

# Individual variation in resource use by opossums leading to nested fruit consumption

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Despite recent findings on the ecological relevance of within population diet variation far less attention has been devoted to the role diet variation for ecological services. Seed dispersal is a key ecological service, affecting plant fitness and regeneration based on foraging by fruit-eating vertebrates. Here we used a network approach, widely used to understand how seed-dispersal is organized at the species level, to gain insights into the patterns that emerge at the individual-level. We studied the individual fruit consumption behavior of a South American didelphid *Didelphis albiventris*, during the cool-dry and warm-wet seasons. In species–species networks the heterogeneity in specialization levels generates patterns such as nestedness and asymmetry. Because generalist populations may be comprised of specialized individuals, we hypothesized that network structural properties, such as nestedness, should also emerge at the individual level. We detected variation in fruit consumption that was not related to resource availability, ontogenetic or sexual factors or sampling biases. Such variation resulted in the structural patterns often found in species–species seed-dispersal networks: low connectance, a high degree of nestedness and the absence of modules. Moreover structure varied between the warm-wet and cool-dry seasons, presumably as a consequence of seasonal fluctuation in fruit availability. Our findings suggest individuals may differ in selectivity causing asymmetries in seed dispersal efficiency within the population. In this sense the realized dispersal would differ from the expected dispersal estimated from their average dispersal potential. Additionally the results suggest possible frequency-dependent effects on seed dispersal that might affect individual plant performance and plant community composition.

The study of the structure of interaction networks between species sheds light on the underlying ecological and evolutionary processes that shape and organize species interactions (Bascompte et al. 2003, 2006, Jordano et al. 2003, Vázquez et al. 2005, Olesen et al. 2007). Mutualistic networks typically incorporate structural patterns, which have been reported for several distinct systems (Bascompte et al. 2003, Guimarães et al. 2007, Olesen et al. 2007), such as asymmetry in the number of interactions (Vázquez and Aizen 2004), low connectance (i.e. low proportion of realized interactions; Jordano 1987), nestedness (i.e. the interacting assemblage of a species is a subset of the interacting assemblage of species with more interactions; Bascompte et al. 2003), the presence of modules (i.e. distinct subsets of highly interacting species (Olesen et al. 2007) and right-skewed distributions of the number of interactions per species (Jordano et al. 2003). However, such species networks do not explicitly address variation within populations (Araújo et al. 2008, 2009, 2010, Pires et al. 2011, Bolnick

et al. 2011) because the networks are based on a mean-field approach, which considers conspecific individuals as ecologically equivalent. However, there is an increasing body of theory and empirical evidence showing that many generalist populations are in fact heterogeneous (Bolnick et al. 2003, 2007).

Some populations are divided into groups of individuals that consume similar sets of resources (Araújo et al. 2008), whereas others are comprised of individuals with different degrees of selectivity (Araújo et al. 2010, Pires et al. 2011, Tinker et al. 2012). Recently, the use of networks to depict individual resource-use patterns has proven useful for understanding the basis and implications of interindividual diet variation (Araújo et al. 2008, 2009, 2010, Pires et al. 2011). The structures of individual-level networks that depict mutualistic relationships have just begun to be unraveled (Dupont et al. 2010, Gómez et al. 2011). We now have evidence that such heterogeneity within populations may affect ecological processes at different levels (Bolnick et al. 2011). For instance, the features of individual-level networks may scale up and become drivers of the structure and dynamics of species-level networks

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(Dupont et al. 2010). Recent work on mutualistic individual-based networks of plants and their pollinators demonstrated that network structure was coupled with its functioning and had great implications for the performance of plant populations (Gómez et al. 2011). Similarly, the study of individual-level networks representing other kinds of mutualistic interactions should reveal non-random patterns with implications for ecosystem functioning.

Seed dispersal is one of the most important ecosystem services provided by animals (Kremen 2005). Many species of vertebrates are generalist frugivores that may roam over wide areas dispersing a large variety of plant species (Herrera 1995, Jordano 2000). Seed dispersal by animals facilitates the escape from high mortality rates near parental trees, the colonization of distant areas and the germination of seeds in suitable places (Jordano 2000). Therefore, seed-dispersal by frugivores largely affects plant fitness and recruitment (Jordano and Herrera 1995, Jordano 2000). However, if a population of a seed-dispersing species is comprised of groups of individuals that differ in their diet preferences, one may expect that the realized seed dispersal differs from the dispersal expected when assuming a homogeneous population. Therefore, different network structures of the interactions among individual dispersers and plant species would have implications on the patterns of seed dispersal in a given location.

Here, we studied the fruit consumption behavior of the white-eared opossum *Didelphis albiventris*, a South American didelphid regarded as a generalist forager (Emmons and Feer 1990). We hypothesized that the network structural properties described for species–species networks, such as nestedness and modularity, also emerge in a network depicting fruit consumption at the individual level. Our hypothesis is based on the knowledge that generalist populations may be composed of specialized individuals (Bolnick et al. 2003), so that structure might emerge from this heterogeneity, as happens with consumer–resource relationships. Mutualisms, such as seed dispersal, are essentially consumer–resource relationships (Holland and DeAngelis 2010), and recent work has revealed that modularity (Araújo et al. 2008) and nestedness (Araújo et al. 2010, Pires et al. 2011) are structural patterns that emerge in networks depicting interactions among individual consumers and resource categories. Nestedness emerges if the individuals have different levels of resource selectivity (Araújo et al. 2010, Pires et al. 2011). In the context of seed dispersal networks, nestedness would mean that more selective individuals feed on and disperse subsets of the broader frugivorous diet of the more opportunistic individuals, which consequently disperse a higher diversity of seeds. Alternatively, if subgroups of individuals use distinct subsets of the available resources, the network would be highly modular (Araújo et al. 2008, Pires et al. 2011). When individuals are clustered based on the similar frugivorous diet, the plant species within each module would be dispersed together. In addition, the dispersal efficiency may depend on the module size: large modules would represent more individuals dispersing a subset of plant species, while the plants within smaller modules would be dispersed by fewer individuals.

## Material and methods

### Studied model

We studied the fruit consumption behavior of the white-eared opossum *Didelphis albiventris*, which is regarded as a generalist forager and opportunistic frugivore that has an important role in seed dispersal dynamics during several successional stages (Cáceres 2002, Cantor et al. 2010). *Didelphis albiventris* is a good model species because of its high abundance (Fonseca and Robinson 1990) and ease of capture in different habitats (Cerqueira 1985). Moreover, consumed plant species can be determined by sampling the opossum fecal content because the seeds are usually defecated intact and viable for germination (Cáceres 2002).

### Study area

The study area covered an urban park (13.44 ha), which included a secondary swamp forest fragment around an artificial dam, in a region of tropical, seasonal semi-deciduous forest (22°48'S, 47°04'W, southeastern Brazil). The swamp forest fragments in the region are characterized by a well-developed shrub-herbaceous stratum, a medium-height canopy (6–12 m) and species with relatively thin trunks (mean diameter at chest height: 9–21 cm). The Clusiaceae, Euphorbiaceae, Annonaceae, Magnoliaceae and Salicaceae families are represented by species that characterize the swamp forests in the region (Santin 1999). Myrtaceae and Lauraceae usually have high species richness in this region. The Köppen–Geiger climate of this region is CW2, i.e. highland sub-humid, with two seasons, namely, a cool-dry (April to September) season and a warm-wet season (October to March).

### Sampling design

We sampled the marsupials along a 1600 m elliptical sampling track, using a set of 100 Young live traps (40 × 20 × 20 cm). Every 40 m, a trap was placed on the ground and another in the understorey (1.6 to 2 m high); every 80 m, a third trap was placed in the canopy, about 5 m above the ground. During 12 months (November 2006 to November 2007), the sampling was performed for three consecutive nights per month (sampling effort: 2868 trap-nights). We baited the traps with a mix of bananas, cornmeal, peanut butter, vanilla flavoring and cod-liver oil over a slice of manioc and checked in the morning of the following days.

The captured individuals were marked with numbered metal ear tags, and their sex, age and geographic position (GPS) were recorded. The animals were released in the same location. The age was inferred based on the sequence of tooth eruption, and individuals were considered adults when the third and fourth molar teeth were erupted (modified from Cordero and Nicolas 1987). We obtained seed-dispersal data by collecting the seeds of consumed fruits from fecal samples, which were previously dissolved and filtered with a 1-mm mesh sieve. The seeds were identified based on surveys of the local flora (Santin 1999) and a reference collection, obtained by monthly collections of

fruits in the region (for sampling details, see Cantor et al. 2010). The mean recapture rate was  $5.8 \pm 3.8$  SD per individual and  $6.1 \pm 4.4$  per trap. A total of 187 fecal samples (88 from the warm–wet and 99 from the cool–dry season) were obtained from 41 captured and recaptured adult opossums (16 males and 25 females). We found 34 individuals that consumed fruits in the warm–wet season and 21 in the cool–dry season.

## Data analysis

The interactions between *D. albiventris* individuals and the consumed plant species were described as individual–resource networks (Pires et al. 2011). This dispersal network was defined as an incidence matrix  $A$  describing the trophic interactions between individuals (depicted in rows) and resources (in columns), where the element  $a_{ij}$  of the matrix is 1 if the consumption of the fruit of plant species  $j$  by individual  $i$  was recorded and zero otherwise. In the network representation, nodes representing individuals were linked to those representing plant species whenever seeds were found in an individual fecal sample. Although individual resource networks can be built with quantitative data, we relied here on qualitative data for four main reasons. First, using the number of seeds found in feces to study the network structure in this case could be misleading. Weighted network measures such as dependence of individuals on fruits or the strength (Bascompte et al. 2006) of each fruit type in the individual–resource networks would be biased by those fruit species that have more seeds per fruit. Second, although we are aware that quantitative information can give us a more complete depiction of how important a given interaction might be, using the number of seeds to assess seed dispersal effectiveness can lead one to overestimate the benefits of dispersal for the plant reproductive output. For instance, seeds that are dispersed altogether might have a low per capita success due to intraspecific competition and density-dependent seed predation (Clark et al. 2005). Third, although interaction frequencies are often assumed to be a good proxy for interaction strength in species–species networks (Vázquez et al. 2007), in the context of individual–resource networks abundant resources will strongly affect estimates. In this sense, estimating interactions strengths are more meaningful if resulting from an assessment of the mutual benefits of the interaction for each individual and plant species, which is a difficult task. Fourth, despite a handful of studies that have described patterns in quantitative networks (Bascompte et al. 2006, Vázquez et al. 2007) most of the previous descriptions of the structure of species–species networks were patterns that emerge in non-weighted networks (Bascompte et al. 2003, Olesen et al. 2007, Vázquez et al. 2009). Because we are interested in the cross-scale generality of network patterns using the non-weighted networks is the natural choice.

Fruiting is highly affected by the climatic seasonality in the study area (Morellato 1991). Most of the plant species that are highly consumed by the opossums, such as *P. guajava*, *P. amalago*, *Passiflora* spp. and *C. pachystachia*, have their highest yield during the warm–wet season. Knowing that seasonality affects fruit availability, we also

investigated if the seed-dispersal network structures were consistent throughout the year. To accomplish the seasonal analysis, we built separate individual–resource dispersal networks representing the interactions recorded in the cool–dry and the warm–wet seasons.

To describe the seed-dispersal network structure, we measured the following network structural properties: 1) connectance, 2) nestedness and 3) modularity. 1) Connectance is the proportion of realized interactions (Jordano 1987), i.e. the proportion of fruit resources used by the individuals. One may expect higher connectance when the individuals have a broad and similar frugivorous diet, while lower values may be a consequence of heterogeneity in fruit consumption. 2) Nestedness is a particular network property describing asymmetric interactions, with a core of nodes with many interactions and other less-connected nodes that usually interact only with the densely connected subset of nodes (Bascompte et al. 2003, Guimarães et al. 2006). In our case, such a structure may point out that selective individuals would interact with subsets of the fruit species that are more consumed by the more opportunistic individuals (Araújo et al. 2010, Pires et al. 2011). We used the nestedness metric based on overlap and decrease fill (NODF; Almeida-Neto et al. 2008) to verify the degree of nestedness for each network using the software ANINHADO (Guimarães and Guimarães 2006). The NODF metric will tend to 100 for highly nested matrices and tend to zero when individuals show other nonrandom patterns of resource use, such as overdispersed and modular diets. 3) Modularity measures the tendency of the nodes to cluster into cohesive groups. A modular network would consist of weakly interlinked groups of individuals that internally are strongly connected due to the use of the same resource plants (Olesen et al. 2007, Fortuna et al. 2010). We used the modularity index,  $M$ , to estimate the degree of modularity of the individual–resource networks. For a given partition of a network in modules,  $M$  measures the difference between the number of interactions among nodes in the same module and among nodes in different modules (Guimerà and Amaral 2005a, b). Therefore a network with high degree of modularity has many within-module interactions and a few between-module interactions. This metric was calculated using the NETCARTO program (Guimerà et al. 2004), which uses a simulated annealing algorithm to find the partition of a network into modules that yields the largest degree of modularity (Guimerà and Amaral 2005a, b). Since the simulated annealing is a stochastic optimization technique, the results might vary among different runs. To explore the consistency of the modular topology, we ran the analysis 100 times for all the empirical matrices (for a similar approach see Donatti et al. 2011) to evaluate the variation of the modularity degree across different runs, and compared this distribution of values with a null distribution generated by a null model. Although this procedure did not consider that the network is composed of two sets of elements (individuals and plant species) and that interactions only occur among elements of distinct sets, any potential effect of this two-mode structure was taken into account by our null model, which produced theoretical bipartite networks (Pires et al. 2011).

All of the metrics were calculated for the individual-resource dispersal networks in the cool-dry and the warm-wet seasons separately. To allow for cross-network comparisons, we used the relative nestedness (Bascompte et al. 2003), a measure that corrects for variation in the size of the networks, i.e. the number of nodes and links. The relative nestedness was defined as  $N^* = (N - \bar{N}_R) / \bar{N}_R$ , where  $N$  is the nestedness of the actual matrix and  $\bar{N}_R$  is the average nestedness of random replicates generated from the null model analysis. Similarly, we also calculated the relative modularity,  $M^* = (M - \bar{M}_R) / \bar{M}_R$ .

Network patterns such as nestedness and modularity could emerge as a result of limited sampling. To overcome this potential sampling bias, we checked the significance of nestedness and modularity by comparing their empirical values to that of random networks of the same size, i.e. same number of nodes, and connectance. We created random networks by randomly resorting the 1's among the matrix cells according to marginal totals of rows and columns (Bascompte et al. 2003). Each cell has a probability of being filled that is proportional to the number of interactions of both individuals and plants:  $c_{ij} = \frac{1}{2} \left( \frac{P_i}{C} + \frac{P_j}{R} \right)$ , where  $P_i$  = number of fruit species consumed by the individual  $i$  (row sums);  $P_j$  = number of individuals that have consumed the species  $j$  (column sums);  $C$  = number of fruit resources (columns); and  $R$  = number of individuals (rows). Because the model uses real data to build the theoretical networks it accounts for possible sampling biases and differences in availability among fruits from the different plant species in the study area. Next, the significance of each metric was evaluated by checking if the observed values were within the 95% confidence intervals generated from 1000 randomized networks.

## Caveats

The study area had a heterogeneous distribution of fruit plants. To evaluate the effect of spatial resource heterogeneity on the resource-use patterns of individuals we used two tests. Because our diet samples involve a short temporal window after the feeding event, the fine-scale resource spatial heterogeneity could be a factor determining the individual variation in fruit consumption. Thus, we first tested if the seeds found in a given fecal sample came from plant species distributed in the vicinity (20-m radius) of the trap at which the fecal sample was collected. We created two dissimilarity matrices of the capture stations using the probabilistic Raup-Crick index based on the Monte Carlo randomization procedure (Raup and Crick 1979): 1) one based on the fecal samples obtained at each station, and 2) the other on the distribution of the more representative fruit resources in the vicinity of each capture station. For the latter, we recorded the presence of the five most-consumed fruit plant species within the 20-m radius around the traps, which were present in 78.4% of the fecal samples from the warm-wet season and in 50.7% of the cool-dry season samples (Table 1). We tested the correlation among the dissimilarity matrices using a Mantel test with 1000 permutations. A high correlation would indicate the content of

each sample was highly influenced by the surroundings suggesting a strong spatial component.

Second, we tested if the differences in the food resource use were a consequence of individual differences in spatial use. If ranging behavior affects diet, we should expect that individuals with a higher individual range overlap have a more similar fruit diet. We used the average Euclidian distance of geographic positions of all of the traps where individuals were captured as a proxy for ranging overlap between pairs of individuals. Then, we tested the correlation between matrices of pairwise diet similarity and ranging overlap using a Mantel test with 1000 permutations. Individual diet matrices were created using the Jaccard similarity index applied to presence-absence data of all of the fruit plant species consumed during the entire study and separated by the seasons. Even though this test is not a thorough analysis of spatial patterns, a high correlation in the diets would suggest that the spatial component is highly affecting network patterns.

Dietary differences related to gender could interfere with individual fruit consumption, and by doing so, the differences might give rise to structure in the individual-resource network. If this hypothesis was true, differences in seed species composition in feces would be expected between males and females. We checked for sexual differences in fruit consumption using a multi-dimensional scaling (MDS) ordination and cluster analysis applied to a standardized matrix of the Jaccard similarity of the fruit diet among individuals. An ANOSIM test was applied to determine the significance of the differences among groups (males and females). Individuals that had only one plant species in their feces (four cases) were excluded from this analysis. Moreover, to exclude ontogenetic differences, we removed juveniles from the dataset.

Finally, because network patterns could emerge as a result of limited sampling we double-checked the reliability of our sample size with two additional tests. First we performed a rarefaction analysis to test how sensitive the main pattern we found, was to sampling effort. We used a rarefaction approach with 1000 replicates for 95%, 90%, 85%, ..., 25% of the total number of fecal samples. For each replicate of each fraction of the total sample we redefined the diet of each individual and recalculated the network metric. If sample size highly affected the network topology we would expect that the values would not converge asymptotically to the value obtained for the whole sample. Additionally, we performed a sensitivity analysis to test how sensitive the pattern was to the number of individuals in the dataset. In this analysis we randomly removed a fraction of individuals from the total and recalculated the metric. Again the pattern would be sensitive to the number of individuals if there was no asymptotic behaviour (Supplementary material Appendix 1).

## Results

A total of 29 plant species was identified in the feces: 21 in the warm-wet season and 27 in the cool-dry season. In the warm-wet season, *Psidium guajava* (Myrtaceae) and *Piper amalago* (Piperaceae) were found in nearly 25% of the



Table 1. Percentage of the occurrence of plant species in the feces of *Didelphis albiventris* during each season.

Species	Family	Warm-wet (%)	Cool-dry (%)	Occurrence in the feces
<i>Psidium guajava</i>	Myrtaceae	28.8	4.0	Dec–Mar, Aug–Sep
<i>Piper amalago</i>	Piperaceae	25.6	2.7	Nov–May
<i>Morus nigra</i>	Moraceae	4.0	24.0	Sep–Oct
<i>Passiflora edulis</i>	Passifloraceae	8.8	16.0	Jan–Sep
<i>Cecropia pachystachya</i>	Cecropiaceae	11.2	4.0	Nov–May
<i>Brachiaria decumbens</i>	Poaceae	0	12.0	Mar–Aug
<i>Leucaena leucocephala</i>	Fabaceae	0	4.0	Feb–Jun and Nov
<i>Cyperus</i> sp.	Cyperaceae	0	4.0	Apr–Aug
<i>Solanum aculeatissimum</i>	Solanaceae	2.4	1.3	Nov–Jun
<i>Turnera ulmifolia</i>	Turneraceae	1.6	1.3	Feb–Jun
<i>Hovenia dulcis</i>	Rhamnaceae	0	2.7	Apr–Jun
<i>Polygonum</i> sp.	Polygonaceae	0	2.7	Jun
<i>Amaranthus hybridus</i>	Amaranthaceae	0.8	1.3	Feb and Jul
<i>Stylosanthes</i> sp.	Fabaceae	0.8	1.3	Jun
<i>Solanum</i> sp.	Solanaceae	0	1.3	Apr–Jun
<i>Carica papaya</i>	Caricaceae	0	1.3	Jun
<i>Luziola</i> sp.	Poaceae	0	1.3	May
<i>Paspalum</i> sp.	Poaceae	0	1.3	May
<i>Sida</i> sp.	Malvaceae	0	1.3	Aug
<i>Sapindus saponaria</i>	Sapindaceae	0.8	0	Mar
Unidentified (11 morphospecies)	–	10.4	10.7	–

samples, whereas in the cool-dry season, the diets were more variable with only *Morus nigra* (Moraceae) being found in a high proportion (Table 1). During the whole sampling period, 16 of the 41 individuals captured consumed only one to two fruit species, whereas nine individuals consumed more than six fruit species. Similarly, the distribution of the number of fruit species consumed per individual was also skewed towards narrow diets in each season. The majority of the individuals consumed only one or two fruits (warm-wet = 50%; cool-dry = 42%) whereas few individuals consumed more than six species (warm-wet = 9.5%; cool-dry = 8.8%). However the distribution of the number of interactions per individual is less uneven in the cool dry season (Fig. 1).

If the fruit consumption was affected by the fine-scale local fruit availability, one may expect individuals feeding in the proximity of their capture location, and so their feces would contain more seeds from the fruit plant species distributed around the trap in which they were captured. However, we found no correlation (total sampling period:  $r = -0.134$ ,  $p = 0.928$ ; warm-wet season:  $r = -0.219$ ,  $p = 0.993$ ; cool-dry season:  $r = 0.107$ ,  $p = 0.132$ ), suggesting that the resource spatial heterogeneity was not the major determining factor of individual variation in fruit consumption. The mean Euclidian distance between pairs of individuals was also not correlated with the similarity of their fruit diet (entire study:  $r = -0.133$ ,  $p = 0.895$ ; warm-wet season:  $r = -0.010$ ,  $p = 0.857$ ; cool-dry season:  $r = 0.019$ ,  $p = 0.467$ ). This finding suggests that individual differences in ranging behavior use did not affect the individual fruit consumption. Moreover, no differences were detected between females and males in the seeds present in the feces (ANOSIM,  $R = -0.013$ ,  $p = 0.528$ ). The MDS analysis (stress = 0.15) indicated a clear overlap between the fecal samples from individuals of different sexes. The cluster analysis suggested that individual difference in frugivory was not related to gender, because only six distinct pairs of

individuals of different sexes showed more than 60% of similarity in their fruit diets.

The network comprising the total dataset ( $C = 0.096$ ) showed a lower connectance than the two seasonal networks (warm-wet:  $C = 0.123$ ; cool-dry:  $C = 0.113$ ) (Fig. 2A). The networks for the total sampling period and for the warm-wet season had a high degree of nestedness (Fig. 2B), in which opossums with few interactions were frequently linked to the core of highly consumed plant species, i.e. those plants with many interactions (total study:  $N^* = 0.891$ ,  $p < 0.0001$ ; NODF = 29.63, 95% CI = 12.92–19.19; warm-wet season:  $N^* = 0.948$ ,  $p < 0.0001$ ; NODF = 38.19, 95% CI = 15.13–24.85). The rarefaction and sensitivity analyses showed that the significant nestedness observed in the warm-wet season was robust to limited sampling (Supplementary material Appendix 1, Fig. A1–A2). During the cool-dry season, nestedness was not detected ( $N^* = 0.301$ ,  $p = 0.025$ ; NODF = 19.20, 95% CI = 11.33–19.21, Fig. 2B). Moreover, all the networks were less modular than expected by chance (Fig. 2C) (total sampling period:  $M^* = -0.057$ ,  $p < 0.0001$ ;  $M = 0.406$ , 95% CI = 0.438–0.489; warm-wet:  $M^* = -0.050$ ,  $p = 0.001$ ;  $M = 0.409$ , 95% CI = 0.443–0.504; cool-dry:  $M^* = -0.011$ ,  $p = 0.020$ ;  $M = 0.517$ , 95% CI = 0.518–0.580). The low variance in empirical modularity values across different runs (mean  $\pm$  SD, total sampling period:  $M = 0.406 \pm 0.004$ ; warm-wet:  $M = 0.409 \pm 0.004$ ; cool-dry:  $M = 0.517 \pm 0.002$ ) suggested that the algorithm did not have problems in identifying the best network partitions.

## Discussion

The patterns in the individual–resource dispersal networks observed here are similar to the structural patterns reported for networks representing mutualism at the community

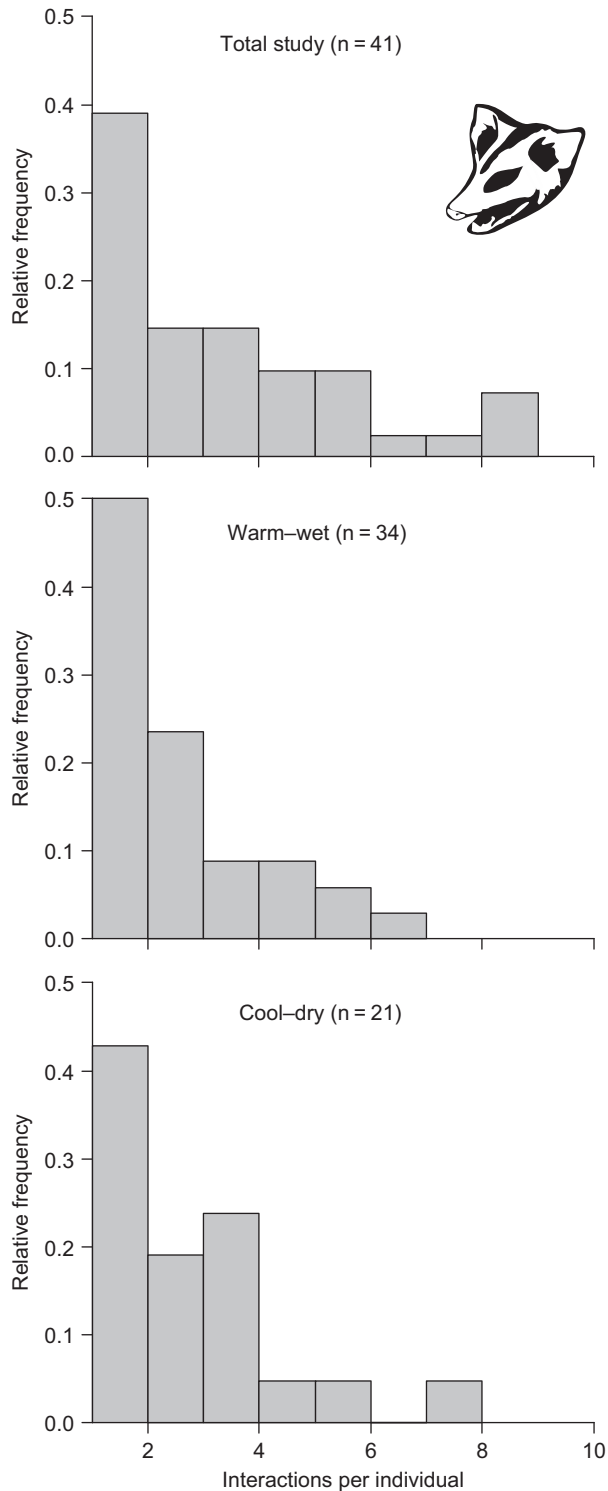


Figure 1. Distribution of the number of interactions per individual of white-eared opossum *Didelphis albiventris* in total sampling period, warm-wet season and cool-dry season.

level. The heterogeneity and nestedness of interactions between white-eared opossums and the seeds that they disperse are also features commonly found in mutualistic networks representing interactions among species (Bascompte et al. 2003, Vázquez et al. 2005, Guimarães et al. 2006, 2007). Thus, this study contributes to the growing evidence

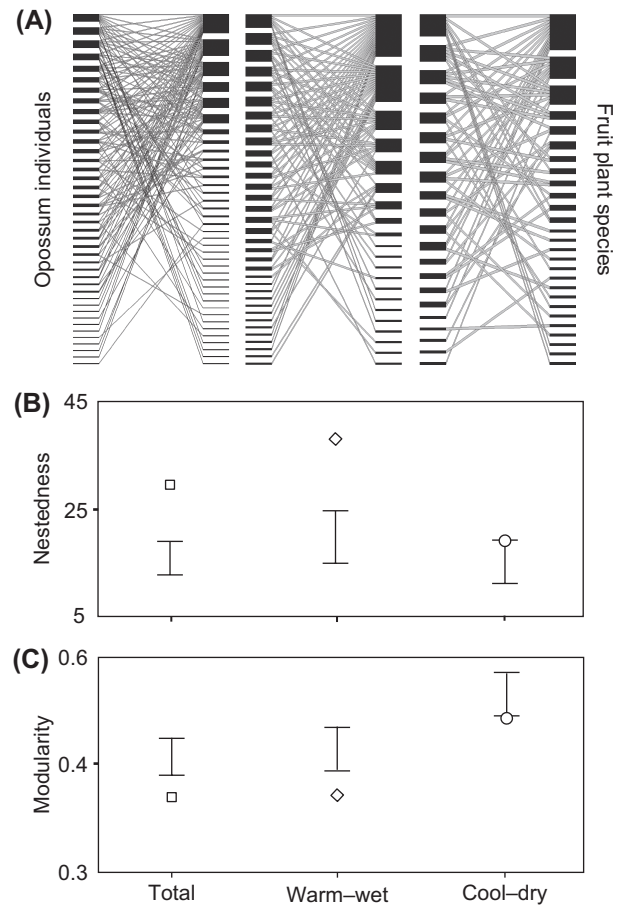


Figure 2. (A) Mutualistic networks depicting interactions between seed-dispersing individuals (left) and the fruit plant species consumed (right). Networks and metrics are presented for the total sampling period and for the warm-wet and cool-dry seasons; (B) nestedness based on overlap and decrease fill; and (C) average modularity based on 100 runs of the simulated annealing algorithm. The whiskers represent the 95% confidence intervals expected for random networks (see the text for further details). The networks were built using the Bipartite package (Dormann et al. 2008) in the R environment (R Development Core Team).

that patterns of resource use among individuals within populations are similar to those observed among species within communities (Dupont et al. 2010, Pires et al. 2011).

In the context of mutualistic community networks, nestedness means there is a core of interactions among generalists and that specialists interact with predictable, ordered subsets of the species that interact with the generalists (Bascompte et al. 2003, Guimarães et al. 2006). Similarly, in the context of individual–resource networks, nestedness means that there are individuals with broad and narrow diets within the same population, what could be the result of different levels of dietary selectivity. In this sense, in a nested individual–resource network the diets of selective individuals are predictable subsets of the diets of the more opportunistic ones, a pattern predicted by optimum diet theory models (Araújo et al. 2010, Pires et al. 2011). In such models nestedness is expected if individuals have identical rank preferences for different resources but differ in their willingness to include the lower-ranked resources

in their diets (the 'shared preference model'; Svanbäck and Bolnick 2005). This is a potential underlying mechanism to explain the nestedness in resource use we report here.

Nonetheless, the structure of the observed individual-resource network changed across seasons. We found differences between the connectance of the network, describing the interactions within each season and in the network for the whole period. Such differences are expected since some plant species fruit only in one season. Therefore when the data for both seasons are pooled together in the same network, links between non-temporally overlapping species and individuals increase both the fruit richness and number of individuals, leading to lower connectance.

Similarly, seasonality affected the network degree of nestedness. The interactions between white-eared opossums and plants were nested only in the warm-wet season. The change in the network pattern probably results from the seasonal fluctuation in fruit availability. In the cool-dry season, fruits become scarce in the study area (Morellato 1991). Because fruits are a key food resource, accounting for more than 75% of the white-eared opossum's diet (Cáceres 2002), individuals must add alternative resources to their diets to cope with the scarcity of top-ranked resources. Although we did not consider other food resources, such as insects and small vertebrates that could be consumed to supplement the diet, we did find a higher diversity of fruits consumed in the cool-dry season. Moreover the distribution of number of interactions per individuals was less uneven in the cool-dry season in comparison to the warm-wet season. This suggests that during the cool-dry season, a larger proportion of individuals used a broader range of available fruits to meet their energetic requirements. As a result, dietary overlap increases and the diets of all individuals become similar. This increase in diet similarity reduces the differences between individuals with broader and narrower diet, reducing the degree of nestedness as a consequence. The increase in dietary similarity is also a prediction of the shared-preference model in a scenario in which resource availability is reduced (Svanbäck and Bolnick 2005).

Different optimum diet theory models assume that individuals have distinct preferences, predicting a modular structure at high or intermediate levels of resource availability (Svanbäck and Bolnick 2005, Pires et al. 2011). However, we found no evidence of a modular structure. Nestedness was recently reported in individual-resource networks for two additional didelphid species and also other groups, such as anurans (Araújo et al. 2010, Pires et al. 2011). Our results add to the body of empirical evidence suggesting that nestedness is the prevalent pattern in individual-resource networks even when considering only mutualistic interactions.

Several mechanisms may contribute to intrapopulational variation in resource use, such as resource spatial heterogeneity, home range or sex- and age-related preferences (Bolnick et al. 2003), and consequently lead to structural patterns in individual-resource networks (Pires et al. 2011). Our findings suggest that the fruit consumption by white-eared opossum individuals was not simply the result of patchiness in resource availability or individual

ranging behavior. The seeds present in a given fecal sample were not related to the fruit resources distributed within the vicinity of each trap in which the sample was collected. In addition, considering that opossums generally have a large spatial range (Sunquist et al. 1987), all individuals of the population could forage throughout the entire area. Therefore, because all individuals probably had access to most of available fruit resources, potential differences in individual ranges and in the frequency distribution of trees would not be enough to explain the individual differences in frugivory. Furthermore, the diet variation was not influenced by gender, and by restricting our dataset only to adults, the effect of age was also discarded. Therefore, the differences in the fruit consumption and the nested pattern observed in this study possibly result from differences in individual feeding strategies. Nestedness would also emerge if some individuals that rely more on animal prey than fruits consume only a subset of the fruit diet of the more frugivore individuals. Regardless of whether nestedness results from differences in the degree of frugivory or the degree of selectivity of individuals for feeding on distinct fruits the underlying mechanism is still related to interindividual diet variation. Diet variation among individuals is usually related to functional tradeoffs that prevent any given individual from exploiting the whole set of available resources and therefore constrain individual niche widths (Robinson 2000, Bolnick et al. 2003). Future studies are needed to evaluate whether functional tradeoffs indeed occur in the population studied.

We are aware that other mechanisms, such as resource-switching behavior, sampling artifacts or even mechanisms related to species abundance, may also contribute to generate non-random structural patterns in mutualistic networks (Vázquez and Aizen 2004, Vázquez et al. 2007, 2009). However, in this study, we worked on a small fragment to enable the sampling effort applied to cover the whole area, collecting a great part of the fruiting plants and capturing almost the entire opossum population. In addition, by splitting the entire network into seasons, we could consider temporal variation due to the plant phenology. Finally, the null model we chose already incorporates the heterogeneity in resource use that may result from differences in resource availability or sampling bias (Bascompte et al. 2003).

The white-eared opossum and other didelphids are often regarded as important seed dispersers (Medellín 1994, Cantor et al. 2010). *Didelphis albiventris* is particularly common in urban and disturbed environments (Fonseca and Robinson 1990, Alho 2005) where reforestation is often required. Because of that, white-eared opossums have been considered as a management tool for forest regeneration due to their seed-dispersal potential (Cáceres and Monteiro-Filho 2007). Here, we showed that the white-eared opossum may display different degrees of selectivity in fruit consumption, which might lead to differences on how each individual impact the plant community. In a context in which the disperser population comprises individuals with non-equivalent frugivorous diets, the realized dispersal would differ from the dispersal expected when considering their average dispersal potential.

Due to the nested pattern of fruit consumption, some plant species will always have their seeds dispersed because they are dispersed by individuals with all degrees of selectivity. In contrast, some plant species would be relying upon dispersal by individuals with larger diet breadth. Thus, we suggest that the heterogeneity in fruit consumption would imply a frequency-dependent seed dispersal process. In this sense, depending on the proportion of selective and opportunistic individuals in the population, at least two distinct ecological implications can be hypothesized. In a selective-skewed scenario, those plant species whose fruits are consumed by most individuals might show a disproportionately large dispersal potential since they are benefited by the dispersal of both opportunistic and selective individuals, but also due to the lower seed diversity carried in the dispersers' feces. Conversely, if the frequency of opportunists is higher, dispersal would not be biased towards such plant species. Such frequency dependence may be one of the contributing factors to the composition of local plant communities. For instance, a more homogeneous dispersal potential across plant species, provided by the opportunistic-skewed scenario, would favor the persistence of a higher diversity in the local plant community.

Estimating disperser effectiveness is challenging and requires knowledge on both the quantity and quality component (Jordano and Schupp 2000). Future studies using quantitative data that adequately take into account the quality component, by testing how dispersal by individuals with different diets benefit germination and plant establishment, for example, are needed to improve our understanding on the robustness of our results and the implications of the patterns found for system dynamics. Our findings reveal for the first time evidence of network structural patterns emerging at an individual level in a seed-dispersal context that might have important implications for the composition of plant communities.

Recent studies have highlighted the means by which intraspecific variation may affect community structure and dynamics (Bolnick et al. 2011, Rudolf and Lafferty 2011). Here, we suggested that the proportion of opportunistic and selective individuals in the population may play an important role in the seed-dispersal potential of the population. One question that emerges from our study is how information on individual-resource networks can help us to understand the patterns and processes at other scales of biological organization. For instance, in populations where the variation among individuals is large, average interaction strengths are not informative, and dynamic models that do not consider such variation would produce unrealistic results (Bolnick et al. 2011). Moreover, by demonstrating a seasonal variation in network structure, we reinforce the need for considering the temporal scale when characterizing interaction networks, as suggested by the recent findings on mutualistic interactions at the community level (Petanidou et al. 2008, Díaz-Castelazo et al. 2010). Developing biologically realistic models that encompass intrapopulation variation and seasonal effects and that consider individual-resource network structural patterns would be the first step for generating more straightforward predictions of the ecological consequences of the patterns described here.

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## References

- Alho, C. J. R. 2005. Intergradation of habitats of non-volant small mammals in the patchy Cerrado landscape. – *Arq. Mus. Nac. Rio de Janeiro* 63: 41–48.
- Almeida-Neto, M. et al. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. – *Oikos* 117: 1227–1239.
- Araújo, M. S. et al. 2008. Network analysis reveals contrasting effects of intraspecific competitions on individual vs population diets. – *Ecology* 89: 1981–1993.
- Araújo, M. S. et al. 2009. Individual-level diet variation in four species of Brazilian frogs. – *J. Anim. Ecol.* 78: 848–856.
- Araújo, M. S. et al. 2010. Nested diets: a novel pattern of individual-level resource use. – *Oikos* 119: 81–88.
- Bascompte, J. et al. 2003. The nested assembly of plant-animal mutualistic networks. – *Proc. Natl Acad. Sci. USA* 100: 9383–9387.
- Bascompte, J. et al. 2006. Asymmetric coevolutionary networks facilitate biodiversity maintenance. – *Science* 312: 431–433.
- Bolnick, D. I. et al. 2003. The ecology of individuals: incidence and implications of individual specialization. – *Am. Nat.* 61: 1–28.
- Bolnick, D. I. et al. 2007. Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. – *Proc. Natl Acad. Sci. USA* 104: 10075–10079.
- Bolnick, D. I. et al. 2011. Why intraspecific trait variation matters in community ecology. – *Trends Ecol. Evol.* 26: 183–192.
- Cáceres, N. C. 2002. Food habits and seed dispersal by the white-eared opossum, *Didelphis albiventris*, in southern Brazil. – *Stud. Neotrop. Fauna Environ.* 37: 97–104.
- Cáceres, N. C. and Monteiro-Filho, E. L. A. 2007. Germination in seed species ingested by opossums: implications for seed dispersal and forest conservation. – *Braz. Arch. Biol. Tech.* 50: 921–928.
- Cantor, M. et al. 2010. Potential seed dispersal by *Didelphis albiventris* (Marsupialia, Didelphidae) in highly disturbed environment. – *Biot. Neotrop.* 10: 45–51.
- Cerqueira, R. 1985. The distribution of *Didelphis* in South America (Polyprotodontia, Didelphidae). – *J. Biogeogr.* 12: 135–145.
- Clark, C. J. et al. 2005. Comparative seed shadows of bird-, monkey- and wind-dispersed trees. – *Ecology* 86: 2684–2694.
- Cordero, A. and Nicolas, A. 1987. Feeding habits of the opossum (*Didelphis marsupialis*) in northern Venezuela. – *Field. Zool.* 39: 125–131.
- Díaz-Castelazo, C. et al. 2010. Changes of a mutualistic network over time: reanalysis over a 10-year period. – *Ecology* 91: 793–801.
- Donatti, C. I. et al. 2011. Analysis of a hyper-diverse seed dispersal network: modularity and underlying mechanisms. – *Ecol. Lett.* 14: 773–781.



- Dormann, C. F. et al. 2008. Introducing the bipartite package: analysing ecological networks. – *R News* 8: 8–11.
- Dupont, Y. L. et al. 2010. Scaling down from species to individuals: a flower–visitation network between individual honeybees and thistle plants. – *Oikos* 120: 170–177.
- Emmons, L. H. and Feer, F. 1990. Neotropical rainforest mammals: a field guide. – Univ. of Chicago Press.
- Fonseca, G. A. B. and Robinson, J. G. 1990. Forest size and structure: competitive and predatory effects on small mammal communities. – *Biol. Conserv.* 53: 265–294.
- Fortuna, M. A. et al. 2010. Nestedness versus modularity in ecological networks: two sides of the same coin? – *J. Anim. Ecol.* 79: 811–817.
- Gómez, J. M. et al. 2011. The functional consequences of mutualistic network architecture. – *PLoS One* 6: e16143.
- Guimarães, P. R. and Guimarães, P. 2006. Improving the analyses of nestedness for large sets of matrices. – *Environ. Modell. Soft.* 21: 1512–1513.
- Guimarães, P. R. et al. 2006. Asymmetries in specialization in ant-plant mutualistic networks. – *Proc. R. Soc. B* 273: 2041–2047.
- Guimarães, P. R. et al. 2007. The nested structure of marine cleaning symbiosis: is it like flowers and bees? – *Biol. Lett.* 3: 51–54.
- Guimerà, R. and Amaral, L. A. N. 2005a. Functional cartography of complex metabolic networks. – *Nature* 433: 895–900.
- Guimerà, R. and Amaral, L. A. N. 2005b. Cartography of complex networks: modules and universal roles. – *J. Stat. Mech. Theor. Exp.* P02001.
- Guimerà, R. et al. 2004. Modularity from fluctuations in random graphs and complex networks. – *Phys. Rev. E* 70: 025101.
- Herrera, C. M. 1995. Plant–vertebrate seed dispersal in the Mediterranean: ecological, evolutionary and historical determinants. – *Annu. Rev. Ecol. Evol. Syst.* 26: 705–727.
- Holland, J. N. and DeAngelis, D. L. 2010. A consumer–resource approach to the density-dependent population dynamics of mutualism. – *Ecology* 91: 1286–1295.
- Jordano, P. 1987. Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries and coevolution. – *Am. Nat.* 129: 657–677.
- Jordano, P. 2000. Fruits and frugivory. – In: Fenner, M. (ed.), *Seeds: the ecology of regeneration in natural plant communities*, 2nd edn. CABI, pp. 125–166.
- Jordano, P. and Herrera, C. M. 1995. Shuffling the offspring: uncoupling and spatial discordance of multiple stages in vertebrate seed dispersal. – *Ecoscience* 2: 230–237.
- Jordano, P. and Schupp, E. W. 2000. Seed disperser effectiveness: the quantity component and patterns of seed rain for *Prunus mahaleb*. – *Ecol. Monogr.* 70: 591–615.
- Jordano, P. et al. 2003. Invariant properties in coevolutionary networks of plant–animal interactions. – *Ecol. Lett.* 6: 69–81.
- Kremen, C. 2005. Managing ecosystem services: what do we need to know about their ecology? – *Ecol. Lett.* 8: 468–479.
- Medellín, R. A. 1994. Seed dispersal of *Cecropia obtusifolia* by two species of opossums in the Selva Lacandona, Chiapas, Mexico. – *Biotropica* 26: 400–407.
- Morellato, L. P. C. 1991. Estudo da fenologia de árvores, arbustos e lianas de uma floresta semidecídua no sudeste do Brasil. – PhD thesis, Univ. Estadual de Campinas, São Paulo.
- Olesen, J. M. et al. 2007. The modularity of pollination networks. – *Proc. Natl Acad. Sci. USA* 104: 19891–19896.
- Petanidou, T. et al. 2008. Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. – *Ecol. Lett.* 11: 564–575.
- Pires, M. M. et al. 2011. The nested assembly of individual–resource networks. – *J. Anim. Ecol.* 80: 896–903.
- Raup, D. M. and Crick, R. E. 1979. Measurement of faunal similarity in paleontology. – *J. Paleont.* 53: 1213–1227.
- Robinson, B. W. 2000. Trade offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. – *Behaviour* 137: 865–888.
- Rudolf, V. H. W. and Lafferty, K. D. 2011. Stage structure alters how complexity affects stability of ecological networks. – *Ecol. Lett.* 14: 75–79.
- Santin, D. A. 1999. A vegetação remanescente do município de Campinas (SP): mapeamento, caracterização fisionômica e florística, visando à conservação. – PhD thesis, Univ. Estadual de Campinas, São Paulo.
- Sunquist, M. E. et al. 1987. Movement patterns and home range in the common opossum (*Didelphis marsupialis*). – *J. Mamm.* 68: 173–176.
- Svanbäck, R. and Bolnick, D. I. 2005. Intraspecific competition affects the strength of individual specialization: an optimal diet theory method. – *Evol. Ecol. Res.* 7: 993–1012.
- Tinker, M. T. et al. 2012. Structure and mechanism of diet specialisation: testing models of individual variation in resource use with sea otters. – *Ecol. Lett.* 15: 475–483.
- Vázquez, D. P. and Aizen, M. A. 2004. Asymmetric specialization: a pervasive feature of plant–pollinator interactions. – *Ecology* 85: 1251–1257.
- Vázquez, D. P. et al. 2005. Species abundance patterns and the distribution of specialization in host–parasite interaction networks. – *J. Anim. Ecol.* 74: 946–955.
- Vázquez, D. P. et al. 2007. Species abundance and asymmetric interaction strength in ecological networks. – *Oikos* 116: 1120–1127.
- Vázquez, D. P. et al. 2009. Evaluating multiple determinants of the structure of plant–animal mutualistic networks. – *Ecology* 90: 2039–2046.

Supplementary material (available online as Appendix oik-00070 at <[www.oikosoffice.lu.se/appendix](http://www.oikosoffice.lu.se/appendix)>). Appendix 1.