite networks (e.g., host-parasite,

487 plant-animal mutualisms, plant-herbivore interactions- with two distinct sets of

488 unrelated higher taxa), are shaped by interspecific encounters among individuals

489 of the partner species (Fig. 2). A crucial ecological aspect limiting these inter-

490 actions is the probability of interspecific encounter, i.e., the probability that two

491 individuals of the partner species actually encounter each other in nature.

492 Given log-normally distributed abundances of the two species groups, the ex-

493 pected probabilities of interspecific encounter ($PIE$) would be simply the product

494 of the two lognormal distributions. Thus, we might expect that for low $PIE$ values,

495 pairwise interactions would be either extremely difficult to sample, or simply do

496 not occur in nature. Consider the Nava de las Correhuelas interaction web (NCH,

497 Table 2), with $A = 36$, $P = 25$, $I = 181$, and almost half of the unobserved inter-

498 actions not accounted for by forbidden links, thus $M = 53.1\%$. Given the robust

499 sampling of this network (Jordano, Vázquez & Bascompte, 2009), a sizable fraction

500 of these possible but missing links would be simply not occurring in nature, most

likely due to 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 $PIE_{median} < 1.4 \ 10^{-4}$ we may safely expect (note the quantile estimate $Q_{75\%} = 3.27 \ 10^{-4}$) 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). 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:

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

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.

523 Strictly speaking Equation (1) gives the probability that the next interaction type

524 recorded will be $X$, after sampling a given assemblage of interacting species. In

525 other words, we scale down the maximum-likelihood estimator $\frac{n}{T}$ by a factor of

526 $\frac{1-E(1)}{T}$. This reduces all the probabilities for interactions we have recorded, and

527 makes room for interactions we haven't seen. If we sum over the interactions we

528 have seen, then the sum of $P(X)$ is $1 - \frac{1-E(1)}{T}$. Because probabilities sum to one,

529 we have the left-over probability of $P_{new} = \frac{E(1)}{T}$ of seeing something new, where

530 new means that we sample a new pairwise interaction. Note, however, that Good-

531 Turing estimators, the traditional asymptotic estimators, do not account in our

532 case for the forbidden interactions.

# Discussion

534 Recent work has inferred that most data available for interaction networks are

535 incomplete due to undersampling, resulting in a variety of biased parameters and

536 network patterns (Chacoff *et al.*, 2012). It is important to note, however, that in

537 practice, most surveyed networks to date have been subnets of much larger net-

538 works. This is also true for protein interaction, gene regulation, and metabolic

539 networks, where only a subset of the molecular entities in a cell have been sam-

540 pled (Stumpf, Wiuf & May, 2005). Despite recent attempts to document whole

541 ecosystem meta-networks (Pocock, Evans & Memmott, 2012), it is likely that most

542 ecological interaction networks will illustrate just major ecosystem compartments.

543 Due to their high generalization, high temporal and spatial turnover, and high

544 complexity of association patterns, adequate sampling of ecological interaction

545 networks is challenging and requires extremely large sampling effort. Undersam-

pling 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; thus, we need to assess how robust is the characterization of the adjacency matrix $\Delta$. 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

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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 accessiblity

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`.

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# 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 $\Delta$ 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).
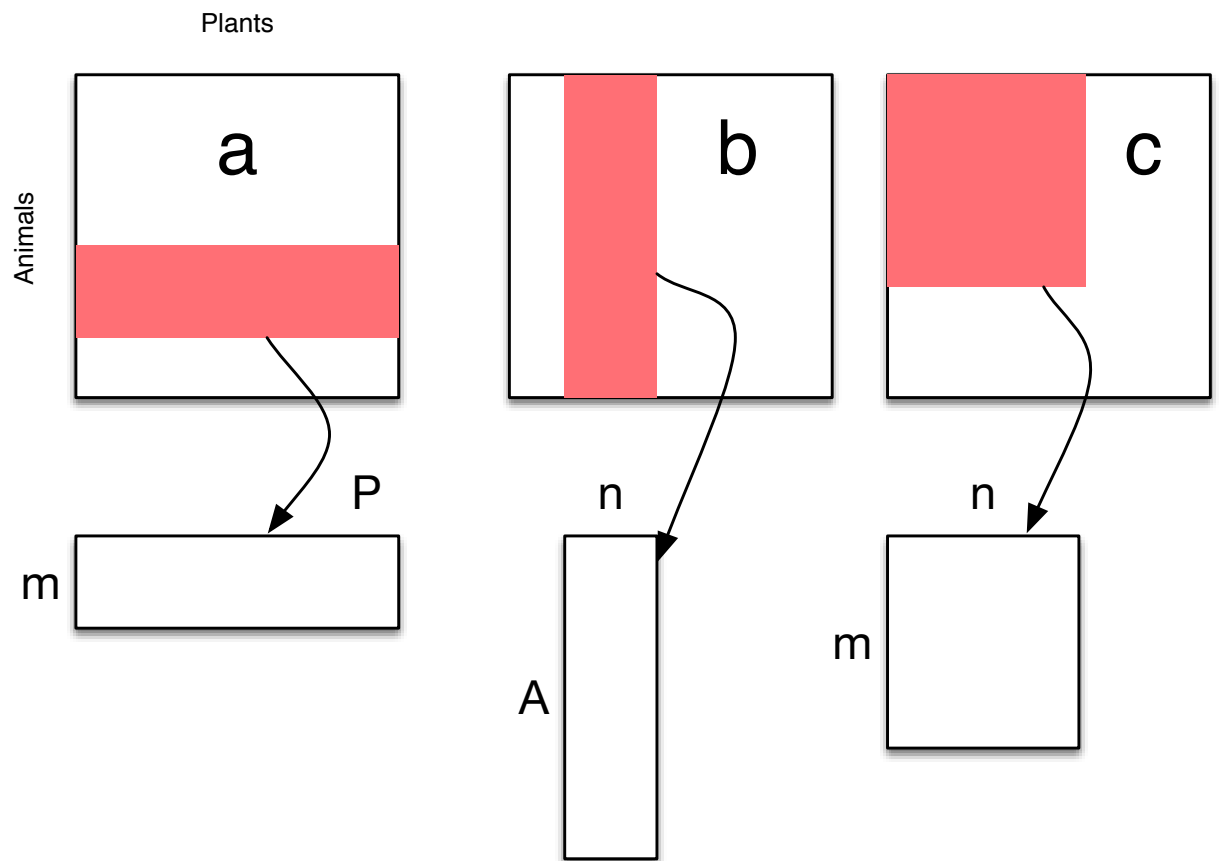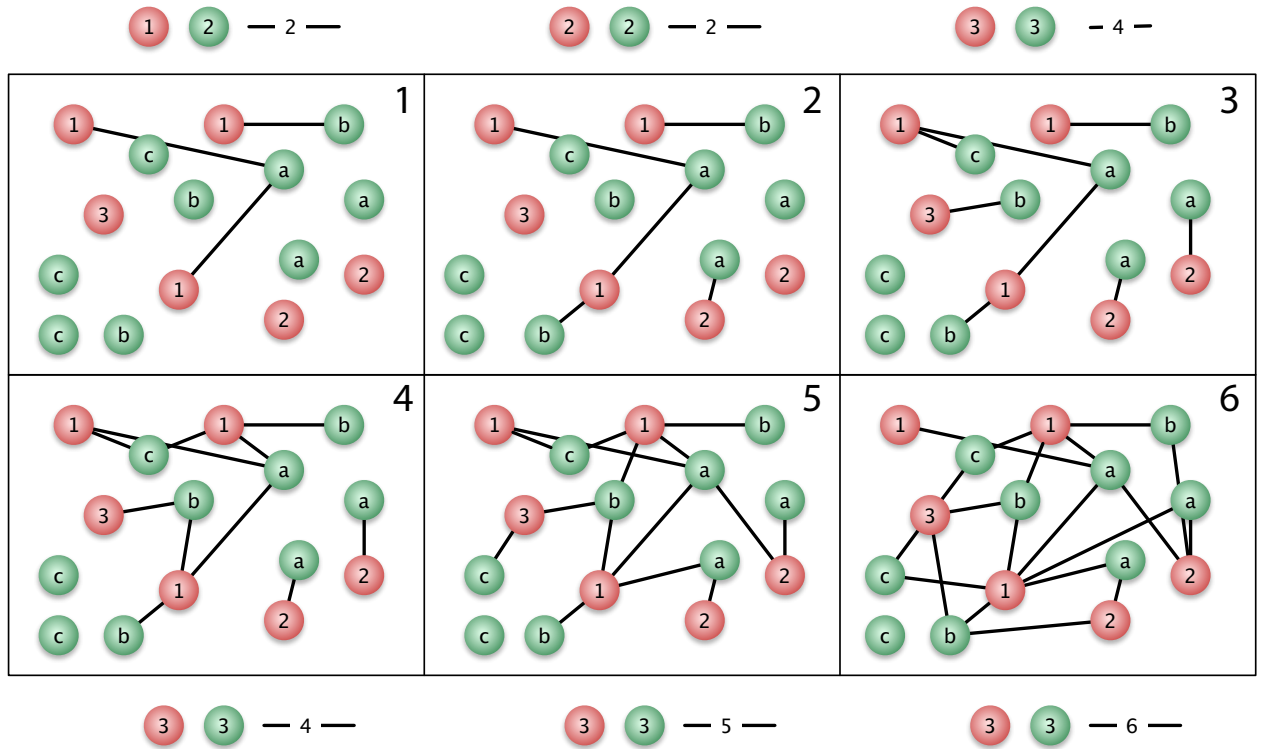
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# 837 Figures

Figure 1:

Plants

Animals

a

b

c

P

m

n

A

n

m

41

Figure 2:

# 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

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862 *et al.* 2006), and their standard errors; $C$, sample coverage for rare interactions

863 (Chao & Jost 2012). Scaled asymptotic estimators and their confidence intervals

864 ($CI$) were calculated by weighting $Chao1$ and $ACE$ with the observed frequencies

865 of forbidden links.

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# 867 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. |

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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) |
| $FL_S$ | $\cdots (\cdots)$ | 8 (0.0184) | 30 (0.0890) | 140 (0.1894) | 31 (0.1834) | 46 (0.0714) |
| $FL_A$ | $\cdots (\cdots)$ | 5 (0.0115) | 150 $(0.445)^a$ | $\cdots (\cdots)$ | 20 (0.1183) | 61 (0.0947) |
| $FL_O$ | $\cdots (\cdots)$ | $\cdots (\cdots)$ | 38 $(0.1128)^b$ | $\cdots (\cdots)$ | $\cdots (\cdots)$ | 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 |
| | | | |
| $Chao1$ | $263.1 \pm 70.9$ | $231.4 \pm 14.2$ | $509.6 \pm 54.7$ |
| $ACE$ | $240.3 \pm 8.9$ | $241.3 \pm 7.9$ | $566.1 \pm 14.8$ |
| | | | |
| $\%$ $unobserved^a$ | 8.33 | 15.38 | 47.80 |

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