

Morphological and Spatio-Temporal Mismatches Shape a Neotropical Savanna Plant-Hummingbird Network

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ABSTRACT

Complex networks of species interactions might be determined by species traits but also by simple chance meetings governed by species abundances. Although the idea that species traits structure mutualistic networks is appealing, most studies have found abundance to be a major structuring mechanism underlying interaction frequencies. With a well-resolved plant–hummingbird interaction network from the Neotropical savanna in Brazil, we asked whether species morphology, phenology, nectar availability and habitat occupancy and/or abundance best predicted the frequency of interactions. For this, we constructed interaction probability matrices and compared them to the observed plant–hummingbird matrix through a likelihood approach. Furthermore, a recently proposed modularity algorithm for weighted bipartite networks was employed to evaluate whether these factors also scale-up to the formation of modules in the network. Interaction frequencies were best predicted by species morphology, phenology and habitat occupancy, while species abundances and nectar availability performed poorly. The plant–hummingbird network was modular, and modules were associated to morphological specialization and habitat occupancy. Our findings highlight the importance of traits as determinants of interaction frequencies and network structure, corroborating the results of a previous study on a plant–hummingbird network from the Brazilian Atlantic Forest. Thus, we propose that traits matter more in tropical plant–hummingbird networks than in less specialized systems. To test the generality of this hypothesis, future research could employ geographic or taxonomic cross-system comparisons contrasting networks with known differences in level of specialization.

Abstract in Portuguese is available in the online version of this article.

Key words: Cerrado; forbidden links; habitats; modules; phenology; pollination; QuanBiMo.

SPECIES ARE PART OF COMPLEX NETWORKS OF INTERACTIONS THAT STRUCTURE ECOLOGICAL COMMUNITIES. However, the mechanisms determining the occurrence and strength of species interactions in local communities remain debated (Vázquez *et al.* 2009b, Olesen *et al.* 2011, Junker *et al.* 2013, Vizentin-Bugoni *et al.* 2014). Much of this debate has been centered on the importance of species traits, such as floral corolla and pollinator mouthpart length or fruit size and the bill gape width of frugivore birds (Olesen *et al.* 2011, Vizentin-Bugoni *et al.* 2014). Furthermore, because species should occur in the same location and at the same time to interact, spatio-temporal mismatches among species may also determine the structure and dynamics of ecological networks (Morales & Vázquez 2008, Vázquez *et al.* 2009a,b, Olesen *et al.* 2011, Vizentin-Bugoni *et al.* 2014). In ecological networks, species traits constraining interactions are often referred to as ‘forbidden links’ (Olesen *et al.* 2011), although this term may better relate to the incidence of interactions (binary networks), and not necessar-

ily their strength (weighted networks). Several recent studies, however, have shown that species abundances can be as important, or even more important, than species traits in structuring ecological interaction networks, including plant–frugivore (Krishna *et al.* 2008), plant–pollinator (Vázquez *et al.* 2009b), host plant–epiphyte (Sáyago *et al.* 2013) and plant–ant interaction networks (Dáttilo *et al.* 2014).

Although current evidence supports a large importance of abundance in shaping interaction networks, it is noteworthy that a recent study of a specialized plant–hummingbird network in the Brazilian Atlantic Forest showed mismatches in species morphology and phenology as the major factors structuring interactions (Vizentin-Bugoni *et al.* 2014). It remains to be investigated if this unique result is due to the intrinsic nature of the system considered. However, the result is consistent with natural history knowledge that interactions between tropical plants and hummingbirds are indeed determined by species traits, including plant and hummingbird morphology, nectar availability, and hummingbird foraging behavior (Stiles 1975, Feinsinger & Colwell 1978, Dalsgaard *et al.* 2009). Here, we use

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a plant-hummingbird network to further understand the factors shaping plant-pollinator interaction networks. Besides species morphology and phenology, we also evaluated the effect of floral nectar availability, which commonly affects hummingbird visitation pattern (e.g., Justino *et al.* 2012) and spatial co-occurrence via habitat occupancy (see also Jordano *et al.* 2006, Morales & Vázquez 2008, Vázquez *et al.* 2009a).

By limiting the occurrence of pairwise interactions, morphological traits, as well as phenological and spatial constraints might also 'scale-up' to the formation of sub-community structure within an ecological network, *i.e.*, modules characterized by high within-module prevalence over between-module interactions (Dormann & Strauss 2014). Modules in pollination networks are proposed to reflect specialized functional groups of pollinators and floral traits, which may determine the subset of preferentially interacting species (Olesen *et al.* 2007, Danieli-Silva *et al.* 2012) or seasonality in floral and pollinator appearance, *i.e.*, phenological matching (Martín González *et al.* 2012). Although modularity is common in plant-pollinator networks, we know surprisingly little about the role of species traits and spatio-temporal occurrence as determinants of modules. Furthermore, virtually all information is based upon binary networks (e.g., Olesen *et al.* 2007, Danieli-Silva *et al.* 2012, Martín González *et al.* 2012, Dalsgaard *et al.* 2013).

In this study, we used data on the interactions between hummingbirds and their flowers from one locality in the Cerrado, the Neotropical savanna ecosystem in the central portion of Brazil. The Cerrado is a clear example of a complex and patchy ecosystem (Silva & Bates 2002), in which the mosaic of distinct habitats is connected by the movement of species, especially highly mobile avian species (Tubelis *et al.* 2004, Maruyama *et al.* 2013a). In this sense, it provides a good model system to test if species' spatial distribution is an important factor in determining patterns of species interactions in ecological communities in addition to species traits, phenology and abundance. We use the recently proposed QuanBiMo algorithm to compute and define modules in weighted bipartite networks (Dormann & Strauss 2014) and probability matrices to predict the important factors influencing interaction frequencies (Vázquez *et al.* 2009b). We addressed two questions: (i) what is the relative importance of species abundance, morphological matching, phenological overlap, habitat occupancy, and floral energy/nectar production in determining interaction frequencies? (ii) Do the observed modules associate to the same factors relevant for predicting interaction frequencies?

METHODS

STUDY SITE.—We collected data at Panga Ecological Station (hereafter 'Panga'; 19°10'27"S, 48°23'51"W) in Brazil. Panga covers approximately 400 ha and includes many plant formations that characterize the Cerrado ecosystem, from grasslands and open savannas to dense forest formations. Climate is seasonal, characterized by a warm rainy season from October to March and a cooler dry season from April to September. Mean monthly temperature is 22.8°C and mean annual precipitation is 1482 mm (Cardoso *et al.* 2009).

PLANT-HUMMINGBIRD INTERACTIONS AND ABUNDANCE.—Sampling took place every other week from November 1996 to November 1997, mostly from 0600 to 1200 h. The overall flower availability, hummingbird abundance, and all plant-hummingbird interactions were quantified. We collected data on flower-hummingbird interactions along transects separated from each other by at least 25 m. We placed these transects in open savanna (11 transects of 50 m × 8 m) and in forest formations (10 transects of 50 m × 8 m). In addition, we placed two transects at the forest edge, one in the forest-savanna transition (165 m × 8 m), and another (200 m × 8 m) along the stream bordering the reserve. Sampled area varied between habitats according to their relative area: a total of 4400 m² in open savanna, 4000 m² inside the forest, and 2920 m² on the forest edge. We defined a visit by a hummingbird to a plant as the moment the hummingbird started probing the flowers until the moment it left the plant. Plant species were included in the network as long as they received legitimate visits by hummingbirds, regardless of whether they conformed to the classical ornithophilous syndrome (Maruyama *et al.* 2013b). To ensure that our sampling was sufficient, we performed an individual-based rarefaction analysis, replacing the number of individuals and species by the number of interactions and each pairwise combination of species (Gotelli & Colwell 2001).

We quantified plant abundance as the total number of flowers produced by each plant species, over the study period, in the same transects interaction data were collected. We estimated hummingbird abundance visually while walking along transects and following the 'line transect count' method (Bibby *et al.* 2000). Counting was mostly restricted to records obtained within the transect width, ensuring comparability among habitats. More details on the sampling procedures, including morphological traits assessments and total focal hours spent on each plant species, can be found in Maruyama *et al.* (2013b).

CONSTRUCTING AND CONTRASTING PROBABILITY MATRICES OF INTERACTIONS.—We evaluated which factors contributed in structuring the observed interactions between flowers and hummingbirds by constructing interaction probability matrices and comparing those with the observed interaction matrix through a likelihood approach, as proposed by Vázquez *et al.* (2009b) and following the modifications in Vizentin-Bugoni *et al.* (2014). The observed matrix (O) is a quantitative plant-pollinator interaction matrix with rows corresponding to plant species (*i*) and columns to pollinators (*j*). Each cell entry is the number of interactions (visits) recorded between a given hummingbird and plant species (*o_{ij}*). The probability matrix based on abundance (A) was constructed as the product of flower abundance per plant species by the abundance of each hummingbird species. The cell values in this matrix are the pairwise product of each plant-hummingbird pair.

To determine the role of temporal match, we constructed the probability matrix based on phenological overlap (F) with cell entries expressing the number of months a plant and a hummingbird co-occurred over the sampling period. Hummingbird bill

length and flower corolla length were used to construct the probability matrix based on morphological match (M). An interaction was considered as possible, and the corresponding cell filled with one, if a given hummingbird species have a bill equal or longer than the flower corolla length. To account for hummingbird tongue extension capacity, we calibrated this measure by adding a conservative value of one-third to the actual bill length (as in Vizentin-Bugoni *et al.* 2014). Data on hummingbird bill length and floral corolla length were extracted from previous studies from the same region (Grantsau 1989, Justino *et al.* 2012, Araújo *et al.* 2013, Maruyama *et al.* 2013b). Two species of plants (*Heliconia psittacorum* L.f. and *Ruellia brevifolia* (Pohl) C. Ezcurra) had longer corollas than the bill length of one of the hummingbird species they interacted with (*Thalurania furcata* [Gmelin, 1788]). In these two cases, we believe that the broader corolla opening in the flowers allows visits of hummingbirds with shorter bills than the corolla length (see also Araújo *et al.* 2013). Therefore, in the matrix M, interaction of these two plant species with all other non-hermit hummingbirds were allowed, since they all have similar bill length as *T. furcata*. The sole hermit hummingbird in our study, *Phaethornis pretrei* (Lesson & Delattre, 1839), has a longer bill length and also visited these two species.

In addition to the above-mentioned parameters previously evaluated by Vizentin-Bugoni *et al.* (2014) for another plant-hummingbird network, we also considered the potential role of floral nectar production and habitat (spatial) overlap on species interactions. The effect of nectar availability on hummingbird behavior can be complex, with unique responses of each hummingbird species at different scales (Dalsgaard *et al.* 2009, Justino *et al.* 2012, Maruyama *et al.* 2013b). Thus, we constructed several different probability matrices to evaluate the role of nectar availability (N1–N4). Data on nectar and flower production for the plant assemblage can be found elsewhere (Maruyama *et al.* 2013b) and sugar content was estimated from these nectar parameters following Galetto and Bernardello (2005). The first nectar availability probability matrix (N1) was constructed based on the average amount of sugar a single flower of each plant species produced, while for the second matrix (N2) we multiplied this value by the number of flowers produced per day for an average plant individual during flowering peak. In these two matrices, all plant species have a unique value corresponding to their resource availability, *i.e.*, all hummingbird species have the same probability to interact with a given plant species. The more resource a plant produce, the higher the probability of interaction, which is consistent with empirical data for hummingbird-flower relationships (*e.g.*, Justino *et al.* 2012).

We also constructed two more matrices (N3, N4) combining the hummingbird weight to nectar availability in an attempt to incorporate species-specific differences among hummingbird species in the probability of the interactions. For this, we took the two previously mentioned nectar matrices and multiplied their cell entries by the corresponding hummingbird species' weight. Larger hummingbirds therefore had higher probability of interacting with plants, especially those with flowers providing greater rewards. Smaller hummingbirds had lower probability of interaction, but

interacted more frequently with more rewarding flowers. The underlying assumption here is that larger hummingbirds requires more energy and are also able to exclude, through aggressive behavior, smaller hummingbirds from more rewarding plants (*e.g.*, Feinsinger & Colwell 1978, Justino *et al.* 2012). Thus, we had four probability matrices considering nectar production, two considering only plant nectar production data (N1, N2) and two incorporating hummingbird weight (N3, N4) that differed in the scale of the nectar availability considered: at flower (N1, N3) or plant individual level (N2, N4). The habitat/spatial overlap matrix (H) was constructed by calculating the relative abundance of each species in the three habitats (savanna, forest interior and forest edge) from the species total abundances (Table S1). Then, for each hummingbird–plant species pair, we calculated the Pianka's index of niche overlap using the package *spaa* (Zhang 2013) for the R programming language (R Core Team 2014). The entry in each cell in the matrix is the pairwise value of Pianka's index, with 0 indicating no overlap and 1 expressing total overlap in habitat use.

All matrices (A, F, M, N, H) were normalized by dividing each cell by the matrix sum so as to minimize the difference in the variation on the cell entries among different matrices. Based on above-mentioned matrices, we also constructed probability matrices using combinations among them by the Hadamard (element-wise) product, which were likewise normalized after the multiplication. Finally, a null matrix (NULL) in which all plant and hummingbird species have the same probability of interaction was considered as a benchmark for comparison with all other probability matrices. The ability of individual parameters and parameter combinations to predict the observed interaction frequencies was evaluated through a likelihood approach with the calculation of Akaike Information Criteria (AIC) and Δ AIC, assuming that the probability of interaction between a given plant and hummingbird species followed a multinomial distribution (Vázquez *et al.* 2009b). The likelihood was calculated using the function *dmultinom* in the *stats* package of R (R Core Team 2014). For nectar matrices (N1–N4) the AIC values were first calculated separately, and the model that performed best was used for subsequent analysis. Following Vizentin-Bugoni *et al.* (2014), the number of parameters used to weight different model complexities was defined as the sum of the number of species of each probability matrix included in the given model, with the exception of the 'NULL' matrix, which was assigned with one parameter since it was not properly based on a matrix. A model matrix was considered to better predict the observed matrix when having a smaller value of AIC, and models with Δ AIC <14 as equivalents (Burnham *et al.* 2011).

SPECIALIZATION AND MODULES IN THE NETWORK.—To calculate the network level specialization, we calculated the index H_2' , which characterizes the degree of specialization among species in the entire network (Blüthgen *et al.* 2006). The observed H_2' value was contrasted to 10,000 randomized networks to assess its significance, using the null models generated by functions *r2dtable* and *vaznull* in R-package bipartite (Dormann *et al.* 2008). In the

first model, which uses the Patefield's algorithm, the marginal totals are constrained in the randomizations. The second is more 'conservative' by also keeping the connectance constant, thus keeping the proportion of unrealized interactions, which might represent forbidden links (Dormann *et al.* 2008).

We next sought to evaluate if the factors that determine interaction frequencies also scaled-up to determine modules within the network. To examine this, we first evaluated if the studied hummingbird-plant interaction network was organized into modules. To test for modularity, we used the QuanBiMo algorithm, which was specifically developed for weighted (quantitative) bipartite networks (Dormann & Strauss 2014) and is implemented in the R-package bipartite (Dormann *et al.* 2008). The QuanBiMo algorithm computes modules based on a hierarchical representation of species link weight and optimal allocation to modules through swapping in a Simulated Annealing-Monte Carlo approach (Dormann & Strauss 2014). The level of modularity (Q) measures the extent to which species interact mainly within their module, ranging from 0 to 1. The higher the Q value, the stronger the data support the division of a network into modules. Modularity was calculated with the function *com-*

puteModules, setting the number of Markov Chain Monte Carlo (MCMC) moves to yield no improvement before the algorithm stops to 10^6 steps, which is the default option adequate for our network size (Dormann & Strauss 2014). Since the algorithm is stochastic, module arrangement can vary between each run, thus we retained the module conformation with the highest Q value as the optimum after 50 independent runs. To assess the significance of Q of the observed network, null model expectations from 100 randomized networks were computed using the functions *r2dtable* and *vaznull* in bipartite package. Values of Q in the randomizations were then used to calculate the z -score, which is the number of standard deviations a datum is above the mean of the 100 randomized networks. Z -score values of ≥ 2 are considered significantly modular (Dormann & Strauss 2014). Having identified how species separate into modules, we examined if modules associate with species abundance, morphology, nectar availability, phenology and spatial co-occupancy (habitat occupancy). Floral traits such as flower corolla length, nectar volume, concentration and sugar content of plants belonging to different modules were compared with ANOVA and post-hoc Tukey tests. We also tested this for flower abundance per plant species using

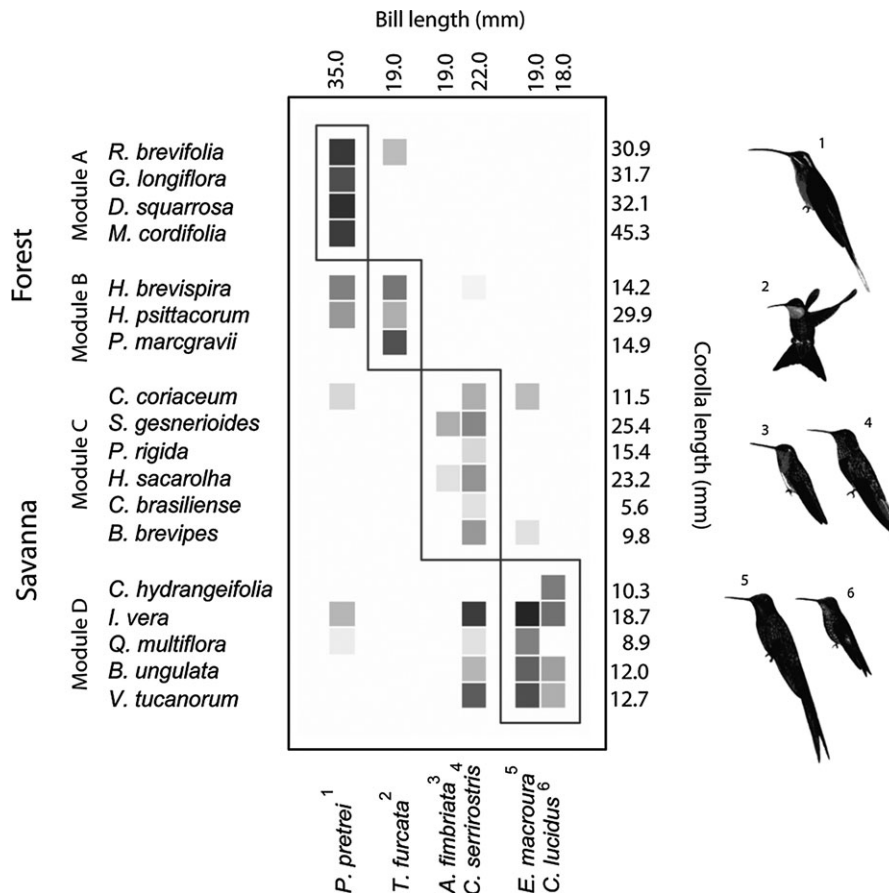


FIGURE 1. Plant-hummingbird interaction matrix from Panga Ecological Station in Brazil's Cerrado. The matrix shows the most common module conformation through 50 runs, using the algorithm QuanBiMo. Intensity of gray-shading represents the interaction frequency. Corolla and bill length for each plant and hummingbird species are shown opposite to their names. For corolla length, we show the effective measurement of flower restriction to hummingbird visitors. The silhouette of hummingbirds shows their relative size (adapted from Grantsau 1989).

a Kruskal–Wallis rank sum test. As each module consisted of only one or two hummingbird species (see Results), formal tests associating modules with hummingbird traits were not conducted.

RESULTS

The savanna hummingbird–plant network was formed by six hummingbird and 18 plant species that interacted 554 times in total through 34 pairwise combinations (Fig. 1, also see Maruyama *et al.* 2013b). Rarefaction indicated our sampling was sufficient for detection of most pairwise interaction in the community (Fig. S1). The best predictor model for the interactions, *i.e.*, the model with lower AIC, was the one combining the matrices M (morphology), F (phenology) and H (habitat), followed by the pairwise combinations among them and then each of these single matrices (Fig. 2). In contrast, all models incorporating species abundances performed poorly, with worse fit than the benchmark NULL matrix (Fig. 2). Likewise, none of the matrices based on nectar availability (N1–N4) performed better than the NULL matrix (Fig. S2); not even the best performing N3 matrix, which was used in all trait combining analyses.

Network level specialization was considerable ($H_2' = 0.598$), and higher than the values observed in the randomized networks (\pm SE; $r2dtable: 0.045 \pm 0.001$; $vaznull: 0.372 \pm 0.001$). The plant–hummingbird network had a modularity value of $Q = 0.484 \pm 0.001$ (\pm SE) and high Z -score ($r2dtable: 35.14$; $vaznull: 5.54$), which indicate significant modularity. Four modules were detected, which were overall consistent across the 50 runs (Fig. 1; Table S2): Module A was formed by the sole hermit hummingbird *P. pretrei* and the four flowers with the longest corollas that occurred mostly in the forest habitats, including interior and edge (Fig. 1, Table 1). Module B comprised of the other hummingbird species that was mostly found in forest habitats, *T. furcata*, and shorter corolla flowers, also for the most part found in this habitat. Modules C and D included plant and hummingbird species mostly occurring in the open savanna habitat but which also occurred at the edge. The flowers in module C and D did not differ significantly in traits from those in module B. The identity of species composing modules A and B were consistent across all 50 runs, whereas modules C and D changed in 14 of the 50 runs (Table S2). Module C was in 14 runs formed only by the hummingbird *Amazilia fimbriata* (Gmelin, 1788) and the plant *Stachytarpheta gesnerioides* Cham. contrasting to the most common module conformation, in which module C consisted of two hummingbird and six plant species (Fig. 1).

DISCUSSION

We have shown that spatio-temporal overlap and species morphology, but not species abundance and floral energy, predict interaction frequencies in a plant–hummingbird network from the Brazilian Cerrado; morphology and spatial distribution also related to the formation of modules. Our results are similar to those observed in the Atlantic Forest (Vizentin-Bugoni *et al.*

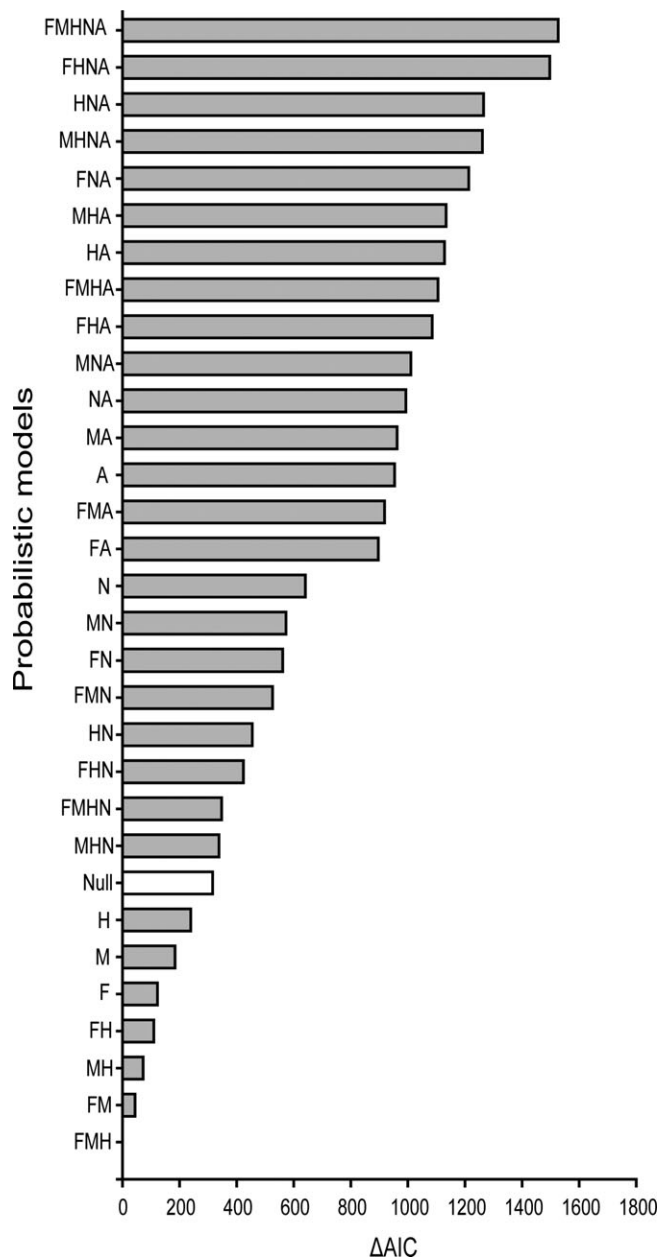


FIGURE 2. Δ AIC values of the probabilistic models (matrices) constructed incorporating species abundance (A), phenology (F), morphology (M), nectar (N) and habitat occupancy (H), and all possible combinations among them in relation to the best model (FMH) fitted to the observed matrix; NULL is the model in which all pairwise interactions have the same probability (white bar). Shorter bars indicate better fit of a given model in relation to model FMH, which presented the best fit to the observed network (*i.e.*, lowest AIC value).

2014), even though floral traits suggest plant communities in the Cerrado are less specialized for hummingbird pollination (Maruyama *et al.* 2013b). Furthermore, the transect method we used should increase the influence of abundance on the interaction records compared to the timed observations carried out by Vizentin-Bugoni *et al.* (2014) in the Atlantic Forest, since it samples

TABLE 1. Floral traits and flower abundance of plants from the four modules found in the savanna plant-hummingbird network. Differences were tested with ANOVA and post-hoc Tukey test, but Kruskal–Wallis test for flower abundance. Values express the mean \pm standard error.

Traits	Modules				$F_{3,14}/K$	P-value
	A	B	C	D		
Corolla (mm)	35.0 \pm 1.6*	19.7 \pm 2.1	15.2 \pm 1.8	12.5 \pm 0.89	9.33	0.001
Nectar volume (μ L)	14.4 \pm 1.6	20.5 \pm 2.9	34.1 \pm 6.4	12.2 \pm 2.2	1.67	0.220
Nectar concentration (%)	22.3 \pm 0.6	16.9 \pm 1.5	20.2 \pm 1.3	22.6 \pm 1.2	0.92	0.456
Nectar sugar (mg)	3.4 \pm 0.4	3.7 \pm 0.7	7.6 \pm 1.5	3.1 \pm 0.7	1.28	0.319
Flower abundance	755.2 \pm 84.1	442.0 \pm 113.8	387.8 \pm 70.3	5839.2 \pm 1216.3	3.28	0.350

*Significantly different from other modules.

the interactions per plant species relative to their abundances (see Gibson *et al.* 2011). Taken together, this suggests species morphology and spatio-temporal mismatches are relatively more important than abundance in organizing plant–hummingbird interactions.

The role of species morphology in structuring the interactions and therefore forming modules is supported by the separation of the long-billed hermit hummingbird *P. pretrei* from the other hummingbird species (Fig. 1). Hermit hummingbirds are often associated with morphologically specialized flowers (Feinsinger & Colwell 1978, Sazima *et al.* 1995, Maruyama *et al.* in press), which may lead to the formation of distinct sub-units in plant–hummingbird networks. Moreover, plant and pollinator distribution over time and space can be important drivers of network structure by constraining species interactions (Vázquez *et al.* 2009a,b, Martín González *et al.* 2012, Vizentin-Bugoni *et al.* 2014). In our network, there is an example of the lack of phenological overlap creating forbidden links—the hummingbird *A. fimbriata* (Gmelin, 1788) and the few plant species it visits. Although this is one of the most common hummingbird pollinators in the open habitats of the Cerrado (Araújo *et al.* 2013), it was recorded only for 3 mo during our study period (Fig. S3). This ‘forbid’ many of the morphologically possible pairwise interaction between this hummingbird and local plant species. Yet, although seasonality may be an important driver of modularity for some ecological networks (Martín González *et al.* 2012, Schleuning *et al.* 2014), we did not observe an association between modules and the seasonality (*i.e.*, dry and wet seasons) that is so characteristic of the Cerrado (Fig. S3). Hence, even though phenological overlap is important in determining interaction frequency between species of hummingbirds and plants, it did not scale-up to also determine modules within the network. Most hummingbirds, in contrast to plants, were distributed through the year and this inhibited the formation of seasonal modules. This suggests that seasonality is a more important driver of modularity for plants interacting with mutualists with high within-year turnover, *e.g.*, insects with shorter life or activity spans (Martín González *et al.* 2012) or migrant frugivorous birds (Schleuning *et al.* 2014).

In contrast to seasonality, the spatial distribution into forest and savanna habitats clearly delimited some of the modules we identified (Fig. 1). Species interactions are inherently spatial, since

individuals must meet in space to interact (Morales & Vázquez 2008). For example, in the forests of Trinidad, a major generator of forbidden links in a plant-hummingbird network is the vertical decoupling of habitat, *i.e.*, canopy vs. understory (Snow & Snow 1972, Jordano *et al.* 2006). In the Cerrado, the patchy distribution of habitats creates a spatially heterogeneous landscape, and plant–animal interactions are probably constrained by species preferences for one of these habitats. Specifically, two species of hummingbirds are more associated with forest—*P. pretrei* and *T. furcata* (Araújo *et al.* 2013)—and each belonged to separate modules from the hummingbirds primarily found in open habitats. While the module for the hermit *P. pretrei* can be easily explained by morphological specialization (*i.e.*, longer bill and corolla length), *T. furcata* is very similar to other hummingbirds occurring in the savanna. The formation of its own module is therefore best explained by its preference for forest. Our results suggest that in addition to morphological traits and phenology, habitat preference is an additional form of spatial complexity that can constrain interactions (Morales & Vázquez 2008, Vázquez *et al.* 2009b) and determine network structure.

Although in general species morphology and spatio-temporal mismatches performed well in determining interactions and network structure, we were surprised to find that nectar performed poorly (Fig. 2; Table 1). The inability of nectar-based matrices in predicting interaction frequencies might indicate a need to incorporate a threshold at which larger hummingbirds do not interact with flowers producing less rewards (Dalsgaard *et al.* 2009, Justino *et al.* 2012). To do so, however, would require more detailed information on the energetic requirements of each hummingbird species (Feinsinger & Colwell 1978, Altshuler *et al.* 2004). Of course, it could also be possible that nectar availability is indeed less important than other traits in determining interaction frequencies. This seems especially likely if traits operate in a hierarchical manner such that one (*e.g.*, nectar) becomes relevant only if others (*e.g.*, corolla length) have already permitted a given pairwise interaction to occur (Junker *et al.* 2013).

Recent studies have provided additional evidence that species traits play a relatively more important role than abundance in structuring interaction networks (Junker *et al.* 2013, Vizentin-Bugoni *et al.* 2014, and this study). Interestingly, these studies were all characterized by networks with relatively higher levels of

specialization, *i.e.*, $H_2' > 0.51$ (Blüthgen *et al.* 2007). Furthermore, some of the studies showing higher importance of abundance have been conducted using more generalized systems, such as plant–frugivorous bird and plant–ant networks (Blüthgen *et al.* 2007, Krishna *et al.* 2008, Dáttilo *et al.* 2014). We propose that future research would benefit of cross-network comparisons testing the hypothesis that traits have greater effects in specialized than in generalized systems. This could be tested across large spatial gradients with a single system, for instance by contrasting more specialized tropical hummingbird–plant networks with more generalized temperate ones (Dalsgaard *et al.* 2011). Alternatively, one could compare the performance of traits and abundance in structuring different types of mutualistic systems in the same location, *e.g.*, by comparing more generalized tropical plant–frugivorous bird networks with more specialized plant–pollinator ones.

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SUPPORTING INFORMATION

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

TABLE S1. Total abundances of hummingbirds and plants according to habitat in Panga Ecological Station, Brazil.

TABLE S2. Frequency of plant and hummingbird species in each one of the four modules.

FIGURE S1. Rarefaction curve for plant–hummingbird pairwise interactions in relation to the number of visits recorded in the Panga Ecological Station, Brazil.

FIGURE S2. AIC values of the four nectar probabilistic models (matrices), the benchmark NULL model and the observed interaction matrix (O) in relation to the interaction data.

FIGURE S3. Phenology of plants and hummingbirds from November 1996 to November 1997 in Panga Ecological Station, Brazil.

LITERATURE CITED

ALTSHULER, D. L., F. G. STILES, AND R. DUDLEY. 2004. Of hummingbirds and helicopters: Hovering costs, competitive ability, and foraging strategies. *Am. Nat.* 163: 16–25.

- ARAÚJO, F. P., M. SAZIMA, AND P. E. OLIVEIRA. 2013. The assembly of plants used as nectar sources by hummingbirds in a Cerrado area of Central Brazil. *Plant Syst. Evol.* 299: 1119–1133.
- BIBBY, C. J., N. D. BURGESS, AND D. A. HILL. 2000. *Bird census techniques*. 2nd edn. Academic Press, London, UK.
- BLÜTHGEN, N., F. MENZEL, AND N. BLÜTHGEN. 2006. Measuring specialization in species interaction networks. *BMC Ecol.* 6: 9.
- BLÜTHGEN, N., F. MENZEL, T. HOVESTADT, B. FIALA, AND N. BLÜTHGEN. 2007. Specialization, constraints, and conflicting interests in mutualistic networks. *Curr. Biol.* 17: 341–346.
- BURNHAM, K. P., D. R. ANDERSON, AND K. P. HUYVAERT. 2011. AIC model selection and multimodel inference in behavioral ecology: Some background, observations and comparisons. *Behav. Ecol. Sociobiol.* 65: 23–35.
- CARDOSO, E., M. I. C. MORENO, E. M. BRUNA, AND H. L. VASCONCELOS. 2009. Mudanças fitofisionômicas no Cerrado: 18 anos de sucessão ecológica na Estação Ecológica do Panga, Uberlândia – MG. *Caminhos Geogr.* 10: 254–268.
- DALSGAARD, B., E. MAGÅRD, J. FJELDSÅ, A. M. MARTÍN GONZÁLEZ, C. RAHBEK, J. M. OLESEN, J. OLLERTON, R. ALARCÓN, A. C. ARAUJO, P. A. COTTON, C. LARA, C. G. MACHADO, I. SAZIMA, M. SAZIMA, A. TIMMERMANN, S. WATTS, B. SANDEL, W. J. SUTHERLAND, AND J. C. SVENNING. 2011. Specialization in plant-hummingbird networks is associated with species richness, contemporary precipitation and Quaternary climate-change velocity. *PLoS ONE* 6: e25891.
- DALSGAARD, B., A. M. MARTÍN GONZÁLEZ, J. M. OLESEN, J. OLLERTON, A. TIMMERMANN, L. H. ANDERSEN, AND A. G. TOSSAS. 2009. Plant-hummingbird interactions in the West Indies: floral specialization gradients associated with environment and hummingbird size. *Oecologia* 159: 757–766.
- DALSGAARD, B., K. TRØJELSGAARD, A. M. MARTÍN GONZÁLEZ, D. NOGUÉS-BRAGO, J. OLLERTON, T. PETANIDOU, B. SANDEL, M. SCHLEUNING, Z. WANG, C. RAHBEK, W. J. SUTHERLAND, J. C. SVENNING, AND J. M. OLESEN. 2013. Historical climate-change influences modularity and nestedness of pollination networks. *Ecography* 36: 1331–1340.
- DANIELI-SILVA, A., J. M. T. DE SOUZA, A. J. DONATTI, R. P. CAMPOS, J. VICENTE-SILVA, L. FREITAS, AND I. G. VARASSIN. 2012. Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands? *Oikos* 121: 35–43.
- DÁTILLO, W., F. M. D. MARQUETTI, P. R. GUIMARÃES, JR, AND T. J. IZZO. 2014. The structure of ant-plant ecological networks: Is abundance enough? *Ecology* 95: 475–485.
- DORMANN, C. F., B. GRUBER, AND J. FRUEND. 2008. Introducing the bipartite Package: Analysing Ecological Networks. *R News* 8/2: 8–11.
- DORMANN, C. F., AND R. STRAUSS. 2014. A method for detecting modules in quantitative bipartite networks. *Methods Ecol. Evol.* 5: 90–98.
- FEINSINGER, P., AND R. K. COLWELL. 1978. Community organization among Neotropical nectar-feeding birds. *Am. Zool.* 18: 779–795.
- GALETTO, L., AND G. BERNARDELLO. 2005. Nectar. In A. Dafni, P. G. Kevan, and B. C. Husband (Eds.). *Pollination ecology: A practical approach*, pp. 156–212. Enviroquest Ltd., Cambridge, ON.
- GIBSON, R. H., B. KNOTT, T. EBERLEIN, AND J. MEMMOTT. 2011. Sampling method influences the structure of plant–pollinator networks. *Oikos* 120: 822–831.
- GOTELLI, N., AND R. K. COLWELL. 2001. Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. *Ecol. Lett.* 4: 379–391.
- GRANTSAU, R. 1989. *Os Beija-flores do Brasil*. Editora Expressão e Cultura, Rio de Janeiro, Brazil.
- JORDANO, P., J. BASCOMPTE, AND J. M. OLESEN. 2006. The ecological consequences of complex topology and nested structure in pollination webs. In N. M. Waser, and J. Ollerton (Eds.). *Plant-pollinator interactions: From specialization to generalization*, pp. 173–199. University of Chicago Press, Chicago, IL.
- JUNKER, R. R., N. BLÜTHGEN, T. BREHM, J. BINKENSTEIN, J. PAULUS, H. M. SCHAEFER, AND M. STANG. 2013. Specialization on traits as basis for

- the niche-breadth of flower visitors and as structuring mechanism of ecological networks. *Funct. Ecol.* 27: 329–341.
- JUSTINO, D. G., P. K. MARUYAMA, AND P. E. OLIVEIRA. 2012. Floral resource availability and hummingbird territorial behaviour on a Neotropical savanna shrub. *J. Ornithol.* 153: 189–197.
- KRISHNA, A., P. R. GUIMARÃES, JR., P. JORDANO, AND J. BASCOMPTE. 2008. A neutral-niche theory of nestedness in mutualistic networks. *Oikos* 117: 1609–1618.
- MARTÍN GONZÁLEZ, A. M., S. ALLESINA, A. RODRIGO, AND J. BOSCH. 2012. Drivers of compartmentalization in a Mediterranean pollination network. *Oikos* 121: 2001–2013.
- MARUYAMA, P. K., M. R. BORGES, P. A. SILVA, K. C. BURNS, AND C. MELO. 2013a. Avian frugivory in *Miconia* (Melastomataceae): Contrasting fruiting times promote habitat complementarity between savanna and palm swamp. *J. Trop. Ecol.* 29: 99–109.
- MARUYAMA, P. K., G. M. OLIVEIRA, C. FERREIRA, B. DALSGAARD, AND P. E. OLIVEIRA. 2013b. Pollination syndromes ignored: Importance of non-ornithophilous flowers to Neotropical savanna hummingbirds. *Naturwissenschaften* 100: 1061–1068.
- MARUYAMA, P. K., J. VIZENTIN-BUGONI, B. DALSGAARD, AND M. SAZIMA. in press. Pollination and breeding system of *Canna paniculata* (Cannaceae) in a montane Atlantic Rainforest: Asymmetric dependence on a hermit hummingbird. *Acta Bot. Bras.* DOI: 10.1590/0102-33062015abb3590.
- MORALES, J. M., AND D. P. VÁZQUEZ. 2008. The effect of space in plant-animal mutualistic networks: Insights from a simulation study. *Oikos* 117: 1362–1370.
- OLESEN, J. M., J. BASCOMPTE, Y. L. DUPONT, H. ELBERLING, C. RASMUSSEN, AND P. JORDANO. 2011. Missing and forbidden links in mutualistic networks. *Proc. Biol. Sci.* 278: 725–732.
- OLESEN, J. M., J. BASCOMPTE, Y. L. DUPONT, AND P. JORDANO. 2007. The modularity of pollination networks. *Proc. Natl Acad. Sci. USA* 104: 19891–19896.
- R CORE TEAM. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.
- SÁYAGO, R., M. LOPEZARAIZA-MIKEL, M. QUESADA, M. Y. ÁLVAREZ-ÁÑOVE, A. CASCANTE-MARÍN, AND J. M. BASTIDA. 2013. Evaluating factors that predict the structure of a commensalistic epiphyte-phytophyte network. *Proc. Biol. Sci.* 280: 20122821.
- SAZIMA, I., S. BUZATO, AND M. SAZIMA. 1995. The saw-billed hermit *Rampodon naevius* and its flowers in southeastern Brazil. *J. Ornithol.* 136: 195–206.
- SCHLEUNING, M., L. INGMANN, R. STRAUß, S. A. FRITZ, B. DALSGAARD, D. M. DEHLING, M. PLEIN, F. SAAVEDRA, B. SANDEL, J. C. SVENNING, K. BÖHNING-GAESE, AND C. F. DORMANN. 2014. Ecological, historical and evolutionary determinants of modularity in weighted seed-dispersal networks. *Ecol. Lett.* 17: 454–463.
- SILVA, J. M. C., AND J. M. BATES. 2002. Biogeographic patterns and conservation in the South American Cerrado: A tropical savanna hotspot. *Bio-science* 52: 225–234.
- SNOW, B. K., AND D. W. SNOW. 1972. Feeding niches of hummingbirds in a Trinidad valley. *J. Anim. Ecol.* 41: 471–485.
- STILES, F. G. 1975. Ecology, flowering phenology, and hummingbird pollination of some Costa Rican *Heliconia* species. *Ecology* 56: 285–301.
- TUBELIS, D. P., A. COWLING, AND C. DONNELLY. 2004. Landscape supplementation in adjacent savannas and its implications for the design of corridors for forest birds in the central Cerrado, Brazil. *Biol. Conserv.* 118: 353–364.
- VÁZQUEZ, D. P., N. BLÜTHGEN, L. CAGNOLO, AND N. P. CHACOFF. 2009a. Uniting pattern and process in plant–animal mutualistic networks: A review. *Ann. Bot.* 103: 1445–1457.
- VÁZQUEZ, D. P., N. P. CHACOFF, AND L. CAGNOLO. 2009b. Evaluating multiple determinants of the structure of mutualistic networks. *Ecology* 90: 2039–2046.
- VIZENTIN-BUGONI, J., P. K. MARUYAMA, AND M. SAZIMA. 2014. Processes entangling interactions in communities: Forbidden links are more important than abundance in a hummingbird–plant network. *Proc. Biol. Sci.* 281: 20132397.
- ZHANG, J. 2013. spaa: Species Association Analysis. R package version 0.2.1. <http://CRAN.R-project.org/package=spaa>.