

## LETTER

# Analysis of a hyper-diverse seed dispersal network: modularity and underlying mechanisms

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### Abstract

Mutualistic interactions involving pollination and ant-plant mutualistic networks typically feature tightly linked species grouped in modules. However, such modularity is infrequent in seed dispersal networks, presumably because research on those networks predominantly includes a single taxonomic animal group (e.g. birds). Herein, for the first time, we examine the pattern of interaction in a network that includes multiple taxonomic groups of seed dispersers, and the mechanisms underlying modularity. We found that the network was nested and modular, with five distinguishable modules. Our examination of the mechanisms underlying such modularity showed that plant and animal trait values were associated with specific modules but phylogenetic effect was limited. Thus, the pattern of interaction in this network is only partially explained by shared evolutionary history. We conclude that the observed modularity emerged by a combination of phylogenetic history and trait convergence of phylogenetically unrelated species, shaped by interactions with particular types of dispersal agents.

### Keywords

Birds, body mass, complex networks, fish, fruit diameter, mammals, nestedness, phylogenetic analyses, reptiles.

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## INTRODUCTION

In mutualistic interactions, species commonly interact with multiple partners, forming a network of interactions. The pattern of these interactions in a community, i.e. the way interactions are organized, can be described using a network approach, which helps to elucidate the complexity of such interactions (Jordano 1987; Bascompte & Jordano 2007). Mutualistic networks are highly structured, with a prevalence of a nested pattern (Bascompte *et al.* 2003; Vázquez *et al.* 2009; Fortuna *et al.* 2010; Joppa *et al.* 2010). That is, the interactions of the specialist species tend to be a subset of the interactions observed among the generalists (Bascompte *et al.* 2003). In addition to being nested, some mutualistic networks are also modular (Dicks *et al.* 2002 and Olesen *et al.* 2007: pollination networks; Fonseca & Ganade 1996 and Guimarães *et al.* 2007: ant-plant networks), whereby subsets of species (modules) more frequently interact with each other than with species in other modules (Olesen *et al.* 2007).

Among mutualisms, modularity has been investigated in depth in pollination networks (Dicks *et al.* 2002; Olesen *et al.* 2007; Fortuna *et al.* 2010), which often include a highly diverse array of animal and plant taxa (e.g. Rezende *et al.* 2007). In contrast, the majority of seed dispersal networks studied includes mainly seed-dispersing birds, which interact with plant species that share similar traits (Rezende *et al.* 2007), leading to a highly nested and low modularity pattern of interaction (see Fortuna *et al.* 2010). The widespread habit of producing fleshy fruits among tropical plant species has been

evolutionarily associated with the diversification of frugivorous vertebrates (Fleming *et al.* 1987). Therefore, the diversity of animals that interact with a particular set of plant species could make them tightly linked within modules. Thus, one can predict networks of interactions in diverse communities, involving plants and several taxonomic groups of seed-dispersing animals, to have low nestedness and high modularity.

Herein, we test if a hyper-diverse seed dispersal network is characterized by low nestedness and high modularity. Beyond such a test, we examine the mechanisms organizing this network through a combination of long-term fieldwork, network theory and phylogenetic analysis. We analyse the structure of the plant–animal interactions in one of the world's last remaining species-rich communities involving large vertebrates: the Pantanal (Harris *et al.* 2005). By investigating the structure of this community of plants and seed dispersers, we are filling an important gap in the studies of species networks, given that most similarly diverse communities worldwide have lost at least some of their vertebrates involved in mutualisms and include only subsets of the major frugivore groups (but see Gautier-Hion *et al.* 1985). To the extent that modular patterns reflect a more diversified network of ecological functions and services, the understanding of the mechanisms that determine modularity could help uncovering the general processes shaping the evolutionary ecology of plant–animal interactions.

Modularity in a broad range of ecological networks is associated with habitat heterogeneity (Pimm & Lawton 1980), phylogenetic

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clustering of closely related species (Lewinsohn *et al.* 2006), convergence towards syndromes (Corbet 2000), and combinations of these factors (Cattin *et al.* 2004; Olesen *et al.* 2007; Rezende *et al.* 2009). For mutualistic networks, a combination of coevolutionary complementarity and convergence appears to draw other species into the interaction over time, creating a coevolutionary vortex (Thompson 2005) reflected in the structure of the network.

Herein, we (1) examine the pattern of interaction in a highly diverse seed dispersal network, including a variety of species from major taxonomic groups of seed dispersers, mammals, birds, fish and reptiles, and the fleshy-fruited species they disperse, and (2) test current hypotheses on mechanisms that may generate the modularity in mutualistic networks. Interactions in this network were sampled in three habitats within a community in the Brazilian Pantanal. We tested the following hypotheses: (1) this network should be modular given the diversity of taxonomic groups of seed dispersers involved, (2) animal and fruit traits, as well as phylogeny should therefore be associated with the modularity of this network, and (3) the habitat types where interactions were recorded should not be associated with modules, given that, although several plant species in this community are habitat specialists, the majority of animal species is not.

We first illustrate that interactions in this network have a combination of nested and modular patterns. Then, we describe how the modules are predominantly associated with fruit and animal traits, and not with the different habitat types where plant species predominantly occur. We show that the modularity in this network is only partially explained by shared evolutionary history because, although modules are related to the different taxonomic groups of animals, phylogeny explains only the assemblage of species in modules associated with birds. We conclude that such modularity likely emerged by a combination of shared phylogenetic history and trait convergence of phylogenetically unrelated species, shaped by interactions with particular types of dispersal agents.

## MATERIAL AND METHODS

### Study sites

This study centred on two neighbouring locations in the Brazilian Pantanal: Rio Negro (19°34'15" S 56°14'43" W) and Barranco Alto farms (19°34'40" S 56°09'08" W), covering 7500 ha and 11 000 ha, respectively, of private land (see Appendix S1 in Supporting Information). The main vegetation types in these locations, where seed dispersal interactions were recorded, include gallery forests, savannas and semi-deciduous forests (Prance & Schaller 1982).

### Seed dispersal interactions

Seed dispersal interactions were recorded using four methodologies. To sample seed dispersal by birds, we carried out focal observations at 14 plant species for 882 h, recording the identity of birds that were unequivocally observed carrying fruits outside the canopy area or swallowed them *in situ*. Seed dispersal by red-footed tortoises (*Geocelone carbonaria*), rheas (*Rhea americana*) and the majority of mammal species, were recorded with camera traps located beneath fruiting trees of 27 plant species, capturing events of fruit ingestion, for a total of 14 800 h. Some terrestrial and semi-terrestrial bird species were also recorded via camera traps. We analysed 716 scats of several species of mammals, rheas and red-footed tortoises, and

identified the intact seeds in them. To record seed dispersal by the pacu fish (*Piaractus mesopotamicus*), we caught 80 individuals and identified the intact seeds in their intestine (see Galetti *et al.* 2008). One event of seed dispersal was considered as such when either: fruits were recorded to have been swallowed or removed from a plant species during focal observations; fruit removal of a particular species by a potential seed disperser was detected with camera traps; a scat pile was found to have at least one intact seed of a particular species in it; or a sampled fish intestine contained at least one intact seed from a particular species.

### Sampling robustness

To assess if we had recorded enough interactions to describe this network, we generated an accumulation curve with the number of interactions as a function of the number of seed dispersal events sampled (Guimarães *et al.* 2007; Jordano *et al.* 2009). We estimated average and standard deviation of the number of interactions for any given fraction of the number of events recorded in 1000 iterations. After generating this curve, we used the *drc* package in R (<http://www.r-project.org/>) and the dose–response model to extrapolate the curve. We then used the Michaelis–Menten equation to assess the asymptotic value of the curve.

### The network structure

To define the pattern of interaction in the network, we organized a qualitative seed dispersal matrix, collectively using the methodologies previously described. In a matrix of plants in columns and animals in rows, an element representing a seed dispersal interaction received the value of 1, and 0 otherwise. We then used this matrix to test for nestedness and modularity.

Nestedness was analysed using the NODF metric (Almeida-Neto *et al.* 2008), through the ANINHADO program (Guimarães & Guimarães 2006). To test if the network is more nested than expected by species richness and heterogeneity of interactions, we compared the recorded NODF value to that of 1000 random matrices generated by a null model that controls for the number of interactions per species in the network ('null model 2', Bascompte *et al.* 2003).

To detect modularity we used the NETCARTO program and an algorithm based on simulated annealing (SA) (Guimerà & Amaral 2005) that identifies modules formed by both plants and animals simultaneously (see Olesen *et al.* 2007). We computed the network modularity index  $M$ , which measures the degree to which the network is organized into clearly defined modules, as well as the level of significance of the modularity in this network by comparing its  $M$  to that of random networks of similar sizes, generated by the same null model used for the nestedness analysis. As the algorithm is based on an optimization process, the outcomes may vary in different runs. Therefore, to assign each species to a particular module, we ran the analysis 50 times. Species that were assigned to a particular module in > 90% of the runs were included in that module. We compared the nestedness and the modularity of this network with those same parameters calculated for other 24 frugivory networks available in the literature (Table S1 in Supporting Information).

We associated each plant species with the habitat type (gallery forest, savanna, semi-deciduous forest) in which it was predominantly recorded in a previous 4-year phenological study (C.I. Donatti,

unpublished work). We used binomial distributions to test the associations of animal taxonomic groups with particular modules, i.e. if phylogeny explains the coarse network's topology, and the associations of plant species that predominantly occur in each habitat type to particular modules, i.e. if habitat type explains the coarse topology of the network. We tested if the presence of species from the same taxonomic group or from the same habitat type is over-represented in a particular module when compared with the null expectation that taxonomy or habitat heterogeneity does not affect the organization of modules. For each module, we estimated the probability of getting, by chance alone, a number of species from the same taxonomic group or habitat type equal or higher than that observed in the real network. We used the number of species in a module (number of trials,  $N$ ), the proportion of species of a given taxonomic group or habitat type in the whole sample (probability of success), and the number of species that belongs to a particular taxonomic group or habitat type that also belongs to a particular module (number of successes), as parameters of binomial distributions.

We gathered animal body mass information from the literature, and measured fruit and seed traits (length, diameter and mass) for all plant species, in at least 30 fruits and seeds from at least five individuals. Values of body mass were log transformed and values of plant traits were Box–Cox transformed using JMP v.5.0 (SAS Institute Inc., Cary, NC, USA). We used ANOVA to compare body mass of animal species among modules, MANOVA to compare all fruit and seed traits among modules, and  $t$ -tests to compare body mass between modules that represented the same taxonomic animal group.

### Phylogenetic signal in animal and plant traits and in the network's pattern

We tested whether animal and plant traits had a significant phylogenetic signal, i.e. a quantitative measure of the degree to which phylogeny predicts the ecological similarity of species. To build the animal phylogenetic tree, we followed Bininda-Emonds *et al.* (2007) for relationships among mammal species and Hackett *et al.* (2008) for relationships among bird species. In addition, we used published work to resolve relationships within Cracidae (Pereira *et al.* 2002), Tyrannidae (Tello *et al.* 2009) and Thraupidae (Klicka *et al.* 2007). We also used two mitochondrial DNA sequences (Cytochrome b and Cytochrome oxidase subunit 1), available on GenBank, to resolve the relationships between and within the Thraupidae and Icteridae. We then generated a phylogenetic tree for those sequences using the program *Méthodes et algorithmes pour la bio-informatique* (<http://www.phylogeny.fr/>) and added those relationships in the tree. The plant phylogenetic tree was built using Phylomatic software (<http://www.phylodiversity.net/phyloomatic/phyloomatic.html>). Relationships within Fabaceae followed Wojciechowski *et al.* (2004), within Rubiaceae followed Brewer & Eriksson (2009) and within Arecaceae followed Asmussen *et al.* (2006). Since all branches in animal and plant trees were set equal to 1, we conducted simulations that showed that using branch lengths equal to 1 is a conservative approach when values of  $K$  are lower than or equal to 1 (see Appendix S2 in Supporting Information).

We assessed  $K$  statistic to measure the phylogenetic signal in animal and plant traits, using the function *phylosignal* in the *picante* package (Kembel *et al.* 2010) of R. To assess phylogenetic signal in body mass, we analysed mammal and bird species independently. The  $K$  statistic

compares the observed signal in a trait to the signal under a Brownian motion model of trait evolution on a phylogeny (Blomberg *et al.* 2003). The statistical significance of phylogenetic signal is evaluated by comparing observed patterns of the variance of independent contrasts of a trait to a null model of shuffling taxa labels across the tips of the phylogenetic tree. To test for evidence of phylogenetic signal in modules, i.e. if phylogeny explains the composition of species within modules, we used a function *ad hoc* in R that corresponds to the 'Fixed Tree, Character Randomly Reshuffled' model proposed in Maddison & Slatkin (1991). This function counts the minimum number of transitions needed to get the distribution of modules observed in the real network, randomizes the modules in the phylogeny and then counts the number of transitions in each randomization. The statistical significance of phylogenetic signal is achieved if there are fewer transitions in the real network than in 95% of the randomizations. Phylogenetic signal in modules was tested using the animal and the plant phylogenetic trees independently. For animals, we run the analyses separately for mammals and birds.

### The role of individual species in the network structure

The SA algorithm also assigns an ecological role to each species in the network based on its interactions within modules ( $\zeta$ ) and on its interactions among modules ( $\epsilon$ ) (Olesen *et al.* 2007). Species with low  $\zeta$  and low  $\epsilon$  are considered peripheral species, i.e. they usually interact with species within their own module. Species with either a high  $\zeta$  or  $\epsilon$  were considered generalists, and either (1) module hubs, i.e. highly connected within their own module (high  $\zeta$  and low  $\epsilon$ ), or (2) connectors, those species that link modules (low  $\zeta$  and high  $\epsilon$ ). Species with a high  $\zeta$  and a high  $\epsilon$  were considered supergeneralists, acting as both module hubs and connectors. To define the role of each species, we used the most common values of  $\zeta$  and  $\epsilon$  generated in the 50 times we run the analysis. We used the values of 2.5 for  $\zeta$  and of 0.62 for  $\epsilon$  to define those categories (cf. Fig. 4), following Olesen *et al.* (2007).

We performed additional analyses using  $\zeta$  and  $\epsilon$  values in order to assess the correlates of these values with species traits. We analysed the values of  $\zeta$  and  $\epsilon$  of each animal species as a function of its body mass and the values of  $\zeta$  and  $\epsilon$  of each plant species as a function of its fruit and seed traits using correlations. We compared the  $\epsilon$  and  $\zeta$  values between animal and plant species using  $t$ -tests and the  $\epsilon$  values among modules using ANOVA.

## RESULTS

### The network structure

The network included 46 plant species and 46 animal species. We recorded 2070 seed dispersal events and 273 seed dispersal interactions (Table S2, in Supporting Information). One plant species (*Sapindus saponaria*) did not show interactions with seed dispersers, probably due to the high level of saponins in the pulp (Pott & Pott 1994). Using the Michaelis–Menten equation we estimated to have sampled 94.5% of the seed dispersal interactions occurring in this community (Figure S1 in Supporting Information). Therefore, we assume that the network described herein is robust to additional sampling.

The Pantanal seed dispersal network was not only significantly nested (NODF = 26.27, expected NODF = 18.04,  $P < 0.001$ ) but also significantly modular ( $M = 0.422$ , expected = 0.341,  $P < 0.001$ ),

with animal and plant species grouped in five statistically different modules (Fig. 1). Module composition was very robust: we detected five modules in all 50 runs. All but two species were assigned to the same module in 100% of the 50 runs. These species, the bird *Crax fasciolata* (Cracidae) and the plant *Guazuma ulmifolia* (Sterculiaceae), were assigned to the same module in 94% of the runs.

### Modularity

Two modules were exclusively represented by bird species and the plant species they interact with (hereafter bird module 1 and bird module 2; green and blue in Fig. 1, respectively). Two other modules were represented mainly by mammal species and by the plant species they interact with. One of these (hereafter mammal-dominated module 1; red in Fig. 1) also included the tortoise and the rhea, whereas the other one (hereafter mammal-dominated module 2; yellow in Fig. 1) also included a ground-foraging bird (*C. fasciolata*). The fifth module (hereafter fish module; purple in Fig. 1) was represented by the fish and plant species it mainly interacts with (Table S3 in Supporting Information). The Pantanal seed dispersal network is the second less nested and more modular of the seed dispersal networks so far studied that show a significant pattern (NODF =  $54.04 \pm 17.15$ ,  $n = 24$ ;  $M = 0.31 \pm 0.092$ ,  $n = 4$ ).

The binomial distributions showed that each module is associated with animal species that belong to a particular animal taxonomic group (bird module 1:  $P = 0.006$ ,  $n = 22$ ; bird module 2:  $P = 0.003$ ,  $n = 22$ ; mammal-dominated module 1:  $P = 0.012$ ,  $n = 25$ ; mammal-dominated module 2:  $P < 0.001$ ,  $n = 18$  and fish module:  $P = 0.02$ ,  $n = 4$ ). However, each module does not include plant species that predominantly occur in a particular habitat type (bird module 1:  $P >$

0.666, bird module 2:  $P > 0.437$ , mammal-dominated module 1:  $P > 0.179$ , mammal-dominated module 2:  $P > 0.659$ , fish module:  $P > 0.168$ ).

Regarding nestedness, the mammal-dominated module 2 showed a significant nested pattern (NODF = 64.73,  $P = 0.005$ ), while the other three modules did not (bird module 1: NODF = 41.20,  $P = 0.169$ ; bird module 2: NODF = 62.83,  $P = 0.061$  and mammal-dominated module 1: NODF = 60.78,  $P = 0.330$ ). The non-detection of a nested pattern within modules could be an artefact of the low number of species (Guimarães *et al.* 2006). The fish module could not be tested, given its low species richness.

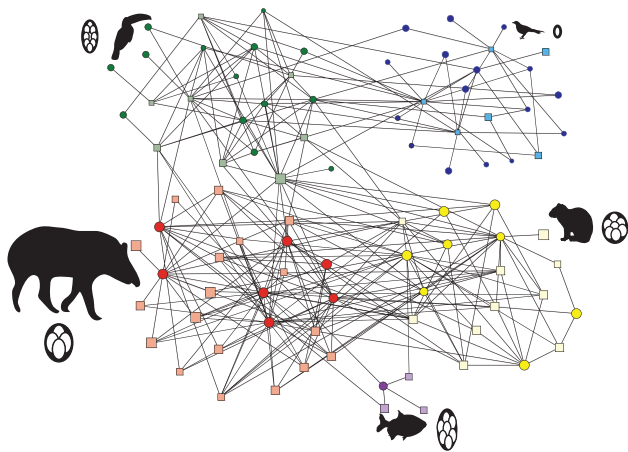
Animal body mass varied across modules ( $F = 64.51$ ,  $P < 0.0001$ , d.f. = 44; mammal-dominated module 1 =  $47.41 \text{ kg} \pm 32.7$ , mean  $\pm$  SD, mammal-dominated module 2 =  $15.38 \text{ kg} \pm 5.11$ , bird module 1 =  $0.24 \text{ kg} \pm 0.07$ , bird module 2 =  $0.06 \text{ kg} \pm 0.01$ ) and explained 82.52% of the variance among modules. Mean animal body mass significantly differed between the two bird modules ( $t = 2.710$ ,  $P = 0.0129$ , d.f. = 29), but not between the two mammal-dominated modules ( $t = 0.501$ ,  $P = 0.6240$ , d.f. = 14), although the low species number may cause low statistical power.

Modules also significantly differed when taking into account all fruit and seed traits (MANOVA  $F = 3.4821$ ,  $P < 0.0001$ , d.f. = 44). Modules were therefore characterized by particular suites of traits. Fruit mass mainly explained the variance among modules (26.65%), followed by fruit diameter (23.13%), fruit length (17.42%) and seed mass (11.98%). Mean values of all plant traits but fruit length and seed length in each module were positively and significantly correlated with mean body mass of seed dispersers in each module (fruit diameter:  $F = 58.48$ ,  $P = 0.004$ ,  $r = 0.97$ ; fruit mass:  $F = 15.06$ ,  $P = 0.03$ ,  $r = 0.91$ ; seed diameter:  $F = 26.09$ ,  $P = 0.014$ ,  $r = 0.94$ ; seed mass:  $F = 19.63$ ,  $P = 0.021$ ,  $r = 0.93$ , d.f. = 4): modules with heavy seed dispersers also had heavy and wide fruits and seeds. When considering all interactions in the network, there were positive associations between the body size of seed dispersers and all fruit and seed traits (Fig. 2) (fruit length:  $F = 50.29$ ,  $P < 0.0001$ ,  $r = 0.39$ , fruit diameter:  $F = 109.4550$ ,  $P < 0.0001$ ,  $r = 0.53$ ; fruit mass:  $F = 115.95$ ,  $P < 0.0001$ ,  $r = 0.54$ , d.f. = 272; seed length:  $F = 29.9$ ,  $P < 0.0001$ ,  $r = 0.31$ ; seed diameter:  $F = 41.25$ ,  $P < 0.0001$ ,  $r = 0.36$ ; seed mass:  $F = 54.76$ ,  $P < 0.0001$ ,  $r = 0.41$ , d.f. = 269).

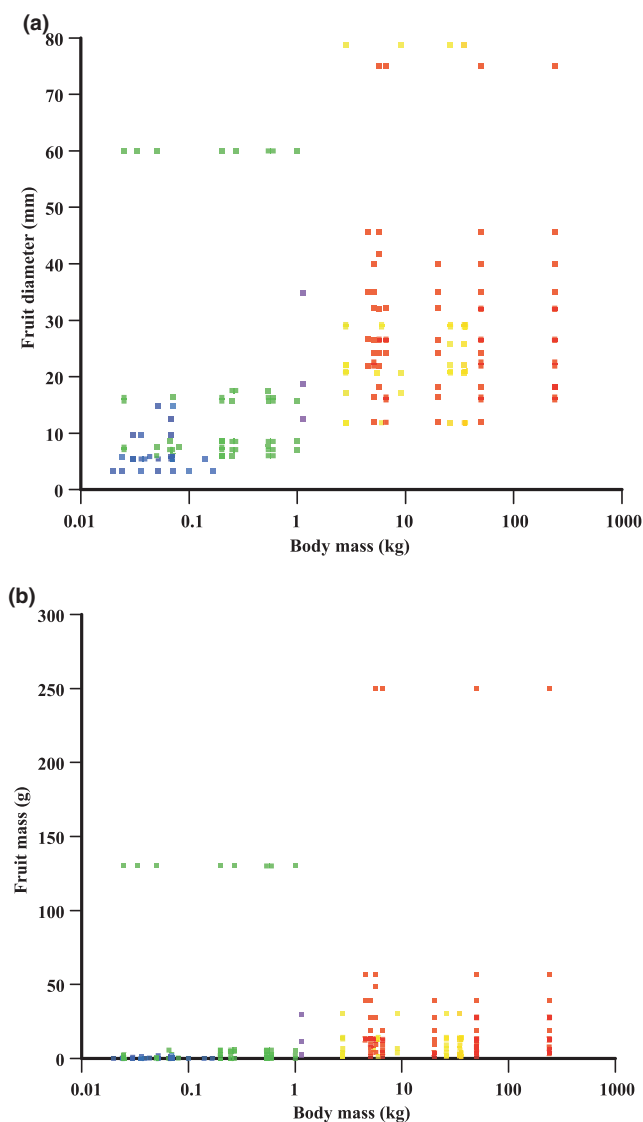
### Phylogenetic signal in animal and plant traits and in the network's pattern

The body mass of closely related mammal species has exactly the amount of signal predicted by Brownian motion ( $K = 1.01$ ,  $P = 0.005$ ). In contrast, the body mass of birds and all seed traits are more divergent than expected under a Brownian model (birds:  $K = 0.778$ ,  $P = 0.001$ , d.f. = 31; seed length:  $K = 0.526$ ,  $P = 0.001$ ; seed diameter:  $K = 0.417$ ,  $P = 0.003$ ; seed mass:  $K = 0.496$ ,  $P = 0.001$ ; d.f. = 43). Fruit traits did not show a significant signal (fruit length:  $K = 0.370$ ,  $P = 0.133$ ; fruit diameter:  $K = 0.372$ ,  $P = 0.248$ ; fruit mass:  $K = 0.392$ ,  $P = 0.159$ ; d.f. = 44).

Modules included phylogenetically related bird species ( $P = 0.006$ ), but not phylogenetically related mammal species ( $P = 0.2$ ) or phylogenetically related plant species ( $P = 0.83$ ) (Fig. 3). Although modules are associated with the major animal taxonomic groups (mammals, birds or fish), only modules associated with birds included phylogenetically related bird species.



**Figure 1** Modularity of the Pantanal seed dispersal network. Each module is identified by a different colour (bird module 1: green, 22 species; bird module 2: blue, 22 species; mammal-dominated module 1: red, 25 species; mammal-dominated 2: yellow, 18 species and fish: purple, 4 species) in which each species was assigned. Circles in dark shades represent animal species and squares in light shades represent plant species. The size of circles refers to animal body mass (with large circles representing species with body mass  $\geq 4.5$  kg), whereas the size of squares refers to the fruit diameter (with large squares representing species with fruit diameter  $\geq 95$  mm). Both body mass and fruit diameters were divided in four size categories exclusively for the purpose of this figure. Fruits and seed sizes are in the same scale in all modules, and represent the relative diameter and length of fruits and seeds in each module. The figure was manually done using the package *pajek* (<http://vlado.fmf.uni-lj.si/pub/networks/pajek/>).



**Figure 2** Association between body mass and values of fruit traits, illustrating trait complementarity underlying the modular structure of the network (bird module 1: green, bird module 2: blue, mammals-dominated module 1: red, mammal-dominated module 2: yellow and fish: purple). (a) fruit diameter and (b) fruit mass. Body mass is in log scale.

### The role of individual species in the network structure

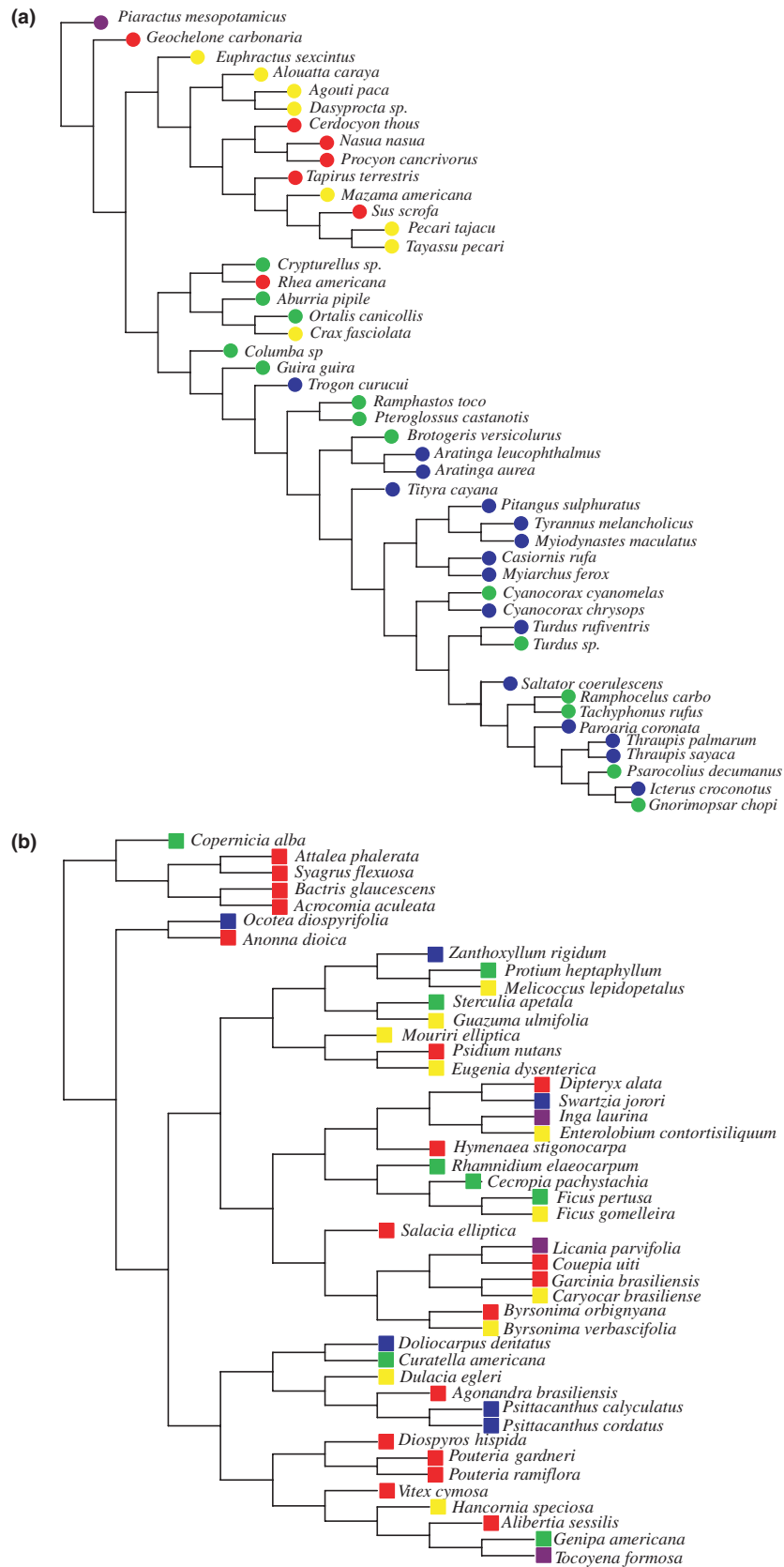
As in pollination networks, the majority of species in this seed dispersal network were peripheral, i.e. they almost always interact with species within their own module (Fig. 4). Three species, the exotic feral pig (*Sus scrofa*, Suidae,  $\bar{z} = 2.7869$ ,  $c = 0.5104$ ), the plant *Dolioscarpus dentatus* (Dilleniaceae,  $\bar{z} = 2.75$ ,  $c = 0.4897$ ) and the tapir (*Tapirus terrestris*, Tapiridae,  $\bar{z} = 2.4954$ ,  $c = 0.4099$ ), were considered module hubs, i.e. species with many interactions within their own module. The howler monkey (*Alouatta caraya*, Cebidae,  $\bar{z} = -1.3687$ ,  $c = 0.6666$ ), one plant (*Genipa americana*, Rubiaceae,  $\bar{z} = 1.3574$ ,  $c = 0.66463$ ) and the chaco chachalaca (*Ortalis canicollis*, Cracidae,  $\bar{z} = 0.55$ ,  $c = 0.625$ ) were considered connectors, i.e. species that had interactions across different modules. None of the species were defined as supergeneralist, indicating a low cohesiveness in this seed dispersal network.

Plant and animal species did not differ in both their interactions inside modules ( $\bar{z}$ ) and in their interactions among modules ( $c$ ) ( $t = 0.351$ ,  $P = 0.726$ , d.f. = 44;  $t = 1.035$ ,  $P = 0.303$ , d.f. = 45, respectively). However, we found that the mean participation of species in the whole network ( $c$ ) differed among modules ( $F = 3.597$ ,  $P = 0.009$ , d.f. = 4; mammal-dominated module 2 =  $0.391 \pm 0.05$ , fish module =  $0.343 \pm 0.11$ , mammal-dominated module 1 =  $0.326 \pm 0.04$ , bird module 1 =  $0.260 \pm 0.04$ , bird module 2 =  $0.139 \pm 0.04$ ). The values of both  $\bar{z}$  and  $c$  increased with the body mass of dispersers ( $\bar{z}$ :  $F = 20.2381$ ,  $P < 0.001$ ,  $r = 0.55$ ;  $c$ :  $F = 45.654$ ,  $P < 0.001$ ,  $r = 0.7$ ; d.f. = 45): a large-bodied seed disperser has more interactions both inside and among modules. In contrast, the values of  $\bar{z}$  decreased with an increment in all fruit and seed traits (fruit length:  $F = 17.74$ ,  $P = 0.0001$ ,  $r = 0.54$ ; fruit diameter:  $F = 20.31$ ,  $P < 0.0001$ ,  $r = 0.56$ ; fruit mass:  $F = 26.65$ ,  $P < 0.0001$ ,  $r = 0.61$ ; d.f. = 44; seed length:  $F = 18.81$ ,  $P < 0.0001$ ,  $r = 0.55$ ; seed diameter:  $F = 18.39$ ,  $P = 0.0001$ ,  $r = 0.55$ , seed mass:  $F = 25.06$ ,  $P < 0.0001$ ,  $r = 0.61$ ; d.f. = 43) and the values of  $c$  decreased with an increment in seed traits (seed length:  $F = 7.18$ ,  $P = 0.01$ ,  $r = 0.38$ ; seed diameter:  $F = 4.95$ ,  $P = 0.031$ ,  $r = 0.32$ ; seed mass:  $F = 6.68$ ,  $P = 0.013$ ,  $r = 0.37$ ; d.f. = 43): a plant species with small fruit and seed trait values has more interactions inside modules and a plant species with small seed trait values has also more interactions among modules. As the body mass of seed dispersers increases, they are able to disperse a variety of plant species, regardless of fruit and seed sizes, whereas large fruits and/or seeds are restricted to a few animal species able to disperse them. These results could reflect the association between plant and animal species traits and the number of interactions, which increases significantly with body mass of the seed dispersers and decreases significantly with fruit length and all seed traits (body mass:  $F = 65.62$ ,  $P < 0.0001$ ,  $r = 0.77$ , d.f. = 45; fruit length:  $F = 4.64$ ,  $P = 0.036$ ,  $r = 0.31$ , d.f. = 44; seed length:  $F = 13.83$ ,  $P = 0.0006$ ,  $r = 0.49$ ; seed diameter:  $F = 10.69$ ,  $P < 0.002$ ,  $r = 0.45$ ; seed mass:  $F = 18.95$ ,  $P < 0.0001$ ,  $r = 0.55$ , d.f. = 43).

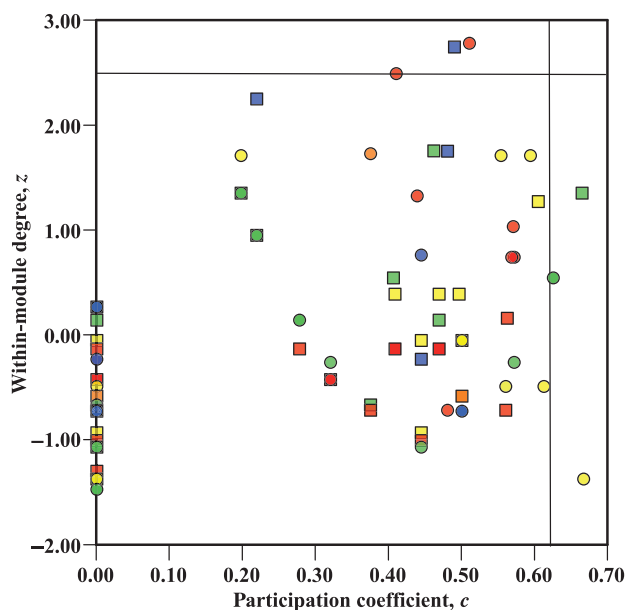
### DISCUSSION

Seed dispersal interactions in this network were nested and modular, as in some pollination and ant-plant mutualistic networks (Fonseca & Ganade 1996; Guimarães *et al.* 2007; Olesen *et al.* 2007). The studied network has a heterogeneous structure that is organized around a modular pattern, which reflects a diversity of taxonomic groups of seed dispersers and of fruit and seed morphological traits. In essence, the major taxonomic groups of seed dispersers separated species in five distinct modules. In addition, seed disperser species within those modules varied in their body mass and interacted with plant species that differed in fruit and seed traits. In fact, there were strong correlations between the body size of the seed dispersers and the size of fruits (especially diameter and mass) in modules. Therefore, specific traits of seed dispersers and fruits help explaining the mixing of major taxa of animals among different modules. Similarly, Gautier-Hion *et al.* (1985) studying a diverse community of fruit and frugivores in an African tropical rainforest found that morphological traits of fruits revealed syndromes associated with consumption by different taxa of vertebrates.

In a coarse-level of taxonomic resolution, the phylogenetic effect on network structure was limited to the over-representation of species from a given animal taxonomic group in each module. In a fine-level



**Figure 3** (a) The phylogenetic tree of animal species and modules (identified by different colours) in which each species was assigned (bird module 1: green, bird module 2: blue, mammals-dominated module 1: red, mammal-dominated 2: yellow and fish: purple). Modules associated with birds showed significant phylogenetic signal. (b) The phylogenetic tree of plant species and modules (identified by different colours) in which each species was assigned (bird module 1: green, bird module 2: blue, mammals-dominated module 1: red, mammal-dominated 2: yellow and fish: purple).



**Figure 4** Role of each species in the seed dispersal network. Each symbol describes the within-module degree ( $z$ ) and the participation coefficient ( $c$ ) of each species. We used the values of 2.5 for  $z$  and of 0.62 for  $c$  to assign a role to each species, which could be: peripheral species (bottom left), module hub (top left), supergeneralist (top right) or connector (bottom right). Species are colour coded according to the module to which they belong (bird module 1: green, bird module 2: blue, mammals-dominated module 1: red, mammals-dominated 2: yellow and fish: purple). Circles represent animal species and squares represent plant species. Lines at  $z = 2.5$  and  $c = 0.62$  define species roles.

of taxonomic resolution, modules associated with birds showed significant phylogenetic signal, whereas modules associated with mammals did not. Although the low number of species in mammal-dominated modules could decrease the statistical power of the phylogenetic test, the mammal composition in modules does not show clear evidence that phylogenetically related species belong to the same modules. For instance, closely related mammal species, such as artiodactyl ungulates, belonged to different modules. In addition, modules did not include phylogenetically related plant species. Therefore, the pattern in this network is only partially explained by shared evolutionary history in the sense that, although modules are related to the major taxonomic groups of animals, the majority of them do not include phylogenetically related species. Given the consistency of our results, we posit that the modularity of this seed dispersal network is not associated with habitat heterogeneity and that phylogeny only determines the existence of bird, mammal and fish modules, and the assemblage of species in bird modules. Therefore, we suggest that modules emerged by a combination of phylogenetic history and trait convergence of phylogenetically unrelated species, shaped by interactions with particular types of dispersal agents (Van der Pijl 1982). However, we do not necessarily imply herein that those tight phenotypic associations between seed dispersers and fruits found in modules are driven by coevolution (e.g., Nuismer *et al.* 2010). We are simply positing that convergence of species towards a similar and predictable set of traits (Thompson 2005), rather than phylogeny alone, is what explains the way this seed dispersal network is organized. In fact, convergence in these networks might be both an outcome of evolutionary processes such as local adaptation and

coadaptation (Thompson 2005) and a consequence of ecological convergence in resource use by subsets of frugivores.

In a broader context, the Pantanal seed dispersal network was less nested than all other seed dispersal networks so far studied, with the exception of one sampled in the Brazilian Atlantic forest (Silva *et al.* 2007) that includes interactions between plants and both mammal and bird species. However, the network analysed herein is more modular than the other four frugivory networks that had significant values of modularity. Modularity of plant-animal networks is expected to increase with trophic specificity, with herbivory and ant-plant networks found to be more modular than pollination and seed dispersal networks (see Fonseca & Ganade 1996; Guimarães *et al.* 2007; Thebault & Fontaine 2010). Pollination networks are likely to have higher and more prevalent modularity than seed dispersal networks because flowers may restrict the range of visitors through morphological barriers (Santamaría & Rodríguez-Gironés 2007; Stang *et al.* 2007), whereas fruit traits may lend them to be more open to interaction with multiple visitors (Blüthgen *et al.* 2007). However, the high degree of modularity in this diverse and well-sampled seed dispersal network suggests that factors other than interaction type are constraining the modularity in frugivory and seed dispersal networks. For instance, the lack of significant modularity in most of these networks could happen because they predominantly include a single taxonomic group. Nevertheless, the phylogenetic diversity only partially explains the modularity of this diverse network and some modules are composed by species from different taxonomic groups. We therefore hypothesize that modularity emerges from the interplay between shared evolutionary history and convergence in patterns of interaction.

One of the values of detecting a modular pattern, other than contributing to elucidate the evolutionary ecology of plant-frugivore interactions, is the identification of the role of species in the network (Olesen *et al.* 2007). This is important because the robustness of the network, i.e. the ability of a species to persist given the extinction of an interacting partner in the community (Jordano *et al.* 2006) may depend on the role of species in the network. For example, the extinction of connectors may cause the network to fragment into isolated modules, but will have a minor impact on the internal structure of modules. In contrast, the extinction of a module hub may cause its module to fragment with minor cascading impact on other modules. Interestingly, only 6.4% of the species in this network, a lower percentage than that found in pollination networks (Olesen *et al.* 2007), are connectors or module hubs.

Some plant species were important in maintaining the pattern of this network. *G. americana* was considered a connector, linking modules together. This plant species exhibits a typical 'megafauna seed dispersal syndrome' (Guimarães *et al.* 2008) in that their seed dispersers are/were large mammals, yet its fruits are also avidly eaten by several bird species. Consequently, fruits of this species were dispersed by animal species from four modules (two bird modules and two mammal-dominated modules).

Although large-bodied seed dispersers such as tapirs and feral pigs showed to be important in linking species within a module, they were not important in linking modules together, maybe because they mainly interact with large- and medium-seeded species, which were not present in all modules of this network. Medium-bodied species, such as howler monkeys and chaco chachalacas, were connectors in this network and therefore structurally important because they link modules together. Consequently, we posit that the loss not only of

large-bodied seed dispersers, the ones that disperse a high number of plant species, but also of some of the medium-bodied species, may change the pattern of this network, given that their absence could cause the network to fragment into isolated modules.

Here, the use of the network approach helped us to understand the structure of a highly diverse seed dispersal network and enabled us to identify the mechanisms that underlie the modular pattern, contributing to elucidate the ecology and evolution of plant–frugivore interactions. In addition, the identification of the modular pattern gave us insights regarding the possible consequences of differential defaunation (cf. Dirzo & Miranda 1991) on the functioning of this seed dispersal network. For example, the presence of few animal species that can link modules together could contribute to the robustness of this network in a scenario of extinction of particular species.

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## REFERENCES

- Almeida-Neto, M., Guimarães, P., Guimarães, P.R. Jr, Loyola, R.D. & Ulrich, W. (2008). A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. *Oikos*, 117(8), 1227–1239.
- Asmussen, C.B., Dransfield, J., Deichmann, V., Barfod, A.S., Pintaud, J.-C. & Baker, W.J. (2006). A new subfamily classification of the palm family (Arecaceae): evidence from plastid DNA phylogeny. *Bot. J. Linn. Soc.*, 151, 15–38.
- Bascompte, J. & Jordano, P. (2007). The structure of plant–animal mutualistic networks: the architecture of biodiversity. *Annu. Rev. Ecol. Syst.*, 38, 567–593.
- Bascompte, J., Jordano, P., Melián, C.J. & Olesen, J.M. (2003). The nested assembly of plant–animal mutualistic networks. *PNAS*, 100(16), 9383–9387.
- Bininda-Emonds, O.R.P., Cardillo, M., Jones, K.E., McPhee, R.D.E., Beck, R.M.D., Grenyer, R. *et al.* (2007). The delayed rise of present-day mammals. *Nature*, 446, 507–512.
- Blomberg, S.P., Garland, T. & Ives, A.R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, 57, 717–745.
- Blüthgen, N., Menzel, F., Hovestadt, T., Fiala, B. & Blüthgen, N. (2007). Specialization, constraints and conflicting interests in mutualistic networks. *Curr. Biol.*, 17, 1–6.
- Brewer, B. & Eriksson, T. (2009). Time tree of Rubiaceae: phylogeny and dating the family, subfamilies, and tribes. *Int. J. Plant Sci.*, 170(6), 766–793.
- Cattin, M.-F., Bersier, L.-F., Banasek-Ritche, C., Baltensperger, R. & Gabriel, J.-P. (2004). Phylogeny constraints and adaptation explain food-web structure. *Nature*, 427, 835–838.
- Corbet, S.A. (2000). Conserving compartments in pollination webs. *Conserv. Biol.*, 14, 1229–1231.
- Dicks, L., Corbet, S.A. & Pywell, R.F. (2002). Compartmentalization in plant–insect flower visitor webs. *J. Anim. Ecol.*, 71, 32–43.
- Dirzo, R. & Miranda, A. (1991). Altered patterns of herbivory and diversity in the forest understory: a case study of the possible consequences of contemporary defaunation. In: *Plant–Animal Interactions: Evolutionary Ecology In Tropical And Temperate Regions* (eds Price, P.W., Lewinsohn, T.M., Fernandes, G.W. & Benson, W.W.). Wiley and Sons Pub., New York, pp. 273–287.
- Fleming, T.H., Breitwisch, R. & Whitesides, G.H. (1987). Patterns of tropical vertebrate frugivore diversity. *Annu. Rev. Ecol. Syst.*, 18, 91–109.
- Fonseca, C.R. & Ganade, G. (1996). Asymmetries, compartments and null interactions in an Amazonian ant–myrmecophyte community. *J. Anim. Ecol.*, 65, 339–347.
- Fortuna, M.A., Stouffer, D.B., Olesen, J.M., Jordano, P., Mouillot, D., Krasnov, B.R. *et al.* (2010). Nesteness versus modularity in ecological networks: two sides of the same coin? *J. Anim. Ecol.*, 79, 811–817.
- Galetti, M., Donatti, C.I., Pizo, M.A. & Giacomini, H.C. (2008). Big fish are the best: seed dispersal of *Bactris glaucescens* by the pacu fish (*Piaractus mesopotamicus*) in the Pantanal, Brazil. *Biotropica*, 40(3), 386–389.
- Gautier-Hion, A., Duplantier, J.M., Quris, R., Feer, F., Sourd, C., Decoux, J.P. *et al.* (1985). Fruit characters as a basis of fruit choice and seed dispersal in a tropical forest vertebrate community. *Oecologia*, 65, 324–337.
- Guimarães, P.R. & Guimarães, P. (2006). Improving the analyses of nestedness for large sets of matrices. *Environ. Model. Softw.*, 21, 1512–1513.
- Guimarães, P.R., Rico-Gray, V., Reis, S.F. & Thompson, J.N. (2006). Asymmetries in specialization in ant–plant mutualistic networks. *Proc. R. Soc. Lond.*, 273, 2041–2047.
- Guimarães, P.R., Rico-Gray, V., Oliveira, P.S., Izzo, T.J., dos Reis, S.F. & Thompson, J.N. (2007). Interaction intimacy affects structure and coevolutionary dynamics in mutualistic networks. *Curr. Biol.*, 17, 1797–1803.
- Guimarães, P.R., Galetti, M. & Jordano, P. (2008). Seed dispersal anachronisms: rethinking the fruits extinct megafauna ate. *PLoS ONE*, 3(3), e1745.
- Guimerà, R.M. & Amaral, L.A.N. (2005). Cartography of complex networks: modules and universal roles. *J. Stat. Mech.-Theory E.*, 02001, 1–13.
- Hackett, S.J., Kimball, R.T., Reddy, S., Bowie, R.C.K., Braun, E.L., Chojnowski, J.L. *et al.* (2008). A phylogenomic study of birds reveals their evolutionary history. *Science*, 320, 1763–1768.
- Harris, M.B., Tomás, W.M., Mourão, G., Da Silva, C.J., Guimarães, E., Sonoda, F. *et al.* (2005). Safeguarding the Pantanal wetlands: threats and conservation initiatives. *Conserv. Biol.*, 19, 714–720.
- Joppa, L.N., Montoya, J.M., Solé, R., Sanderson, J. & Pimm, S. (2010). On nestedness in ecological networks. *Evol. Ecol. Res.*, 12, 35–46.
- Jordano, P. (1987). Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries and coevolution. *Am. Nat.*, 129, 657–677.
- Jordano, P., Bascompte, J. & Olesen, J.M. (2006). The ecological consequences of complex topology and nested structure in pollination webs. In: *Plant–Pollinator Interactions: From Specialization to Generalization* (eds Waser, N.M. & Olferton, J.). The University of Chicago Press, London, pp. 173–199.
- Jordano, P., Vázquez, D. & Bascompte, J. (2009). Redes complejas de interacciones planta–animal. In: *Ecología y Evolución de las Interacciones Planta–Animal: Conceptos y Aplicaciones* (eds Medel, R., Aizen, M. & Zamora, R.). Editorial Universitaria, Santiago, pp. 17–41.
- Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D. *et al.* (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, 26, 1463–1464.
- Klicka, J., Burns, K. & Spellman, G.M. (2007). Defining a monophyletic Cardinalini: a molecular perspective. *Mol. Phylogenet. Evol.*, 45, 1014–1032.
- Lewinsohn, T.M., Prado, P.I., Jordano, P., Bascompte, J. & Olesen, J.M. (2006). Structure in plant–animal interaction assemblages. *Oikos*, 113(1), 174–184.
- Maddison, W.P. & Slatkin, M. (1991). Null models for the number of evolutionary steps in a character on a phylogenetic tree. *Evolution*, 45, 1184–1197.
- Nuismer, S.L., Gomulkiewicz, R. & Ridenhour, B.J. (2010). When is correlation coevolution? *Am. Nat.*, 175(5), 525–537.
- Olesen, J.M., Bascompte, J., Dupont, Y.L. & Jordano, P. (2007). The modularity of pollination networks. *PNAS*, 104(50), 19891–19896.
- Pereira, S.L., Baker, A.J. & Wajntal, A. (2002). Combined nuclear and mitochondrial DNA sequences resolve relationships within the Cracidae (Galliformes, Aves). *Syst. Biol.*, 51(6), 964–958.
- Pimm, S.L. & Lawton, J.H. (1980). Are food webs compartmented? *J. Anim. Ecol.*, 49, 879–898.
- Pott, A. & Pott, V.J. (1994). *Plantas do Pantanal*. Embrapa, Brasília.



- Prance, G.T. & Schaller, G.B. (1982). Preliminary study of some vegetation types of the Pantanal, Mato Grosso, Brazil. *Brittonia*, 34(2), 228–251.
- Rezende, E., Lavabre, J., Guimarães, P.R. & Bascompte, J. (2007). Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature*, 448, 925–928.
- Rezende, E., Albert, M.E., Fortuna, M.A. & Bascompte, J. (2009). Compartments in a marine food web associated with phylogeny, body mass, and habitat structure. *Ecol. Lett.*, 12, 779–788.
- Santamaría, L. & Rodríguez-Gironés, M.A. (2007). Linkage rules for plant-pollination networks: trait complementary or exploitation barriers? *PLoS Biol.*, 5(2), 354–362.
- Silva, W.R., Guimarães, P.R. Jr, dos Reis, S.F. & Guimarães, P. (2007). Investigating the fragility in plant-frugivore networks: a case study of the Atlantic Forest in Brazil. In: *Seed Dispersal: Theory and its Application in a Changing World* (eds Dennis, A.J., Schupp, E.W., Green, R.J. & Westcott, D.A.). CAB International, Wallingford, pp. 561–578.
- Stang, M., Klinkhamer, P.G.L. & van der Meijden, E. (2007). Asymmetric specialization and extinction risk in plant–flower visitor webs: a matter of morphology or abundance? *Oecol.*, 151, 442–453.
- Tello, J.G., Moyle, R.G., Marchese, D.J. & Cracraft, J. (2009). Phylogeny and phylogenetic classification of the tyrant flycatchers, cotingas, manakins, and their allies (Aves: Tyrannidae). *Cladistics*, 25, 429–467.
- Thebault, E. & Fontaine, C. (2010). Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science*, 329, 853–856.
- Thompson, J.N. (2005). *The Geographic Mosaic of Coevolution*. University of Chicago Press, Chicago.
- Van der Pijl, L. (1982). *Principles of Dispersal in Higher Plants*. Springer, Berlin.
- Vázquez, D.P., Chacoff, N.P. & Cagnolo, L. (2009). Evaluating multiple determinants of the structure of plant–animal mutualistic networks. *Ecology*, 90, 2039–2046.
- Wojciechowski, M.F., Lavin, M. & Sanderson, M.J. (2004). A phylogeny of legumes (Leguminosae) based on analysis of the plastid *matK* gene resolves many well-supported subclades within the family. *Am. J. Bot.*, 91, 1846–1862.

## SUPPORTING INFORMATION

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

**Table S1** References that include data on seed dispersal interactions  
\* refers to networks available in Rezende, E., Lavabre, J., Guimarães

Jr., P.R. & Bascompte, J. (2007). Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature*, 448, 925–928) and \*\* refers to networks available in the Interaction Web Database (<http://www.nceas.ucsb.edu/interactionweb>).

**Table S2** Seed dispersal interactions, with animal species in rows and plant species in columns. A matrix element representing a seed dispersal interaction between a plant and an animal species that was recorded received the value of 1, and 0 otherwise.

**Table S3** Module in which each species was assigned (M1: mammal-dominated 1, M2: mammal-dominated 2, B1: bird module 1, B2: bird module 2, F: fish module), family, body mass, fruit length, fruit diameter, fruit mass, seed length, seed diameter, seed mass,  $\alpha$ ,  $c$  and number of interactions in the seed dispersal network.

**Appendix S1** Description of the study sites.

**Appendix S2** Explanation for setting branch lengths equal to 1.

**Figure S1** Accumulation curve with the average (black) and the standard deviation (grey) of the number of seed dispersal interactions in 1000 iterations, as a function of the number of seed dispersal events. Dashed line represents the asymptotic value for the number of seed dispersal interactions, which was estimated to be approximate 289.

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