PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Nectar robbery by a hermit hummingbird: association to floral phenotype and its influence on flowers and network structure

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Abstract Interactions between flowers and their visitors span the spectrum from mutualism to antagonism. The literature is rich in studies focusing on mutualism, but nectar robbery has mostly been investigated using phytocentric approaches focused on only a few plant species. To fill this gap, we studied the interactions between a nectar-robbing hermit hummingbird, *Phaethornis ruber*, and the array of flowers it visits. First, based on a literature review of the interactions involving *P. ruber*, we characterized the association of floral larceny to floral phenotype. We then experimentally examined the effects of nectar robbing on nectar standing crop and number of visits of the pollinators to the flowers of *Canna paniculata*. Finally, we asked whether the incorporation of illegitimate interactions into the analysis affects plant–hummingbird network structure. We identified 97 plant species visited by *P. ruber* and found that *P. ruber* engaged in floral larceny in almost 30 % of these species. Nectar robbery was especially common in flowers with longer corolla. In terms of the effect on *C. paniculata*, the depletion of nectar due to robbery by *P. ruber* was associated with decreased visitation rates of legitimate pollinators. At the community level, the inclusion of the illegitimate visits of *P. ruber* resulted in modifications of how modules within the network were organized, notably giving rise to a new module consisting of *P. ruber* and mostly robbed flowers. However, although illegitimate visits constituted approximately 9 % of all interactions in the network, changes in nestedness, modularity, and network-level specialization were minor. Our results indicate that although a flower robber may have a strong effect on the pollination of a particular plant species, the inclusion of its illegitimate interactions has limited capacity to change overall network structure.

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Introduction

Mutualism, defined as an interaction in which both partner species experience a net positive effect, is one of the major interaction types in nature (Bronstein 2001). Nevertheless, organisms are entangled in multiple interactions that vary

in type and strength. For example, most flowering plants rely on animals for pollination (Ollerton et al. 2011), but not all flower visitors are effective pollinators (Irwin et al. 2010). Floral visitors even engage in floral larceny, i.e., robbing or thieving of floral rewards (Inouye 1980; Irwin et al. 2010). Some floral phenotypes may be especially associated with occurrence of floral larceny, such as longer and more enclosed corollas (Lara and Ornelas 2001; Irwin et al. 2010), but investigations involving the analysis of large datasets are still lacking. Additionally, in a comprehensive review, Irwin et al. (2010) pointed out a number of little explored and fruitful avenues for future research in nectar robbery, advocating studies which would overcome the limitations caused by predominance of a phytocentric approach and noting the lack of community-wide studies.

The same network structural property may have different consequences on network dynamics depending on whether the interaction is mutualistic or antagonistic (Thébault and Fontaine 2010). Therefore, simultaneously considering and merging these two types of interactions could reveal new eco-evolutionary patterns and dynamics that shape ecological communities (Fontaine et al. 2011). For example, theoretical simulations with tripartite networks merging antagonistic and mutualistic sub-networks show that whereas greater connectance of antagonistic interactions lower the resilience of the community, connectance of the mutualistic interactions have an opposite effect (Sauve et al. 2014). If the structural properties of networks change drastically when illegitimate interactions are included, in theory the dynamics and stability of the system should also change. This possibility leads to the notion that merging the interactions of both pollinators and floral larcenists may provide insightful results and also that the inclusion of floral larcenists in an analysis may change the structure of plants and flower-visitor networks (Genini et al. 2010; Fontaine et al. 2011; Yoshikawa and Isagi 2013). Despite the potential importance of simultaneously considering mutualistic and antagonistic flower-visitors, only a few community-wide studies have been conducted, and in two of these studies the simple addition of antagonistic interactions had drastic effects on the overall network structure (Genini et al. 2010; Yoshikawa and Isagi 2013). Specifically, these two studies showed that addition of nectar robbers and flower-eaters increases the modularity of the network, i.e., there seems to be a more distinct sub-community structure when floral larcenists are included (Genini et al. 2010; Yoshikawa and Isagi 2013). Since pollination network structure has been suggested to have important eco-evolutionary consequences, these studies may point to the necessity of considering floral larcenists in evaluations of the ecological dynamics of plant–pollinator communities (Olesen et al. 2007; Thébault and Fontaine 2010; Sauve et al. 2014).

Here, we focus on a hummingbird nectar robber, the Reddish Hermit (*Phaethornis ruber*), as a model organism. This species belongs to the Hermit clade of hummingbirds, which are regarded as specialized and core-pollinators in Neotropical forests (Feinsinger and Colwell 1978; Sazima et al. 1995; Maruyama et al. 2014). However, the small Reddish Hermit has often been recorded as a nectar robber in the lowland Atlantic rainforest where it is commonly found (Buzato et al. 2000), which makes it an ideal model organism to study possible species–species and community-wide effects of nectar robbery. First, we conducted a literature survey of all documented interaction records between *P. ruber* and plants, with the aim to determine whether particular floral traits were associated with the behavior of the hummingbird—i.e., whether the hummingbird acted as a pollinator, nectar robber, or nectar thief. In other words, we used an extensive database comprising a large number of plant species from several families to investigate the association between floral traits and hummingbird behavior. Second, we conducted a case study focusing on the interaction of *P. ruber* with *Canna paniculata* Ruiz & Pav. (Cannaceae), a plant species subjected to intense robber activity by *P. ruber*, in order to examine potential effects of *P. ruber* on plant reproduction. Finally, we collected data on a plant–hummingbird interaction network in a lowland Atlantic rainforest community to explore whether the inclusion of floral larceny interactions influences how we characterize the network structure.

Materials and methods

Literature survey

The literature survey on records of *Phaethornis ruber* interacting with plants was conducted using ISI Web of Science® and Google Scholar®, using "*Phaethornis ruber*" as a search term. For each of the resulting references reporting observations of *P. ruber* visiting a plant, we extracted the following data whenever available: plant species and family; the hummingbird behavior while interacting with the plant, i.e. whether pollinating, robbing, or thieving; flower corolla length; flower color (including secondary attractants such as bracts when present); nectar volume and concentration; pollinator species visiting the plant. The difference between a nectar robber and a thief is based on the observation of whether the floral larcenist damages the flower when accessing the nectar: nectar robbers cause damage, such as by piercing the corolla, whereas nectar thieves illegitimately access the nectar without damaging the flower (Inouye 1980). Missing floral trait data were, whenever possible, complemented with an additional search specific for each plant species, such as by

using studies with the same plant at another location. For species associated with more than one study, the data were averaged: for each plant species, we use only one value for each variable. Plant names were checked for their validity in the Plant list database (http://www.theplantlist.org/) and updated/corrected whenever necessary. Similar data for one community in the lowland Atlantic rainforest (see below) were also included in the survey. Following Wilson et al. (2004) and Dalsgaard et al. (2009), we coded flower color visible to the human eye on a numerical scale of 1 to 4, ranging from short-wave length Hymenoptera syndrome colors (i.e., blue, violet flowers = 1) to an increasing association to specialized hummingbird-pollinated syndromes (i.e. red flowers = 4); 2 and 3 represent intermediate syndrome colors [see Electronic Supplementary Material (ESM) 1 for details]. We also coded the spectrum of legitimate flower visitors other than *P. ruber* according to increasing specialization to hummingbird pollination on a scale of 1–4 where 1 refers to only insect pollinators, 2 refers to insects and hummingbirds, 3 refers to only hummingbirds, and 4 refers to only large hermit hummingbirds (see ESM 1 for details).

To assess the relationship between floral phenotype and *P. ruber* behavior, we analyzed the floral variables using nonmetric multidimensional scaling (NMDS), which is in accordance with data analyses reported in previous studies dealing with similar kind of data (e.g. Wilson et al. 2004; Dalsgaard et al. 2009; Ollerton et al. 2009). For the analysis, each floral variable was standardized by subtracting the minimum value and dividing by the range, so that each variable had the same weight in the analysis. The Euclidian distance between species was calculated from the standardized values. The resulting dissimilarity matrix was used for computing the NMDS ordination with the function *metaMDSiter* in the vegan package in R, which identify a stable solution using several random starts with smaller stress values (Borcard et al. 2011; Oksanen 2013). In our analysis, we set the number of random starts at 200 and examined whether the data was best described by two or three dimensions. The optimal number of dimensions was determined to be three, as the solution with two dimensions increased the stress level considerably, from approximately 10 to approximately 17 (Borcard et al. 2011). After identifying the preferred three-dimensional solution, we rotated axis 1 according to floral corolla length; the variable which best separated *P. ruber* behavior. We then used the function *envfit* to fit the pollinator-visitor score and floral trait variables (as vectors), as well as the *P. ruber* behavior [i.e., pollinator, nectar robber, or nectar thief (as factor–centroid)], into the ordination space. In this sense, we were able to graphically illustrate how floral traits, pollinators, and the behavior of our target hummingbird associate to the dimensions (axes) of the generated NMDS ordination (Borcard

et al. 2011). In the case of the hummingbird behavior, the centroids show the averages of different behaviors in relation to the axes—i.e. to the dimensions representing the traits. The significance of the association between hummingbird behavior and the ordination axes was tested by 999 permutations (Borcard et al. 2011; Oksanen 2013). Finally, we calculated the Pearsons's correlation of each floral variable and the pollinator-visitor score to the resulting three NMDS axes.

Case study

To assess how the nectar robbing behavior of *P. ruber* may affect the availability of floral nectar, we conducted a case study with *Canna paniculata* in the Atlantic rainforest at Núcleo Picinguaba (further referred to as Picinguaba), specifically in Cambucá area. The study site is located in the Serra do Mar State Park in Ubatuba, São Paulo, Brazil (23°19′30″S, 44°56′24″W; approx. 50 m a.s.l.). The mean annual temperature in Picinguaba is 22 °C and the annual precipitation is 2200 mm, and never below 80 mm per month (Joly et al. 2012). *Canna paniculata* is commonly found in the southeast Atlantic rainforest, including the Serra do Mar State Park, occurring in both lowland areas, such as Picinguaba, and higher areas up to an elevation of 1000 m a.s.l. (Maruyama et al. 2015). The data collection period was from February to July in 2012 and 2013, during the main flowering period of *C. paniculata*, and consisted of focal observations (60 h) in which we quantified the number of legitimate and illegitimate visits to *C. paniculata*. Data collected at Picinguaba were compared to data collected at the Santa Virgínia field station (23°20′11″S, 45°8′45″W), a locality also within the Serra do Mar State Park but situated at an elevation of approximately 900 m a.s.l. and located 21 km distant from Picinguaba (Maruyama et al. 2015; see ESM 1). *P. ruber* is absent at the Santa Virgínia field station, but otherwise the hummingbird–plant communities are structured similarly at both sampling sites (Buzato et al. 2000; Vizentin-Bugoni et al. 2014). The sampling protocol (data collection, observation periods, etc.) followed at the Santa Virgínia field station was the same as that at Picinguaba (Maruyama et al. 2015).

We also conducted an experiment to assess the impact of nectar robbing on floral nectar availability, using two treatments and two controls. The treatments consisted of (1) pollinator exclusion, in which legitimate access was prevented by putting a transparent plastic enclosure (approx. 2 cm in length) on the tip of the flower and (2) robber exclusion, in which robbing was prevented by placing a plastic "straw" made of the same plastic material as the enclosure in treatment 1, and with the same dimensions, at the base of the corolla, thereby allowing only legitimate visitors (see ESM 2 video file). The two controls consisted

of (3) bagged flowers, in which flowers were isolated from all visitors with nylon mesh bags and (4) natural conditions, in which all visits to open flowers were allowed ($n = 30$ for each category). All flowers were kept isolated (enclosed in nylon mesh bags) prior to the experiments. Treatments and controls were set before dawn, i.e., before the beginning of visitor activity and at the beginning of floral anthesis, which lasts 1 day. Treatments were dispersed over as many individual plants as possible ($n = 12$ clumps), and the goal was to set different treatments on the same individuals at the same time. After we had made the focal observations, we also noted the presence of stingless bees (*Trigona* sp.) acting as nectar robbers, especially after the first hours in the morning (approx. 09:30 hour). Taking this activity into account, we divided all our treatments into two time intervals so that the remaining amount of nectar in the flowers was measured at 09:00–10:00 hour and at 16:00–17:00 hour ($n = 15$ for each time interval and each category). The remaining nectar volume in the flowers was compared using a linear mixed-effects model in the package *lme4* in R (Bates et al. 2014). We assumed different random intercepts for each individual clump, and first computed the full model with treatment and time interval with an interaction term as fixed effects. We subsequently used the likelihood ratio test to attain *p* values for these factors (e.g., by comparing two models, one with and without the factor "Treatment" to assess its significance). Nectar volume was $\log_{10}$ transformed to fulfill the assumptions of normal distribution of data and variance homoscedasticity. For the experimental categories in which we found a significant result, we also conducted a post hoc Tukey test. Since the "robber marks" left on the flowers differ between *P. ruber* and *Trigona* sp., it was possible to quantify the frequency of nectar robbing by each party, which was assessed in randomly collected flowers throughout the flowering season ($n = 180$ flowers).

Community-wide study

The hummingbird–plant interaction network data were collected in the coastal lowland Atlantic rainforest at Picinguaba between January 2012 and June 2013. Interactions were recorded on focal plants either by direct observation or by video cameras put in front of the plant, with 15–45 h of observation for each plant species (sampling depended on plant abundance). During each observation session, we recorded all visits by hummingbirds, as well as their behavior, such as whether they were visiting legitimately (potential pollination) or illegitimately. To evaluate how the inclusion of nectar larceny by *P. ruber* changes the network structure, we constructed two quantitative plant–floral visitor interaction networks. The first network was constructed taking only legitimate plant–hummingbird visits into

consideration (hereafter the Pollination network), whereas in the second matrix we also included instances in which hummingbirds acted as nectar robbers and thieves (hereafter Visitation network; all but one recorded nectar robbing involved *P. ruber*). For each of the two networks, we calculated distinct metrics illustrating different structural properties of the network:

1. Nestedness quantifies the degree to which interactions of specialized species are subsets of interactions of the more generalist species in the network. It is one of the most recurrent patterns in ecological networks (Bascompte et al. 2003). We calculated the binary and weighted nestedness using the most conceptually consistent metric in the literature (NODF and WNODF, respectively; Almeida-Neto and Ulrich 2011). While binary nestedness accounts for the "plausibility of interaction" (i.e., forbidden links), adding quantitative measures of interactions to the calculations may provide additional insight into species preferences and illustrate whether the core of the network also contains the highest frequencies (Almeida-Neto and Ulrich 2011; Staniczenko et al. 2013).

2. Network-wide specialization can be estimated in both binary and weighted networks. Binary specialization was quantified as connectance, which is the ratio between the number of realized links and the number of possible links in the network. Quantitative specialization was estimated by the $H_2{'}$ index, which describes how species restrict their interactions from those randomly expected based on partner's availability (Blüthgen et al. 2006).

3. Modularity indices quantify the prevalence of interactions within modules, i.e., subunits in the community, in relation to the interactions among modules [Q; binary (Marquitti et al. 2014), weighted (Dormann and Strauss 2014)]. Olesen et al. (2007) showed that smaller plant–floral visitor networks with less than approximately 50 species are rarely multi-modular. However, more recent studies show that when quantitative information in incorporated, i.e., data on the strength of interactions, functional specialization becomes more evident and distinct modules are detected even in smaller networks (Dormann and Strauss 2014; Maruyama et al. 2014; Schleuning et al. 2014).

The modularity algorithms used here are built on optimization procedures that iteratively try to maximize the modularity index of the final solution (Marquitti et al. 2014; Dormann and Strauss 2014). Importantly, as the algorithm is stochastic, module arrangement as well as the value of Q might vary slightly between runs (Marquitti et al. 2014; Maruyama et al. 2014; Dormann and Strauss

2014). However, since the objective of the procedure is to find the solution with the highest value of modularity, this shortcoming can be minimized by repeating the analysis multiple times and retaining the module conformation which yields the highest Q value (see, for example, Schleuning et al. 2014; Maruyama et al. 2014). In this study, we ran the analysis 30 times for each network and kept the module conformation which yielded the highest Q value. Additionally, although the Q value quantifies the support of the modular organization of a network, the information it provides is related to the overall structure of the network and does not reflect a more detailed organization of subunits, such as the actual species composition of the different modules. Nevertheless, the detailed organization of species into modules carries important information, since this organization might reflect functional specialization within communities (e.g., Maruyama et al. 2014); such modules can therefore be regarded as potential co-evolutionary units (Olesen et al. 2007). In this context, it also illustrates the grouping of species with the highest potential to affect each other within the network of interactions. Our evaluation of the detailed organization of modules in networks was only done for quantitative networks, as binary networks did not have significant modularity (see "Results").

Our calculations of both binary and weighted versions of the metrics not only reflect different properties but also allow a better comparison of our results with those of two previous studies which investigated the effect of merging illegitimate interactions within plant–floral visitor networks (binary nestedness and modularity in Genini et al. 2010; binary modularity in Yoshikawa and Isagi 2013). All network metrics were calculated using the package *bipartite* in R (Dormann et al. 2008), with the exception of binary nestedness and modularity, for which we used ANINHADO (Guimarães and Guimarães 2006) and the MODULAR software (Marquitti et al. 2014), respectively, following their default recommendations. Network metrics can be affected by network size, and thus the significance of metrics has to be assessed by comparison with null model networks. For quantitative networks, we used the function *vaznull* in the bipartite package, which generates simulated matrices with the same marginal totals and connectance as the original network. We estimated the 95 % confidence interval for each metric from the simulated values, and a metric value was considered significant if it did not overlap with the confidence interval. The first step in our comparison of the Pollination and Visitation networks was to evaluate whether the incorporation of illegitimate interactions changed the performance of the metrics in relation to the null model (i.e., their significance). We then estimated the magnitude to which the metric

values changed after the incorporation of illegitimate visits. Although no formal tests were conducted for the metric values, which were impaired by the lack of replicates, these procedures are consistent with those of the two previous studies which evaluated similar questions and to which our results are compared (Genini et al. 2010; Yoshikawa and Isagi 2013).

Finally, in order to link the modularity results to floral traits, we conducted NMDS for the plant species found in the lowland Atlantic rainforest following the same protocol as for the literature survey data. Traits used in this second ordination were floral corolla length, color score, nectar volume, and concentration. As in the first NMDS, we kept the three-dimensional solution based on the stress value. We also used the function *envfit* to fit the module identity as a factor into the ordination space. First, we fit the module identity of the Pollination network and assessed whether the modules could be separated by traits; then the same procedure was conducted for the same ordination, but using the module identity defined by the Visitation network.

Results

Literature survey

Our literature search identified 114 case studies reporting visits of *P. ruber* to flowers of 100 plant species, of which three were excluded from further analysis due to lack of information on floral phenotype. In 16 (16.5 %) of the 97 remaining plant species, we had incomplete data on nectar; these data were therefore treated as missing values in our analyses. The final dataset included 97 plant species from 27 families, with Bromeliaceae (24 species), Rubiaceae (11), and Acanthaceae (10) being the most common families (see ESM 1 for details). *P. ruber* was reported to act as a pollinator in 70 (72.2 %) species, as a robber in 16 (16.5 %) species, and as both pollinator and robber in six (6.2 %) species; in five (5.1 %) species *P. ruber* was reported to be a nectar thief. The NMDS ordination resulted in a solution with a stress of 10.19 ($r^2 = 0.93$), with axis 1 associated mainly to floral corolla length and nectar volume, axis 2 to color and visitor score, and axis 3 to nectar volume and concentration (Fig. 1). Distinct roles of *P. ruber* (pollinator, robber, thief) in relation to flowers had the best fit to the two-dimensional plot of axis 1 and axis 3, in which the robbing behavior's centroid was clearly separated from the centroids of the other behaviors along axis 1 (goodness of fit, $r^2 = 0.22$, $p < 0.001$). Since axis 1 is best correlated to corolla length and nectar volume (Pearson's $r > 0.66$; Fig. 1), especially the former, nectar robbing associated best to flowers with longer corollas.
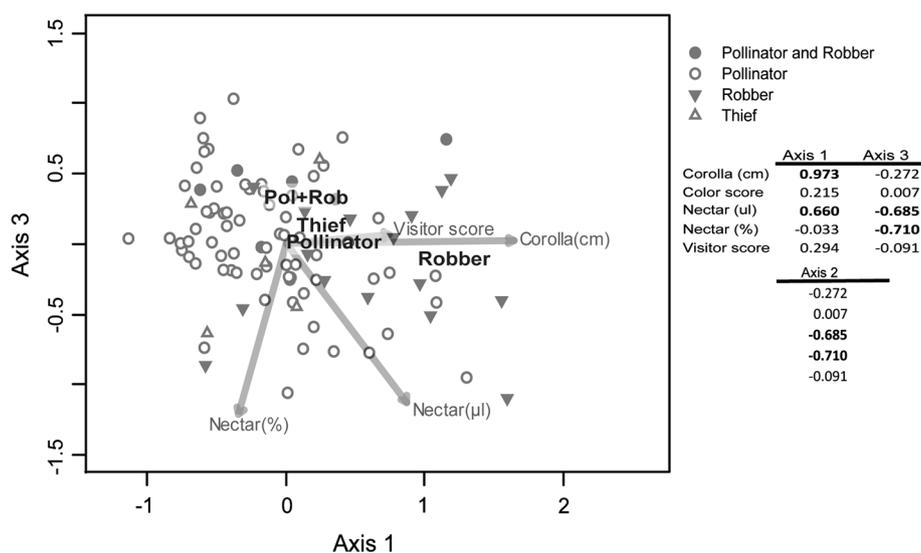
Fig. 1 Nonmetric multidimensional scaling (NMDS) of 97 plant species for which records on interaction with *Phaethornis ruber* were identified in our literature survey (stress level 10.19, $r^2 = 0.93$). *Axis 1* was rotated according to floral corolla length and is shown together with *axis 3*. *Right* Pearson's correlation coefficient of the floral traits and visitor scores in relation to all three axes, with *values in bold* indicating significant and strong correlations ($|r| > 0.65$). The plot with *axes 1* and *3* is shown since it better illustrates the separation of *P. ruber* behaviors (*Pol* Pollinator, *Rob* robber, thief)

Case study

Our experiment showed that *P. ruber* had a dramatic effect on the availability of nectar in *C. paniculata* flowers (Fig. 2a, b). It depleted almost all nectar available in the flowers in the early morning (treatments: $\chi^2 = 205.22$, $df = 2$, $p < 0.001$), even before the activity of the other nectar robber, the stingless bee (time interval: $\chi^2 = 0.5952$, $df = 1$, $p = 0.4404$). No interaction between the factors was observed (treatment × time interval: $\chi^2 = 0.092$, $df = 3$, $p = 0.9928$). Consequently, legitimate visits by hummingbirds to *C. paniculata* were extremely rare and were recorded only four times during our observations (two visits by the hermit hummingbird *Ramphodon naevius* and two visits by the emerald hummingbird *Thalurania glaucopis*). Of the *C. paniculata* flowers sampled throughout the flowering season, 48.9 % had robber marks left by *P. ruber* only, 8.3 % had robber marks left by *Trigona* sp. only, and 42.8 % had robber marks left by both *P. ruber* and *Trigona* sp.; we did not record any intact flowers.

Community-wide study

The hummingbird-plant network from the lowland Atlantic rainforest in Picinguaba comprised of 15 hummingbird and 44 plant species (Fig. 3). In the Pollination network, *P. ruber* ranked fifth considering the number of plant species visited (degree of 14) and third in number of individual visits (180), in a network totaling 1225 records/visits. In the Visitation network, however, *P. ruber* ranked third in relation to number of partners (23) and second in terms of number of visits (300) in a total of 1346 interaction records. Although illegitimate visits constituted approximately 9 % of all interactions, the addition of these to the analysis had only small effects on network metrics (Table 1). Whether or not the metrics departed from randomly obtained values did not change from the Pollination to the Visitation network. Within metrics with significant values, binary nestedness increased by 10.4 %, while specialization (H_2') and quantitative modularity increased by 2.0 % and 2.2 %, respectively (Table 1). The small increase in weighted modularity was associated to the increase in the number of modules, which increased from five to six (Fig. 3). The major change in the module conformation was the emergence of a module containing *P. ruber* as the only hummingbird species, separating it from other hermit hummingbirds with which it composed a single module in Pollination network (Fig. 3).

The NMDS of the floral traits from the lowland Atlantic rainforest plant community had a stress value of 5.95 ($r^2 = 0.98$), with axis 1 associated to corolla length and axis 2 associated to color score and nectar concentration (Fig. 4). Module identities as factors in the multidimensional ordination can be statistically separated when the Pollination network is considered (axes 1 and 2: $r^2 = 0.24$, $p = 0.007$; axes 1 and 3: $r^2 = 0.20$, $p = 0.027$). However, modules are not distinct in the ordination when illegitimate interactions are included (axes 1 and 2: $r^2 = 0.19$, $p = 0.077$; axes 1 and 3: $r^2 = 0.17$, $p = 0.109$).
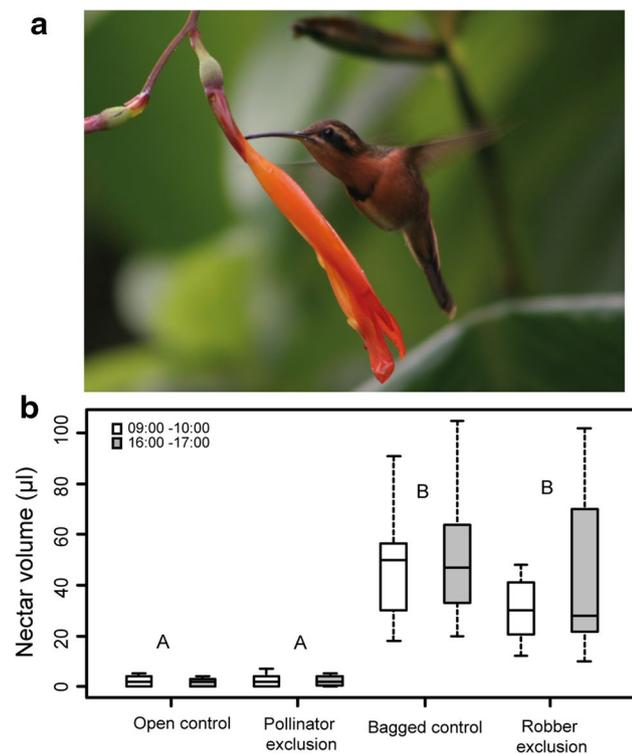
Fig. 2 a Nectar robbing of *C. paniculata* flowers by *P. ruber*. **b** Results from nectar robber exclusion experiments in *C. paniculata*, showing the intensity of nectar volume depletion measured at two time intervals during the day (*upper-left*). *Different uppercase letters* indicate significant differences by the post hoc Tukey test. For explanation of each treatment, see text ("Case study"). The horizontal line in the boxes indicate the median for each treatment with upper and lower limits of the boxes indicating the lower and upper quartiles (25 % and 75 % respectively)

Discussion

Our results show that nectar robbing by *P. ruber* is common, especially in flowers with a long corolla, and that this activity has a strong effect on individual plants and their associations with pollinating hummingbirds. Nevertheless, in our study, the incorporation of illegitimate interactions had only small effects on network metrics. Our literature survey—identifying 97 plant species visited by *P. ruber*—shows that in approximately 28 % of these plants the hummingbird engages in nectar robbery or theft, i.e., illegitimate interactions. These findings show that although the main behavioral strategy of *P. ruber* is to have legitimate and mutualistic associations with plants, exploitation is an important strategy to obtain nectar. Floral larceny is a commonly reported behavior for hummingbirds of the Mangoes, Coquette, and Emerald clades (Lara and Ornelas 2001 and references therein). The high proportion of plant species for which *P. ruber* acts as an exploiter is surprising for a hummingbird belonging to the Hermit clade, which

is often considered to be the most specialized group of pollinators within the hummingbird family (Trochilidae) (Feinsinger and Colwell 1978; Sazima et al. 1995; Vizentin-Bugoni et al. 2014; Maruyama et al. 2014). In this context, our analysis reinforces the notion that nectar use by hummingbirds is more flexible and opportunistic than previously appreciated (e.g. Maruyama et al. 2013).

The illegitimate strategy adopted by *P. ruber* has the potential to greatly affect the reproduction of plants with which it frequently interacts, as we have shown for *C. paniculata*. Nectar robbers may influence plant reproduction negatively by reducing floral attractiveness to pollinators, but they may also have a positive influence by either increasing the number of flowers the pollinators must visit or by increasing traveling distances to obtain their daily energy requirements (Maloof and Inouye 2000; Irwin et al. 2010). The outcome depends on the identity of the legitimate pollinators and their ability to discern and avoid robbed flowers, the amount of nectar removed by the robbers, and the floral neighborhood (i.e., the availability and attractiveness of other floral resources in the surrounding environment of the robbed species), among other factors (McDade and Kinsman 1980; Maloof and Inouye 2000). Moreover, the dependence of plants on biotic vectors for reproduction likely is an important determinant of the effect of floral larcenists, with pollinator-dependent and self-incompatible species being most negatively affected by nectar robbery (Burkle et al. 2007). At Picinguaba, *P. ruber* almost completely depleted the nectar of *C. paniculata*, which most likely explains the few legitimate visits to these flowers (McDade and Kinsman 1980; Justino et al. 2011). The low frequency of legitimate visits recorded at Picinguaba is even more striking when compared to the Santa Virgínia field station, where *P. ruber* is absent and nectar robbers are very infrequent (Maruyama et al. 2015). Using exactly the same sampling procedure, we observed 84 legitimate visits by the Scale-throated Hermit, *Phaethornis eurynome* at the Santa Virgínia field station (Maruyama et al. 2015), compared to only four legitimate visits observed at Picinguaba. Hummingbirds use several cues to avoid less rewarding robbed flowers (Irwin 2000), and the almost complete depletion of nectar by *P. ruber* combined with the high availability of other flower resources in Picinguaba (Fig. 3) reduces the attractiveness of *C. paniculata* to legitimate pollinators. Since *C. paniculata* is a self-compatible but pollinator-dependent species (Maruyama et al. 2015), an expected outcome is that decreased visitation rates due to nectar robbing would decrease plant reproduction (Burkle et al. 2007).

In contrast to the strong effect at the individual plant species level, the incorporation of illegitimate interactions had, overall, small effects on network structure (Table 1). This latter result is in contrast to the findings of Genini
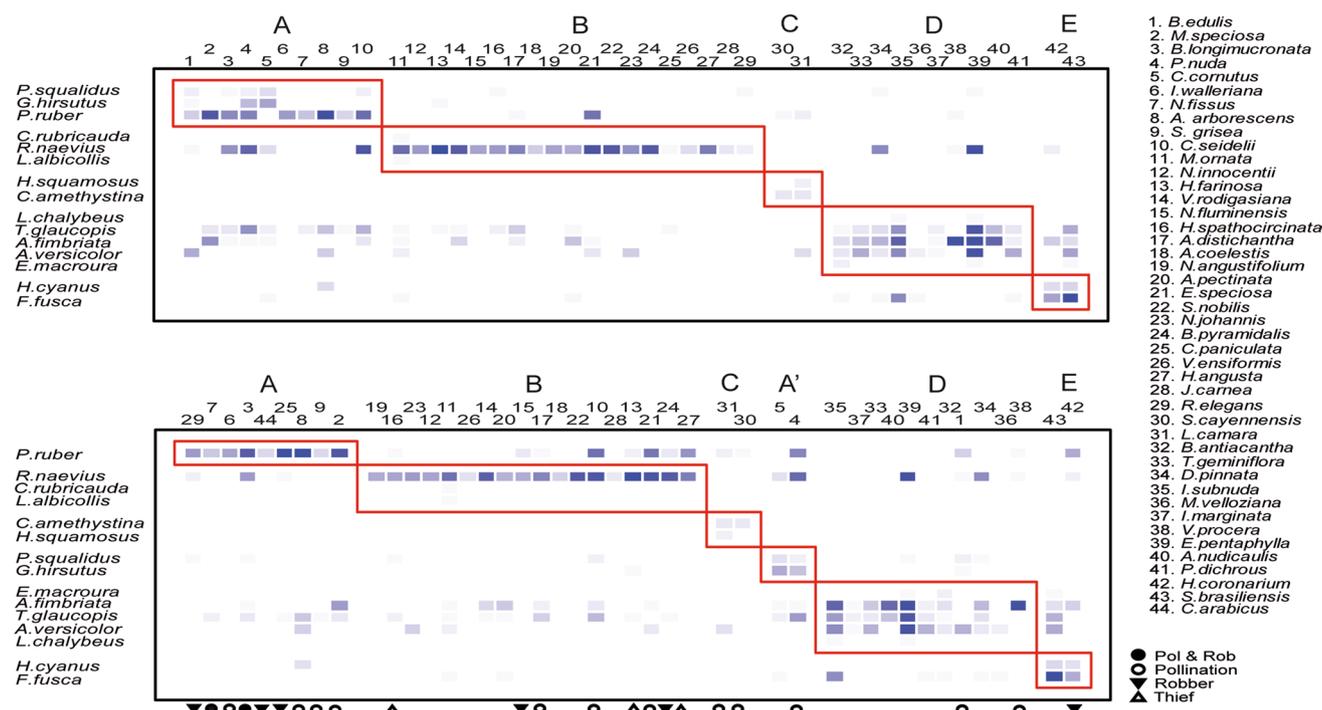
Fig. 3 Modules in the plant–hummingbird visitor network from Núcleo Picinguaba, southeastern Brazil. *Top matrix* Pollination network (PN) with only legitimate interactions, *bottom matrix* Visitation network (VN) also incorporated illegitimate interactions (nectar robbing and thieving). *Uppercase letters above matrices* denote identities of modules in the networks. Note that the original module (*A*) is sepa-rated into two modules (*A*, *A′*) when illegitimate visits are included. Module *A* in the *bottom matrix* has only *P. ruber* as floral visitor. In this new conformation, *P. ruber* is associated to several plants which belonged to modules with other hummingbird species in the *top matrix*

Table 1 Network metrics for the Pollination network, in which only legitimate hummingbird visits were considered, and for the floral Visitation network, in which illegitimate visits, including nectar robbery and theft, were also included

Network metrics	Pollination network	Visitation network	Increase (%)[a]
NODF[b]	29.88[c]	32.98[c]	10.4
WNODF[b]	30.86	32.90	6.6
Connectance	0.21	0.22	4.8
H_2' (specialization)	0.49[c]	0.50[c]	2.0
Modularity (binary)	0.30	0.30	0.0
Modularity (weighted)	0.46[c]	0.47[c]	2.2

[a] Values show the proportional increase of the network metrics in VN in relation to values observed for PN

[b] NODF, WNODF, metrics used to calculate the binary and weighted nestedness, respectively

[c] Network metrics are significant; i.e., metrics that did not overlap to null model expectations (95 % confidence interval)

et al. (2010) and Yoshikawa and Isagi (2013) who inde-pendently compared networks with and without flower exploiters and reported more striking differences than we found in our study. This difference may be related to the relative proportion of exploiters in the networks, as one of the two systems evaluated by Genini et al. (2010) also had few exploiters and fewer changes in the network structure. Previously reported increase in modularity and specializa-tion when mutualistic and antagonistic interactions were merged is attributed to higher specialization of antagonistic interactions, which may contribute to an overall increase in network level functional specialization (Genini et al. 2010; Fontaine et al. 2011; Yoshikawa and Isagi 2013). In our study, binary nestedness increased the most, which likely reflects the increased level of generalization of *P. ruber*, making it one of the "core generalist" species in the Visi-tation network. Our results indicate that one species, even though common and interacting frequently and widely with the plant assemblage, has limited influence on network metrics—i.e., on how we characterize the overall structural properties of a network—especially if the floral visitor has a dual role as both pollinator and exploiter.

Although overall network structural changes were small, the incorporation of illegitimate visits by *P. ruber* neverthe-less changed the conformation of the modules in the net-work (Fig. 3) and trait distribution within modules (Fig. 4). Modules are regarded as "subcommunities within com-munities," such that tightly linked species within a module

Modules
○ A △ A' + B × C ◇ D ▽ E
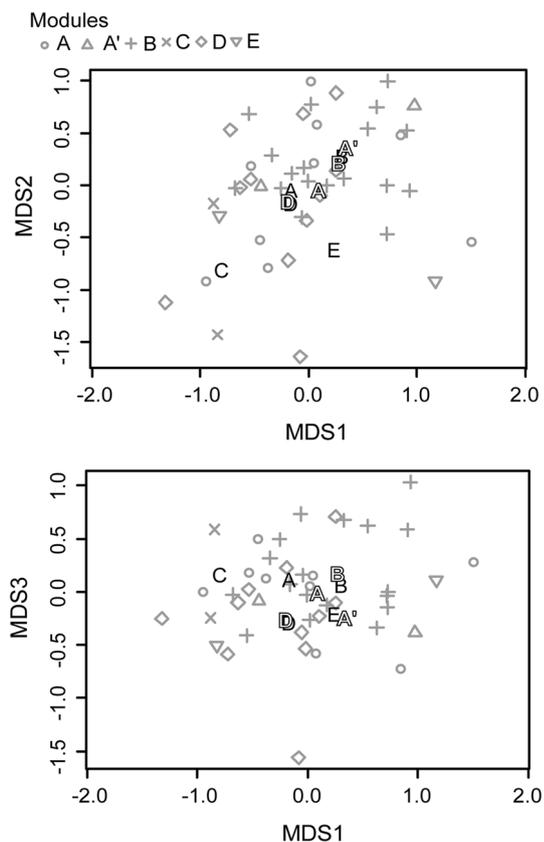
Correlations

	Axis 1	Axis 2	Axis 3
Corolla (cm)	**0.978**	0.284	0.152
Color score	0.152	**0.890**	-0.446
Nectar (ul)	0.400	-0.216	-0.439
Nectar (%)	0.577	**0.693**	0.592

Fig. 4 Nonmetric multidimensional scaling (NMDS) of floral traits of plants from Núcleo Picinguaba, southeastern Brazil (stress = 5.95, $r^2 = 0.93$). *Points* Plant species, *letters* indicate the mean value of floral phenotype of each module. Module identities were fitted as factors in the multidimensional ordination (*uppercase letters*), with *black-filled uppercase letters* indicating modules in Pollination networks (PN) and *open uppercase letters* indicating module identities in the Visitation networks (VN), whenever species composition changed. Modules in PN can be statistically separated (*axes 1* and *2*: $r^2 = 0.24$, $p = 0.007$; *axes 1* and *3*: $r^2 = 0.20$, $p = 0.027$), but those in the VN cannot (*axes 1* and *2*: $r^2 = 0.19$, $p = 0.077$; *axes 1* and *3*: $r^2 = 0.17$, $p = 0.109$). Pearson's correlation coefficient of the floral traits and three axes are shown *below the plots*, with values in *bold* indicating significant and strong correlations ($|r| > 0.65$)

have a stronger effect on each other than on species in other modules (Olesen et al. 2007). Hence, characterization of the modules in the network incorporating illegitimate visits can present complementary information on how species affect each other. For example, it is reasonable to conclude that *P. ruber* has a strong influence on plant species that are "pulled away" from other modules into the new module with *P. ruber* in the Visitation network (module A at the bottom matrix, Fig. 3). Our case study species *C. paniculata* was included

in module B in the Pollination network, but as the interaction frequency with *P. ruber* is much higher than with the legitimate pollinators, in the Visitation network it was assigned to module A. Demonstration of this change may contribute to a better representation of the influence of the floral visitors and potentially of other plant species on our focal species. This interpretation is strengthened by the strong effect of *P. ruber* on the nectar standing crop. Similar effects on nectar availability may be expected for other plant species in which robbing is much more frequent than legitimate interactions (e.g., *Ruellia elegans* Poir, Acanthaceae). Finally, the separation of modules in the ordination space by taking the Pollination network into account, but not the Visitation network, reflects different rules by which specific pairwise interactions occur. While the legitimate pairwise interactions in plant–hummingbird networks are strongly influenced by morphological matching (Maruyama et al. 2014; Vizentin-Bugoni et al. 2014), floral larcenists, by definition, ignore the barriers imposed by plants to floral visitors, possibly explaining why floral traits do not explain modules only in the Visitation network including illegitimate interactions.

Conclusion

Based on our results, we conclude that floral larcenists may exert strong effects on nectar availability and the pollination of specific plants and are often associated with flowers with longer corollas. Since species from several hummingbird clades switch between pollination and nectar robbing, we suggest that hummingbirds may offer an interesting system for studying the evolutionary correlates of mutualism exploitation (see Bronstein 2001; Irwin et al. 2010). In contrast to previous studies, we show that network metrics are not strongly affected when illegitimate interactions are included, although if the outcome for specific interactions or within-module organization are considered in detail, floral larcenists have the potential to affect the functioning of communities (e.g., community-level seed production). To better understand the functioning of flower–floral visitor networks including floral larcenists, future studies could employ experimental manipulations at the community level. Although it is prohibitive to actually manipulate an entire community of pollinators and plants, such studies could employ a subset of species and manipulate the presence of floral larcenists. In such a framework it would be possible to evaluate both how larcenists affect the interaction of other species and their functional outcome, such as fruit set. Only few recent studies have taken such an experimental approach (e.g., Brosi and Briggs 2013; Fründ et al. 2013). However, whether and how floral larcenists affect other species' foraging behavior and consequently the structure of networks remain to be thoroughly investigated.

References

Almeida-Neto M, Ulrich W (2011) A straightforward computational approach for measuring nestedness using quantitative matrices. Environ Model Softw 26:173–178. doi:10.1016/j.envsoft.2010.08.003

Bascompte J, Jordano P, Melián CJ, Olesen JM (2003) The nested assembly of plant-animal mutualistic networks. Proc Natl Acad Sci 100(16):9383–9387

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects model using Eigen and S4. R package version 1.1–6. Available at: http://CRAN.R-project.org/package=lme4

Blüthgen N, Menzel F, Blüthgen N (2006) Measuring specialization in species interaction networks. BMC Ecol 6:9. doi:10.1186/1472-6785-6-9

Borcard D, Gillet F, Legendre P (2011) Numerical ecology with R. Springer, New York

Bronstein JL (2001) The exploitation of mutualisms. Ecol Lett 4:277–287. doi:10.1046/j.1461-0248.2001.00218.x

Brosi BJ, Briggs H (2013) Single pollinator species losses reduce floral fidelity and plant reproductive function. Proc Natl Acad Sci USA 110:13044–13048. doi:10.1073/pnas.1307438110

Burkle LA, Irwin RE, Newman DA (2007) Predicting the effects of nectar robbing on plant reproduction: implications of pollen limitation and plant mating system. Am J Bot 94:1935–1943. doi:10.3732/ajb.94.12.1935

Buzato S, Sazima M, Sazima I (2000) Hummingbird–pollinated floras at three Atlantic forest sites. Biotropica 32:824–841. doi:10.1111/j.1744-7429.2000.tb00621.x

Dalsgaard B, Martín González AM, Olesen JM, Ollerton J, Timmermann A, Andersen LH, Tossas AG (2009) Plant–hummingbird interactions in the West Indies: floral specialisation gradients associated with environment and hummingbird size. Oecologia 159:757–766. doi:10.1007/s00442-008-1255-z

Dormann CF, Strauss R (2014) A method for detecting modules in quantitative bipartite networks. Methods Ecol Evol 5:90–98. doi:10.1111/2041-210X.12139

Dormann CF, Gruber B, Fründ J (2008) Introducing the bipartite package: analysing ecological networks. R News 8:8–11

Feinsinger P, Colwell RK (1978) Community organization among neotropical nectar-feeding birds. Am Zool 18:779–795. doi:10.1093/icb/18.4.779

Fontaine C, Guimarães PR Jr, Kéfi S, Loeuille N, Memmott J, van der Putten WH, van Veen FJF, Thébault E (2011) The ecological and evolutionary implications of merging different types of networks. Ecol Lett 14:1170–1181. doi:10.1111/j.1461-0248.2011.01688.x

Fründ J, Dormann CF, Holzschuh A, Tscharntke T (2013) Bee diversity effects on pollination depend on functional complementarity and niche shifts. Ecology 94:2042–2054. doi:10.1890/12-1620.1

Genini J, Morellato LP, Guimarães PR Jr, Olesen JM (2010) Cheaters in mutualism networks. Biol Lett 6:494–497. doi:10.1098/rsbl.2009.1021

Guimarães PR Jr, Guimarães P (2006) Improving the analyses of nestedness for large sets of matrices. Environ Model Softw 21:1512–1513. doi:10.1016/j.envsoft.2006.04.002

Inouye DW (1980) The terminology of floral larceny. Ecology 61:1251–1253

Irwin RE (2000) Hummingbird avoidance of nectar-robbed plants: spatial location or visual cues. Oikos 91:499–506. doi:10.1034/j.1600-0706.2000.910311.x

Irwin RE, Bronstein JL, Manson JS, Richardson L (2010) Nectar robbing: ecological and evolutionary perspectives. Annu Rev Ecol Evol Syst 41:271–292. doi:10.1146/annurev.ecolsys.110308.120330

Joly CA, Assis MA, Bernacci LC, Tamashiro JY, Campos MCR, Gomes JAMA, Lacerda MS, Santos FAM, Pedroni F, Pereira LS, Padgurschi MCG, Prata EMB, Ramos E, Torres RB, Rochelle A, Martins FR, Alves LF, Vieira SA, Martinelli LA, Camargo PB, Aidar MPM, Eisenlohr PV, Simões E, Villani JP and Belinello R (2012) Florística e fitossociologia em parcelas permanentes da Mata Atlântica do sudeste do Brasil ao longo de um gradiente altitudinal. Biota Neotrop 12(1):123–145. http://www.biotaneotropica.org.br/v12n1/en/abstract?article+bn01812012012

Justino D, Maruyama PK, Oliveira PE (2011) Floral resource availability and hummingbird territorial behaviour on a Neotropical savanna shrub. J Ornithol 153:189–197. doi:10.1007/s10336-011-0726-x

Lara C, Ornelas J (2001) Preferential nectar robbing of flowers with long corollas: experimental studies of two hummingbird species visiting three plant species. Oecologia 128:263–273. doi:10.1007/s004420100640

Maloof JE, Inouye DW (2000) Are nectar robbers cheaters or mutualists? Ecology 81:2651–2661. doi:10.1890/0012-9658(2000)081[2651:ANRCOM]2.0.CO;2

Marquitti FMD, Guimarães PR Jr, Pires MM, Bittencourt LF (2014) MODULAR: software for the autonomous computation of modularity in large network sets. Ecography 37:221–224. doi:10.1111/j.1600-0587.2013.00506.x

Maruyama PK, Oliveira GM, Ferreira C, Dalsgaard B, Oliveira PE (2013) Pollination syndromes ignored: importance of non-ornithophilous flowers to Neotropical savanna hummingbirds. Naturwissenschaften 100:1061–1068. doi:10.1007/s00114-013-1111-9

Maruyama PK, Vizentin-Bugoni J, Oliveira GM, Oliveira PE, Dalsgaard B (2014) Morphological and spatio-temporal mismatches shape a Neotropical savanna plant-hummingbird network. Biotropica 46:740–747. doi:10.1111/btp.12170

Maruyama PK, Vizentin-Bugoni J, Dalsgaard B, Sazima M (2015) Pollination and breeding system of *Canna paniculata* (Cannaceae) in a montane Atlantic rainforest: asymmetric dependence on a hermit hummingbird. Acta Bot Bras 29:157–160. doi:10.1590/0102-33062014abb3590

McDade LA, Kinsman S (1980) The impact of floral parasitism in two neotropical hummingbird–pollinated plant species. Evolution 34:944–958

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) Vegan: community ecology package. R package version 2.0–10. Available at: http://CRAN.R-project.org/package=vegan

Olesen JM, Bascompte J, Dupont YL, Jordano P (2007) The modularity of pollination networks. Proc Natl Acad Sci USA 104:19891–19896. doi:10.1073/pnas.0706375104

Ollerton J, Alarcón R, Waser N, Price M, Watts S, Cranmer L, Hingston A, Peter C, Rotenberry J (2009) A global test of the pollination syndrome hypothesis. Ann Bot 103:1471–1480. doi:10.1093/aob/mcp031

Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? Oikos 120:321–326. doi:10.1111/j.1600-0706.2010.18644.x

Sauve A, Fontaine C, Thébault E (2014) Structure-stability relationships in networks combining mutualistic and antagonistic interactions. Oikos 123:378–384. doi:10.1111/j.1600-0706.2013.00743.x

Sazima I, Buzato S, Sazima M (1995) The saw-billed hermit *Ramphodon naevius* and its flowers in southeastern Brazil. J Ornithol 136:195–206. doi:10.1007/BF01651241

Schleuning M, Ingmann L, Strauß R, Fritz SA, Dalsgaard B, Dehling DM, Plein M, Saavedra F, Sandel B, Svenning J-C, Böhning-Gaese K, Dormann CF (2014) Ecological, historical and evolutionary determinants of modularity in weighted seed-dispersal networks. Ecol Lett 17:454–463. doi:10.1111/ele.12245

Staniczenko PP, Kopp JC, Allesina S (2013) The ghost of nestedness in ecological networks. Nat Commun 4:1391. doi:10.1038/ncomms2422

Thébault E, Fontaine C (2010) Stability of ecological communities and the architecture of mutualistic and trophic networks. Science 329:853–859. doi:10.1126/science.1188321

Vizentin-Bugoni J, Maruyama PK, Sazima M (2014) Processes entangling interactions in communities: forbidden links are more important than abundance in a hummingbird–plant network. Proc Royal Soc B 281:20132397. doi:10.1098/rspb.2013.2397

Wilson P, Castellanos MC, Hogue JN, Thomson JD, Armbruster WS (2004) A multivariate search for pollination syndromes among penstemons. Oikos 104:345–361. doi:10.1111/j.0030-1299.2004.12819.x

Yoshikawa T, Isagi Y (2013) Determination of temperate bird–flower interactions as entangled mutualistic and antagonistic sub-networks: characterization at the network and species levels. J Anim Ecol 83:651–660. doi:10.1111/1365-2656.12161