

Research

The topological differences between visitation and pollen transport networks: a comparison in species rich communities of the Himalaya–Hengduan Mountains

Yan-Hui Zhao, Amparo Lázaro, Zong-Xin Ren, Wei Zhou, Hai-Dong Li, Zhi-Bin Tao, Kun Xu, Zhi-Kun Wu, Lorne M. Wolfe, De-Zhu Li and Hong Wang

Y.-H. Zhao (<http://orcid.org/0000-0002-7569-4591>), Z.-X. Ren, H.-D. Li (<http://orcid.org/0000-0002-0789-7346>), Z.-B. Tao, L. M. Wolfe and H. Wang (wanghong@mail.kib.ac.cn), Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Inst. of Botany, Chinese Academy of Sciences, CN-650201 Kunming, PR China. ZBT also at: Kunming College of Life Sciences, Univ. of Chinese Academy of Sciences, Kunming, PR China. – A. Lázaro, Mediterranean Inst. For Advanced Studies, Esporles, Spain. – W. Zhou and D.-Z. Li, Germplasm Bank of Wild Species, Kunming Inst. of Botany, Chinese Academy of Sciences, Kunming, PR China. – K. Xu and Z.-K. Wu, Lijiang Forest Ecosystem Research Station, Kunming Inst. of Botany, Chinese Academy of Sciences, Kunming, PR China.

Oikos

128: 551–562, 2019

doi: 10.1111/oik.05262

Subject Editor: Paulo Guimarães Jr

Editor-in-Chief: Dries Bonte

Accepted 3 October 2018

Pollination networks are usually constructed and assessed by direct field observations which commonly assume that all flower visitors are true pollinators. However, this assumption is often invalid and the use of data based on mere visitors to flowers may lead to a misunderstanding of intrinsic pollination networks. Here, using a large dataset by both sampling floral visitors and analyzing their pollen loads, we constructed 32 networks pairs (visitation versus pollen transport) across one flowering season at four elevation sites in the Himalaya–Hengduan Mountains region. Pollen analysis was conducted to determine which flower visitors acted as potential pollinators (pollen vectors) or as cheaters (those not carrying pollen of the visited plants). We tested whether there were topological differences between visitation and pollen transport networks and whether different taxonomic groups of insect visitors differed in their ability to carry pollen of the visited plants. Our results indicated that there was a significantly higher degree of specialization at both the network and species levels in the pollen transport networks in contrast to the visitation networks. Modularity was lower but nestedness was higher in the visitation networks compared to the pollen transport networks. All the cheaters were identified as peripheral species and most of them contributed positively to the nested structure. This may explain in part the differences in modularity and nestedness between the two network types. Bees carried the highest proportion of pollen of the visited plants. This was followed by Coleoptera, other Hymenoptera and Diptera. Lepidoptera carried the lowest proportion of pollen of the visited plants. Our study shows that the construction of pollen transport networks could provide a more in-depth understanding of plant–pollinator interactions. Moreover, it suggests that detecting and removing cheater interactions when studying the topology of other mutualistic networks might be also important.

Keywords: cheater, flower visitor, modularity, mutualistic network, nestedness, pollen load analysis



Introduction

Species in ecological communities form networks of interactions (Pocock et al. 2012, Dattilo et al. 2016). Mutualistic networks (e.g. pollination and seed dispersal) have far-reaching effects on the maintenance of ecosystem services. Thus, there have been extensive efforts to describe and compare the topology of mutualistic networks (Bascompte and Jordano 2007, Olesen et al. 2007). Most of the recent studies describing pollination networks are usually constructed under the presumption that all animal species observed visiting angiosperm species in bloom are true pollinators (Cuartas-Hernández and Medel 2015, Traveset et al. 2015). In fact, some flower visitors are really cheaters, taking floral rewards without dispersing pollen and/or depositing viable grains on receptive stigmas (Inouye 1980, Forup and Memmott 2005, Gibson et al. 2006, Zych 2006, Irwin et al. 2010). Therefore, such plant–animal interaction systems, as described above, should really be referred to as plant–flower visitor networks or visitation networks as they only provide essential information on resource use from the perspective of these foraging animals. The same problem also exists in the study of other plant–animal mutualisms (e.g. plant–ant and plant–frugivore; Chamberlain et al. 2014, Jones et al. 2015, Genrich et al. 2017). To construct true mutualistic networks, though, it is crucial to detect and remove cheater interactions.

Specifically, a flower visitor becomes an effective pollinator when it transfers viable pollen from one flower to the receptive stigmas of conspecific and compatible genotypes. In particular, insect flower visitors vary across major taxonomic groups (e.g. Coleoptera, Diptera, Lepidoptera and Hymenoptera) in their ability to remove and carry pollen grains, due to differences in their foraging behaviors response to attractants and their respective morphologies (Schemske and Horvitz 1984, Waser et al. 1996, Ollerton et al. 2007). Compared with other insects, bees often carry significantly higher proportions of pollen of the visited plants on their bodies (Forup et al. 2008, Alarcón 2010). Bees have been reported to actively collect pollen, have long foraging bouts, and often show a high degree of constancy to certain plant species (Chittka et al. 1999, Rader et al. 2011). In addition, the comparatively hairy bodies of most bees increase opportunities for pollen dispersal and lead to the act of pollination (Mayfield et al. 2001, Michener 2007). In contrast, many members of the Order Lepidoptera often carry lesser amounts of pollen of the visited plants (Schemske and Horvitz 1984, Forup and Memmott 2005, Alarcón 2010). This might be related to the fact that most species in the Lepidoptera forage for nectar exclusively and this results in lower frequencies of passive contact with anthers (Ballantyne et al. 2015).

Therefore, including cheaters (i.e. animals that regularly visit flowers but do not carry or deposit pollen of the visited plants) when examining visitation networks may distort our perception of network structure and role of generalization

from the perspective of resident plant species. For example, if only visitors that efficiently transport pollen are included in the pollen transport networks, these networks are smaller, in terms of the overall number of species and pairwise interactions recorded, compared to visitation networks (Alarcón 2010, Popic et al. 2013). In addition, recent studies show that pollination networks excluding cheaters have been shown to be more specialized when using a quantitative index (H_2' ; Alarcón 2010, Ballantyne et al. 2015), whereas more generalized when using a qualitative index (connectance; Forup and Memmott 2005, Gibson et al. 2006).

Other network properties that might be affected by the presence of cheaters include modularity and nestedness. Both occur commonly in ecological networks and appear to be invariant across different ecosystems (Bascompte and Jordano 2007, Olesen et al. 2007). Past research shows that mutualistic networks are more nested but less modular than antagonistic networks (i.e. host–parasite) (Bascompte et al. 2003, Thébault and Fontaine 2010). Therefore, antagonistic interactions in ecological networks might contribute negatively to nestedness but positively to modularity. For example, Genini et al. (2010) showed that the removal of cheaters from a plant–flower visitor network resulted in the disappearance of significantly modular structure.

As field observations alone (without accurately investigating foraging behavior on the flowers or conducting reproductive tests) cannot assess the role of some visitors as pollinators, additional efforts are needed to properly construct more accurate plant–pollinator networks. Until now, two approaches have been used to discriminate between cheaters and pollinators at the community level. First, there is the direct measurement of per visit pollen deposition on stigmas to define the roles of flower visitors as pollinators (King et al. 2013). However, this approach is time consuming and difficult to apply to large, complex networks in species-rich communities (Ballantyne et al. 2015). Second, an examination of plant pollen carried by flower visitors is an alternative approach to explore the effectiveness of visitors as pollen vectors (Forup and Memmott 2005, Gibson et al. 2006, Lopezaraiza-Mikel et al. 2007, Alarcón 2010, Olesen et al. 2011, Jędrzejewska-Szmek and Zych 2013, Popic et al. 2013, Coux et al. 2016, Zhao et al. 2016). Of course, some pollen grains could be lost because insects remove accumulated pollen during bouts by grooming. Currently, the use of pollen load analyses in pollination network studies is relatively infrequent compared with the large number of published studies on visitation networks. In addition, the structural comparison between visitation and pollen transport networks is normally conducted using only a small number of networks (Forup and Memmott 2005, Gibson et al. 2006, Alarcón 2010, Jędrzejewska-Szmek and Zych 2013, Popic et al. 2013). This does not allow for a quantitative comparison of network metrics between network types.

In this study, we evaluate how network structure changed when the cheaters were excluded from visitation networks. For that, we collected flower visitors from four

species-rich communities along a high elevation gradient in the Himalaya–Hengduan Mountains region during the 2012 flowering season. We created a pollen load data base using scanning electron microscopy to identify pollen grains on 3055 flower-visiting insects. This permitted us to identify and define potential pollinators (i.e. insects that visit flowers and also carry their pollen). Using this large and detailed database we constructed 32 visitation networks and 32 pollen transport networks (7–9 networks per site at different sampling times through the season) to do a quantitative comparison of network structure between the two network types. Our specific objectives were threefold: 1) to determine whether network structure (species richness, specialization, modularity and nestedness) differed between visitation networks and pollen transport networks; 2) to examine the role of cheaters in the visitation networks and evaluate how the cheaters affected network structure; and 3) to quantify whether different taxonomic groups of insect visitors (i.e. bees, other Hymenoptera, Coleoptera, Diptera and Lepidoptera) differed in their ability to carry pollen of the visited plants. This will help to discern which of the insects involved in the networks might have a stronger role as pollinators or cheaters, and how this might affect network structure in communities with different composition of flower visitors. Due to the removal of the antagonistic interactions between plants and cheaters in the pollen transport networks, we predicted that specialization and nestedness would be lower, while modularity would be higher in visitation networks compared to pollen transport networks. We expected that differences between network types could be explained by the role of cheaters in the visitation networks. Lastly, we also predicted that bees, which are hairy insects, would show a higher probability of transport pollen of the visited plants compared with Lepidoptera, which are ‘smooth-bodied’ (glabrous, sub-hirsute).

Methods

Study sites

Specimens and data sets were collected in four, subalpine/alpine meadows on the eastern slopes of Yulong Mountain in the Himalaya–Hengduan Mountains, SW China 2012. They are 1) Yushuizhai (YSZ), 2725 m a.s.l., 27°00′10″ N, 100°12′05″ E; 2) Haligu (HLG), 3235 m a.s.l., 27°00′09″ N, 100°10′57″ E; 3) Yakou (YK), 3670 m a.s.l., 27°00′56″ N, 100°10′17″ E; and 4) Diyifeng (DYF, above tree line), 3910 m a.s.l., 27°01′41″ N, 100°11′03″ E (Supplementary material Appendix 1 Fig. A1). The linear distance between neighboring meadows was about 2.0 km. The vegetation surrounding these four meadows differed among sites from forests dominated by *Pinus* species to mixed *Abies* and *Rhododendron* species. Mean temperatures (from 11 May to 29 September 2012) decreased from the lowest to the highest meadows (16.6°C, 12.9°C, 9.6°C and 8.9°C, respectively; Zhao et al. 2016).

Field surveys of flower visitors

Field work was performed during the 2012 flowering season (early May to early October). We conducted nine surveys at two-week intervals for each community. For each survey, we collected flower visitors by walking along pre-arranged transects (150 m in length and 2 m in width) within a 1.5 ha plot from 9:00 to 17:00 h on either sunny days, or during sunny gaps on cloudy or foggy days. Only insects that made contact with plant reproductive organs or foraged for nectar and/or pollen were considered as flower visitors and collected (Memmott 1999, Alarcón 2010). Plant species that received no insect visits (e.g. anemophilous members of the Poaceae) were excluded from all analyses. Voucher specimens of insects and plants were deposited in the Kunming Institute of Botany, Chinese Academy of Sciences.

Insect pollen load analysis

During the study period we collected a total of 6004 insect visitors on flowers of identified species at the four sites. Based on plant–flower visitor interactions observed during each survey at each site we randomly selected five insect specimens that belonged to the same species for pollen load analysis. We used all the insect specimens collected in a specific plant species for pollen load analysis when the total number of them was lower than five.

Each insect specimen was bathed in 95% ethanol in a centrifuge tube using an ultrasonic cleaner for three minutes. The pollen located in specialized pollen-carrying structures of bees, e.g. scopa, may not be available for pollination (Alarcón 2010). Therefore, for the bees carrying pollen grains on their scopae or corbiculae we removed the hind legs (for Halictidae, Colletidae, Andrenidae and Apidae) or metasoma (for Megachilidae) before washing them. Each bathed insect was removed and preserved in a second co-referenced tube in 95% alcohol. The pollen samples remaining in the first tube were centrifuged at 5000 rpm for 10 min and the supernatants were decanted. Each pollen sample was then placed on a SEM stub and viewed under a scanning electron microscope. Pollen grains were identified by comparing them to a pollen reference library constructed from field-collected and identified flowers (Supplementary material Appendix 1 Table A1, Fig. A2). Pollen grains of some phylogenetically related species could not be differentiated to species by a standard combination of size, exine morphology or aperture traits. In these cases, we grouped the grains into types based on the generalized pollen morphology of a known and recognized genus or family (e.g. *Gentiana* type, *Pedicularis* type, and *Ligularia* type). An insect specimen was considered to be a potential pollinator of the plant species it visited if we could identify a minimum of 10 grains of the same pollen morphotype (Bosch et al. 2009). For each unique plant–flower visitor interaction at each site (YSZ: 703, HLG: 445, YK: 322, DYF: 203) we assumed that pollen grains of a specific pollen morphotype belonged to the same plant species on which the insect was captured. In our communities, the

majority of congeners were either distributed at different elevations and/or flowered at different times over the same season (Zhao et al. 2016, Supplementary material Appendix 1 Table A1). Therefore, we identified almost all pollen grains to plant species level by combining this spatial–temporal information.

If the selected insect specimens did not carry pollen of the visited plant species, we kept choosing the remaining specimens until one of them presented pollen of the visited plant species. When none of the insect specimens belonging to the same species carried pollen of the focal plant species we classified that species as a cheater on this plant species. In total, the pollen load of 3055 insect specimens representing 355 insect species were examined.

Network construction and analysis

By using the bipartite package (Dormann et al. 2008) in R ver. 3.2.3 (<www.r-project.org>) we constructed a quantitative visitation network for each survey at the four sites. The strength of each interaction was determined by the number of flower visitors collected from each plant species. At the two higher elevation sites (YK and DYF), there were fewer species in flower at the earliest and latest flowering periods. Therefore, we combined the first and second surveys, as well as the 8th and final surveys. In total, we constructed 32 visitation networks from these four sites.

We then constructed the pollen transport networks by excluding the cheater–plant interactions from each of the 32 visitation networks. The strength of each interaction was calculated as the number of individuals collected carrying pollen of the visited plants. The plant and insect species that had no interaction partners were removed from the pollen transport networks.

To compare the visitation and pollen transport networks we analyzed the most widely used descriptors of the structure of each networks (i.e. species richness, specialization, modularity and nestedness; Tylianakis and Morris 2017). Some of these metrics have been found to differ between networks with and without cheaters (Alarcón 2010, Genini et al. 2010, Popic et al. 2013). We calculated the network specialization index (H_2') and species level specialization index (d') for both insects and plants using the bipartite package in R. Both H_2' and d' measure the deviation of realized interaction frequencies from an expected probability distribution of interactions, based on the observed interaction frequencies (Blüthgen et al. 2006). The values of both metrics range between 0 and 1 for extreme generalization and specialization, respectively.

Modularity refers to the extent to which species within a group (module) interact more frequently with each other than they do with species in other groups (Olesen et al. 2007). We calculated the qualitative (i.e. binary) modularity (M) and the number of modules using the program NcCarto (Guimerà and Amaral 2005). For quantitative modularity (Q) and the number of modules we used the QuanBiMo

algorithm (Dormann and Strauss 2014) in the bipartite package in R. As Q values can vary among different runs we repeated the calculations 10 times with 10^7 swaps and chose the highest value for each network (Vizentin-Bugoni et al. 2016).

For each species in the binary modular networks we assigned a topological role (network hub, module hub, connector or peripheral) based on the standardized within-module degree (c) and among-module connectivity (z ; Guimerà and Amaral 2005, Olesen et al. 2007). A network hub ($c > 0.62$, $z > 2.5$) is highly linked to species within their own module and species of other modules. Therefore, it is important for the connectivity among species in both its own module and within the entire network (Olesen et al. 2007). A module hub ($c \leq 0.62$, $z > 2.5$) plays an important role in connecting species within its own module. A connector species ($c > 0.62$, $z \leq 2.5$) is important for among-module connectivity but plays an inferior role within its own module (Olesen et al. 2007). Peripheral species ($c \leq 0.62$, $z \leq 2.5$) have a few interactions inside their own module and rarely link to any other modules. As such, they play an inferior structural role in these networks (Olesen et al. 2007).

For ecological networks, nestedness occurs when the more specialist species tend to interact with subsets of those species interacting with the more generalists (Bascompte et al. 2003). We calculated both the qualitative nestedness (NODF) and the quantitative nestedness (WNODF) using the bipartite package in R. Following Saavedra et al. (2011), we also estimated the contribution of each species to nestedness in each binary network. This estimate is derived from the NODF metric and positive values of species contribution to nestedness indicate a species that increases the nested pattern. Conversely, negative values of species contribution to nestedness indicate a species that decreases the nested pattern. We performed this analysis using the bipartite package in R.

To test the significance of these network level metrics, we compared the observed values to those generated by null models. For qualitative modularity (M) we generated random networks which constrains the same linkage level ranking as the empirical one (Guimerà and Amaral 2005). For qualitative nestedness (NODF), we used the C_e null model from the software Aninhado (Guimarães and Guimarães 2006). This null model assumes that the probability of an interaction is proportional to the generalization level of both plant and insect species. For quantitative metrics (H_2' , Q and WNODF) we generated random networks using Patefield's algorithm, which fixes the network size and the interaction matrix marginal totals (Patefield 1981). We generated 1000 randomized networks to estimate H_2' , NODF and WNODF (Schleuning et al. 2012, Sebastián-González et al. 2015). We only generated 100 randomized networks to estimate binary and quantitative modularity because of the large computational time required by these algorithms (Schleuning et al. 2014, Zanata et al. 2017). Metric values were considered

significant if they did not overlap 95% of confidence intervals of the randomized values.

We used null model corrections to control network metrics that may be influenced by network size and sampling effort in order to allow comparison of network metrics across networks (Dalsgaard et al. 2017). We standardized network level specialization and modularity by calculating the differences between observed value and the mean value across all randomized networks (Δ -transformation; Schleuning et al. 2012, Dalsgaard et al. 2017). We standardized nestedness by calculating the z-score of NODF and WNODF (z-transformation; Sebastián-González et al. 2015).

Statistical analysis

We performed several generalized linear mixed models (GLMMs) to evaluate the effects of network types (visitation versus pollen transport) on species richness and network metrics. In these GLMMs we included network type as a fixed factor and survey nested within site as a random factor. To compare plant and insect richness between visitation and pollen transport networks we performed two GLMMs fitted with poisson error distributions and log link functions. To evaluate the effects of network types on $\Delta H_2'$, ΔM , ΔQ , z-NODF and z-WNODEF, we conducted GLMMs fitted with gaussian distributions and identity link functions. For networks that were significantly modular we first compared the number of qualitative/quantitative modules between network types by means of GLMMs fitted with poisson error distributions and log link functions. As differences in the number of modules may be mediated by the decreased size of the pollen transport networks (compared to visitation networks) we also included network size (i.e. the total number of plant and insect species) as a covariate in each model. Then we analyzed the effects of network types on the number of network hubs, module hubs, connectors and peripheral species by means of different GLMMs fitted using poisson error distributions and log link functions.

At the plant species level, we performed GLMMs with a gaussian (identity link function) and gamma (log link function) distribution to assess the effects of network types (fixed effect) on d' and species contribution to nestedness, respectively. In these models, each plant species in each specific network was included as a separate value ($n = 841$). The survey nested within site and plant species identity were included as random effects. For insects, we tested the effects of network type, insect taxonomic group and their interaction on d' and species contribution to nestedness using GLMMs with a gamma (log link function) and gaussian (identity link function) distribution, respectively. In these models, each insect species in each specific network was included as a separate value ($n = 2218$). Both, the survey nested within site and insect species identity were included as random effects.

To evaluate whether the proportion of interactions in which insects carried pollen of the visited plants (i.e.

interactions from which pollen was carried effectively/all the interactions observed in the field) differed among taxonomic groups (i.e. bees, other Hymenoptera – ants and wasps –, Coleoptera, Diptera and Lepidoptera), we performed a GLMM with a binomial error distribution and a logit link function. Members of Order Hemiptera were excluded from this analysis because we collected only three species. In this model, the taxonomic group to which each insect species was assigned was set as a categorical explanatory variable, insect abundance (i.e. the total number of insect individuals collected) as a covariate, and site and insect species identity as random factors.

We fitted all GLMMs with the 'glmer' function from the lme4 package (Bates et al. 2014) in R. None of the binomial or poisson models showed overdispersion (Zuur et al. 2009). Full models were simplified by sequentially eliminating non-significant terms and interactions ($p > 0.05$) to establish reduced models following standard, stepwise, deletion procedures. The significance of the fixed effects and their interactions were established using a likelihood ratio test (Zuur et al. 2009). We performed post hoc multiple comparisons among all five insect taxonomic groups (bees, other Hymenoptera, Coleoptera, Diptera and Lepidoptera) using the 'glht' function of the multcomp package (Hothorn et al. 2008). Means are accompanied by their SE throughout the text.

Data deposition

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.7ms1bb5>> (Zhao et al. 2018).

Results

In total, our field surveys recorded 6004 individual, plant–flower visitor interactions involving 355 insect species (Diptera 162; Hymenoptera 121; Lepidoptera 43; Coleoptera 26; Hemiptera 3) and 103 identified plant species (Fig. 1, Supplementary material Appendix 1 Table A1, A2). There was a grand total representing 1622 specific interactions (links; i.e. unique interactions between one plant species and one flower visitor species) within all four sites (Fig. 1). Of the 355 insect species identified, a total of 27 (8%) species classified as cheaters on all plant species they visited due to the complete absence of the pollen of visited plants on study samples. For each site, more than half (57–67%) of the flower visiting species were classified as potential pollinators on all the plant species they visited. In contrast, 27–33% of flower visiting species were classified as potential pollinators for some plant species but cheaters for other plant species. Only 3–16% of all flower-visiting species were cheaters on all plant species they visited (Fig. 2a). The percentage of antagonistic interactions between cheaters and plant species compared to the total unique plant–flower visitor interactions within each site was 11–17% (Fig. 2b).

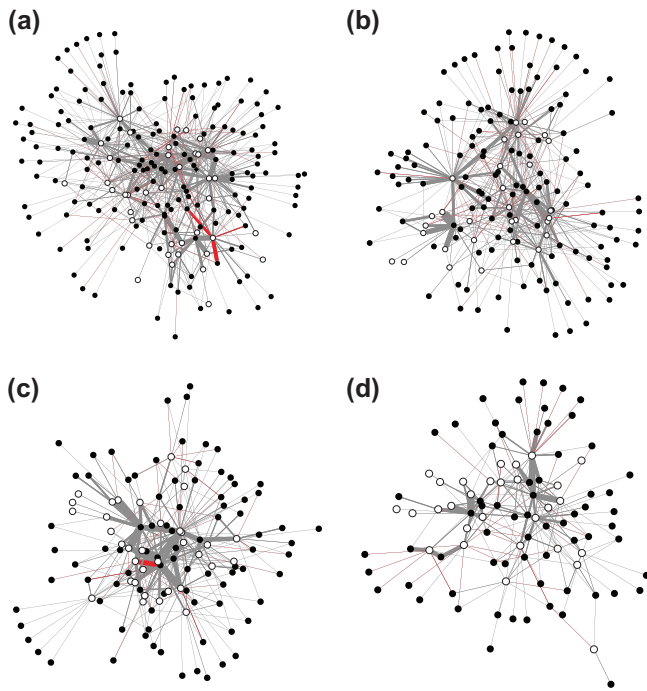


Figure 1. Quantitative visitation networks (gray and red interactions) and pollen transport networks (gray interactions) at four sites on the Yulong Mountain, SW China. (a): YSZ, 2725 m; (b) HLG, 3235 m; (c): YK, 3670 m; (d) DYE, 3910 m. The networks show data pooled across all surveys for each elevation. For each network open circles represent plant species while filled circles represent insect species. Line width is proportional to the frequency of interactions. Gray lines show interactions with insects that carried pollen of the visited plants on their bodies. Red lines show interactions with insects that did not carry pollen of the visited plants. This figure was drawn using the Fruchterman–Reingold algorithm in *sna* package (Butts 2016) in R.

Topological differences between visitation and pollen transport networks

Plant species richness did not differ significantly between the visitation networks and the pollen transport networks ($\chi^2=0.63$, $df=1$, $p=0.43$). However, there were significantly higher insect richness in visitation compared to pollen transport networks ($\chi^2=7.04$, $df=1$, $p=0.008$).

All 64 visitation and pollen transport networks were significantly specialized (Supplementary material Appendix 1 Table A3). However, the degree of specialization changed considerably from visitation to pollen transport networks. Pollen transport networks showed significantly higher specialization ($\Delta H_2'$) than visitation networks ($\chi^2=25.28$, $df=1$, $p<0.001$; Fig. 3a).

For binary networks, 11 were significantly modular in both visitation and pollen transport networks, five were not significant in visitation but were significant in pollen transport networks. The other 16 did not show significant modular structure in any network type (Supplementary material Appendix 1 Table A3). In contrast, all of the 64 quantitative visitation and pollen transport networks showed significant

modular structure (Supplementary material Appendix 1 Table A3). Both qualitative modularity (ΔM) and quantitative modularity (ΔQ) were significantly lower in the visitation networks than in the pollen transport networks ($\chi^2\geq 7.42$, $df=1$, $p\leq 0.006$; Fig. 3b, c). However, the number of modules in both qualitative and quantitative networks did not differ significantly between visitation and pollen transport networks ($\chi^2\leq 0.71$, $df=1$, $p\geq 0.40$) after accounting for variation in network size ($\chi^2\geq 4.16$, $df=1$, $p\leq 0.04$).

For binary networks, 23 were significantly nested in both visitation and pollen transport networks, four were significantly nested in visitation but were not in pollen transport networks, whereas five did not show a significant nested structure in any network type (Supplementary material Appendix 1 Table A3). None of the quantitative visitation and pollen transport networks showed significantly nested structure (Supplementary material Appendix 1 Table A3). The qualitative nestedness values ($z\text{-NODF}$) were significantly higher in the visitation networks than in the pollen transport networks ($\chi^2=22.52$, $df=1$, $p<0.001$; Fig. 3d). In contrast, the quantitative nestedness values ($z\text{-WNODF}$) did not differ significantly between the visitation networks and the pollen transport networks ($\chi^2=0.22$, $df=1$, $p=0.64$).

Comparison of species roles between visitation and pollen transport networks

At the plant species level, we found significantly lower specialization values (d') in visitation compared with their values in pollen transport networks ($\chi^2=5.16$, $df=1$, $p=0.02$; Supplementary material Appendix 1 Fig. A3a). For insect species, there were no significant differences in d' between the two network types ($\chi^2=3.30$, $df=1$, $p=0.069$; Supplementary material Appendix 1 Fig. A3b). However, d' for insect species differed significantly among insect taxonomic groups ($\chi^2=11.03$, $df=4$, $p=0.03$). Bees were more specialized (0.35 ± 0.01) than Diptera (0.25 ± 0.007). Coleoptera, other Hymenoptera, and Lepidoptera showed intermediate levels of specialization at 0.32 ± 0.02 , 0.30 ± 0.02 , and 0.33 ± 0.02 , respectively (Table A4 for Post hoc analyses in Supplementary material Appendix 1). There was no significant two-way interaction between network type and insect taxonomic group affecting specialization of insect species ($\chi^2=0.91$, $df=4$, $p=0.92$).

Three topological roles (module hub, connector and peripheral) were detected in the significantly modular, binary networks. However, we did not detect network hubs in any network (none had $c>0.62$ and $z>2.5$). For insect species, 41 shifted roles from visitation to pollen transport networks in the 11 pairs of significantly modular networks. Most shifts (61%) were from important roles to peripheral roles (connector to peripheral: 15 species, module hub to peripheral: 10 species). Furthermore, 39% of shifts were from peripheral roles to important roles (peripheral to connector: 15 species, peripheral to module hub: one species). The number of module hubs ($\chi^2=1.44$, $df=1$, $p=0.23$; Supplementary material Appendix 1 Fig. A3c) and connectors ($\chi^2=0.01$, $df=1$,

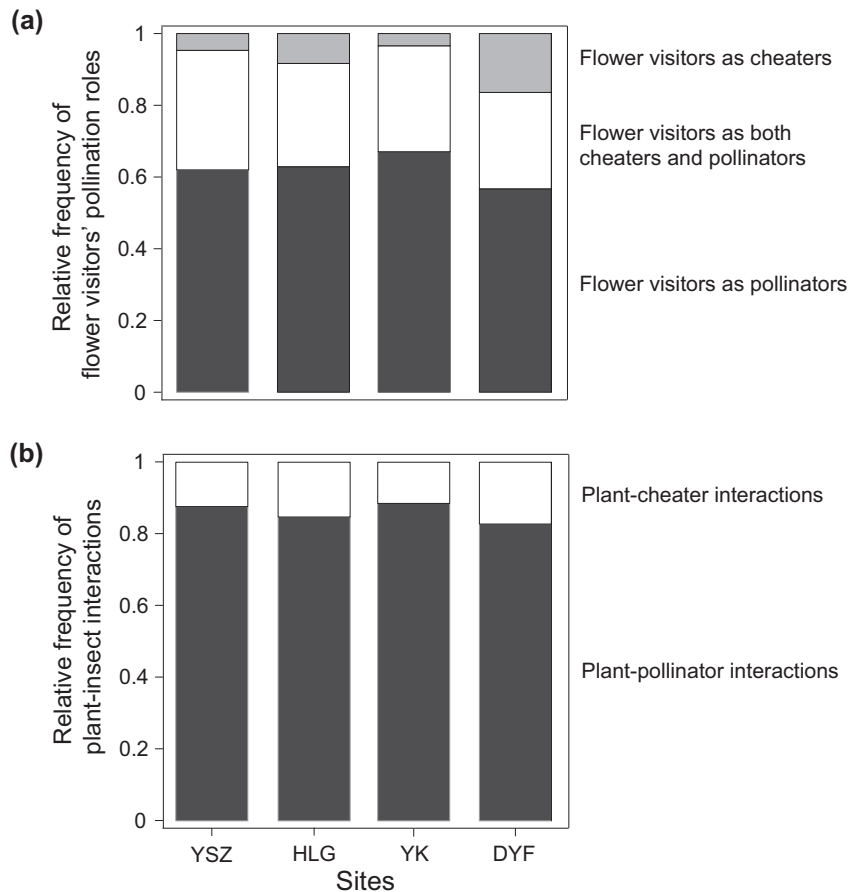


Figure 2. The relative frequency of (a) flower visitors' roles and (b) interaction types between plants and flower visitors at four sites on the Yulong Mountain, SW China.

$p=0.94$; Supplementary material Appendix 1 Fig. A3d) did not differ significantly between the two network types. However, the number of peripheral species tended to be higher in visitation networks compared to pollen transport networks ($\chi^2=3.51$, $df=1$, $p=0.06$; Supplementary material Appendix 1 Fig. A3e), because the 56 cheaters and nine plants found only in visitation networks were all peripheral species.

The contribution of plant species to nestedness did not differ significantly between the visitation networks and the pollen transport networks ($\chi^2=0.09$, $df=1$, $p=0.77$; Supplementary material Appendix 1 Fig. A3f). In contrast, the contribution of insect species to nestedness was higher in visitation networks compared to pollen transport networks ($\chi^2=106.17$, $df=1$, $p<0.001$; Supplementary material Appendix 1 Fig. A3g). In addition, the contribution of insect species to nestedness also showed significant differences among insect taxonomic groups ($\chi^2=12.67$, $df=4$, $p=0.01$). Post hoc analysis (Supplementary material Appendix 1 Table A5) revealed that this was the result of a higher contribution to nestedness in Lepidoptera (0.84 ± 0.07) compared to bees (0.38 ± 0.05) and Coleoptera (0.51 ± 0.08); while other Hymenoptera and Diptera showed intermediate values of contribution to nestedness (0.70 ± 0.08 , and

0.59 ± 0.04 , respectively; Supplementary material Appendix 1 Table A5). There was no significant two-way interaction between network type and insect taxonomic group affecting the contribution of insect species to nestedness ($\chi^2=5.13$, $df=4$, $p=0.27$). Most insect species (88%) occurring only in the visitation networks showed a positive contribution to nestedness, indicating that these species enhanced the nested pattern.

Comparison of insect taxonomic groups carrying pollen of the visited plants

The proportion of plant–insect interactions that resulted in insect-mediated pollen dispersal from total, unique, plant–insect interactions differed significantly among insect taxonomic groups ($\chi^2=32.04$, $df=4$, $p<0.001$) after accounting for variation in abundance ($\chi^2=20.26$, $df=1$, $p<0.001$). Unsurprisingly, bees (0.93 ± 0.01) carried the highest proportion of pollen of the visited plants. The second highest was for members of the Order Coleoptera (0.88 ± 0.03), followed by other Hymenoptera (0.88 ± 0.03), and Diptera (0.84 ± 0.01). Members of Order Lepidoptera (0.74 ± 0.03) carried the lowest proportion of pollen of the visited plants (Fig. 4).

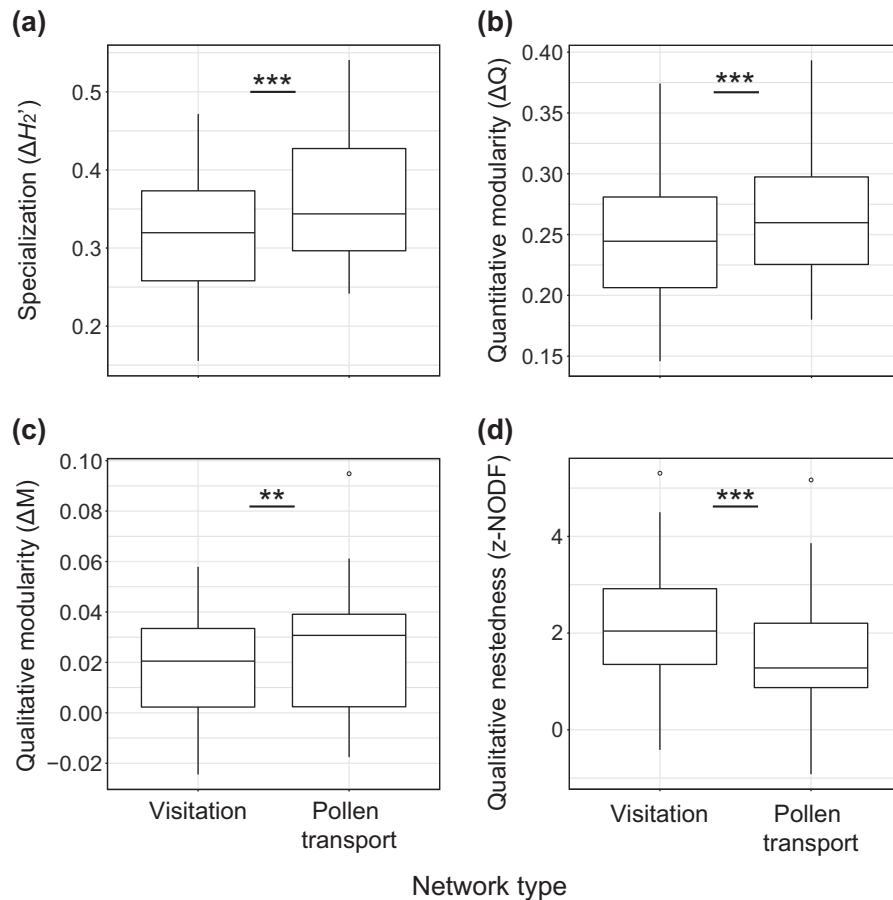


Figure 3. Box plots illustrating the network structure metrics in visitation networks and pollen transport networks. (a) Network-level specialization ($\Delta H_2'$), (b) quantitative modularity (ΔQ), (c) qualitative modularity (ΔM) and (d) qualitative nestedness ($z\text{-NODF}$) for each network types. The horizontal lines across boxes are medians. The bottom and top limits of each box are the lower and upper quartiles (25% and 75%, respectively). Asterisks denote significant statistical differences (p -values): ** $p \leq 0.01$, *** $p \leq 0.001$.

Discussion

In this study, we used a large number of networks created across four sites, and in nine temporally distinct sampling periods, to compare network properties between visitation and pollen transport networks. Excluding cheaters from the visitation networks increased specialization and modularity whereas nestedness decreased. Therefore, these results only partially supported our first prediction on the impact of network types on network topology indicating that true plant–pollinator interactions are more specialized than plant–visitor interactions observed in the field. In line with our second prediction, we found that the roles of cheaters could explain the variation of network structure between visitation and pollen transport networks. Also, we have demonstrated that there were significant differences in the likelihood to carry pollen of the visited plants among the different taxonomic groups of insect visitors. Aligning with our third prediction, bees carried the highest proportion of the visited plants' pollen at all four sites while Lepidoptera were the least effective. This means that removing cheaters from the visitation networks will have a different impact on

network topology depending on the composition of insect communities at each site. Below, we discuss the results in light of our three predictions acknowledging their limitations but highlighting their implications in future studies of mutualistic networks.

Topological differences between visitation and pollen transport networks and the species roles in them

In this study, the exclusion of cheater–plant interactions changed the architecture of the mutualistic networks. As it has been shown in other studies (Alarcón 2010, Popic et al. 2013), species richness decreased significantly when cheaters were excluded from the visitation networks. Some studies have demonstrated that removing cheaters from visitation networks could result in a significant increase in quantitative network specialization (H_2' ; Alarcón 2010, Popic et al. 2013, Ballantyne et al. 2015). Consistent with these studies, in our communities the pollen transport networks exhibited higher specialization compared to the visitation networks. This is because plant species, but not insect species, become more specialized when excluding antagonistic interactions. Thus,

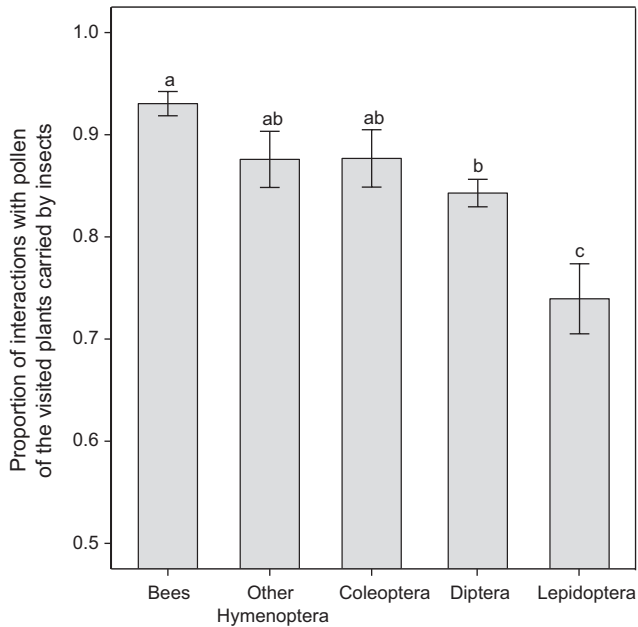


Figure 4. Mean (\pm SE) proportion of interactions with pollen of the visited plants carried by insects in different groups of flower visitors. Different letters above the bars indicate significant differences among taxonomic groups.

our findings indicate that the use of visitation networks may underestimate the levels of specialization inherent to plant–pollinator interactions.

Previous studies have shown that modularity and the number of modules tended to increase with network size in mutualistic networks (Olesen et al. 2007, Trøjelsgaard and Olesen 2013). Therefore, we hypothesized that the exclusion of cheaters from visitation networks may result in a loss of or decrease in modularity in interaction networks (Genini et al. 2010, Maruyama et al. 2015). In contrast, we found a lower number of networks with a significant modular structure among the visitation networks than among the pollen transport networks. In addition, both qualitative and quantitative modularity were lower in the visitation networks than in the pollen transport networks. The roles of species in the network relate to their contribution to the overall network structure (Coux et al. 2016). In this study the number of topological important species (i.e. module hubs and connectors) did not differ significantly between visitation and pollen transport networks, while the number of peripheral species tended to be higher in visitation networks compared to pollen transport networks. This suggests that the differences in modularity between these two network types were mainly caused by variation in peripheral species rather than topological important species. Indeed, all the cheaters found only in visitation networks were peripheral species (Fig. 1). Specifically, they were species with very few interactions so their exclusion played an important role in increasing the modularity of pollen transport networks.

Previous studies reported that nestedness values remained relatively unchanged when comparing visitation and pollen transport networks (Alarcón 2010, Popic et al. 2013). In our study, the quantitative nestedness (z-WNODF) did not differ significantly between visitation networks and pollen transport networks. This indicates that cheaters have only a minor influence on quantitative nestedness as cheaters most probably lacked strong interactions with the plants they visited. However, qualitative nestedness (z-NODF) decreased significantly from visitation networks to pollen transport networks. One explanation for this change in qualitative nestedness was that the contribution to nestedness of insect species occurring in both network types was higher in visitation networks than in pollen transport networks. This difference in nestedness between the two network types could also be at least partly explained by the topological role of cheaters and those plant species visited only by cheaters. As for specialist species in visitation networks, most of them tended to interact with generalist species and this made a positive contribution to nestedness. Of the five insect taxonomic groups, Lepidoptera played an important role in the variation of nestedness because they had the highest proportion of cheater–plant interactions and made the highest contribution to nestedness. Therefore, network metrics might be more biased when estimated by using visitation networks in communities with a higher proportions of species in the Order Lepidoptera.

Overall, removing cheater–plant interactions using pollen load analyses is a useful approach to increase the accuracy of pollination networks. However, we admit that this approach has some limitations. First, although SEM is better than light microscopy to identify pollen to the species level (Jones and Bryant 2007), we could not discriminate between the pollen grains of some closely related species (e.g. *Gentiana* spp., *Pedicularis* spp. and *Ligularia* spp.). In our study, this could be solved only partially by considering the spatiotemporal distribution of each plant species in our communities. For example, a pollen morphotype was considered to belong to a particular plant species in a community when its flowering phenology and spatial distribution did not overlap with those of its congeners. The second limitation of using pollen analysis to discriminate the roles of flower visitors as potential pollinators is that it may treat some cheaters as pollinators since it records pollen transport without showing which insect species consistently deposit viable grains on receptive stigmas. Flower visitors that are most effective at pollen removal may merely represent guilds of pollen robbers (Wilson and Thomson 1991, Aigner 2001, Hargreaves et al. 2009). More controlled experiments to determine the number of viable pollen grains deposited on receptive stigmas by different flower visitors would help to define pollinators versus foragers (King et al. 2013). Third, the network construction approach used in this study neglects that individual insects often vary in foraging behaviors and resource use within a population (Dupont et al. 2014, Tur et al. 2015). For example, a bee species may carry pollen of visited

plants during a pollen foraging bout (act as a pollinator) but fail to carry pollen of the same plant species during a nectar foraging bout (act as a cheater). Consequently, our approach could treat some important pollinators as cheaters if pollen analysis is limited to the capture of species taken during nectar foraging bouts. A high sampling intensity, while using the number of insect individuals captured carrying a given pollen morphotype as the interaction frequency could increase the accuracy of interactions in quantitative pollen transport networks (Ramírez-Burbano et al. 2017).

Comparison of insect taxonomic groups carrying pollen of the visited plants

Any study of pollination networks must first discriminate between effective pollinators and cheaters. Some researchers contend that the identification and location of pollen on a flower visitor's body helps to provide a more accurate and consistent way of determining whether it may function as a pollinator (Forup and Memmott 2005, Alarcón 2010, Popic et al. 2013, Zhao et al. 2016). Alarcón (2010) studied pollen transport networks over two consecutive years in a montane meadow community of southern California. The author found that 23–29% of plant–flower visitor interactions were between cheaters and plants. In our four communities cheater–plant interactions varied from 12 to 17% largely matching Alarcón (2010). In both studies Lepidoptera played the least effective roles as agents of pollen of the visited plants compared to bees. However, compared to the Californian community, a higher proportion of Diptera carried pollen of the visited plants in our Himalayan communities. In addition to show higher pollen dispersal, our specimens of Diptera dominated communities at higher elevations (Fig. 1). This parallels observations at higher elevations on other continents that true flies provide key pollination services in subalpine and alpine meadows (Arroyo et al. 1982, Larson et al. 2001).

Conclusions

The use of pollen analysis permitted detection of a high proportion of antagonistic interactions between cheaters and plants in visitation networks. The inclusion of these antagonistic interactions could overestimate nestedness while underestimating specialization and modularity in pollination networks. This is, in part, because all cheaters in this study were peripheral species and most of them contributed positively to the nested structure by interacting with generalist species. Our results indicate that pollen load analysis is another practical approach to better identify and define the role of flower visitors. The construction of pollen transport networks using both field observations of floral visitors and pollen analysis information could improve our understanding of mutualistic interactions along elevation gradients in montane communities.

Acknowledgements – We thank Prof. Zhi-Shu Xiao for helpful comments. We thank Prof. Ke-Ke Huo and Dr. Gan-Yan Yang for identifying insect specimens. We thank Dr. Ming-Ying Zhang and Mr. Hui Tang for assistance in the field. We are grateful to Dr. Peter Bernhardt, Alan Moss and Dr. Gerardo Camilo (all at Saint Louis University) for addressing English composition and grammar.

Funding – This study was supported by Strategic Priority Research Program of the Chinese Academy of Sciences (XDB31020000), National Key Basic Research Program of China (2014CB954100), Joint Fund of the National Natural Science Foundation of China–Yunnan Province (U1502261), Major International Joint Research Project of NSF China (31320103919), Applied Fundamental Research Foundation of Yunnan Province (2014GA003), National Natural Science Foundation of China (31700361), Yunlin Scholarship of Yunnan Province to H. Wang (YLXL20170001) and CAS ‘Light of West China’ Program to Y.H. Zhao. A. Lázaro was supported by a Ramón Cajal contract financed by the Spanish Ministry of Economy and Competitiveness.

References

- Aigner, P. A. 2001. Optimality modeling and fitness tradeoffs: when should plants become pollinator specialists? – *Oikos* 95: 177–184.
- Alarcón, R. 2010. Congruence between visitation and pollen-transport networks in a California plant–pollinator community. – *Oikos* 119: 35–44.
- Arroyo, M. T. K. et al. 1982. Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. – *Am. J. Bot.* 69: 82–97.
- Ballantyne, G. et al. 2015. Constructing more informative plant–pollinator networks: visitation and pollen deposition networks in a heathland plant community. – *Proc. R. Soc. B* 282: 20151130.
- Bascompte, J. and Jordano, P. 2007. Plant–animal mutualistic networks: the architecture of biodiversity. – *Annu. Rev. Ecol. Evol. Syst.* 38: 567–593.
- Bascompte, J. et al. 2003. The nested assembly of plant–animal mutualistic networks. – *Proc. Natl Acad. Sci. USA* 100: 9383–9387.
- Bates, D. et al. 2014. lme4: linear mixed-effects models using ‘Eigen’ and S4. – R package ver. 1.1–12. <<http://CRAN.R-project.org/package=lme4>>.
- Blüthgen, N. et al. 2006. Measuring specialization in species interaction networks. – *BMC Ecol.* 6: 9.
- Bosch, J. et al. 2009. Plant–pollinator networks: adding the pollinator’s perspective. – *Ecol. Lett.* 12: 409–419.
- Butts, C. T. 2016. sna: tools for social network analysis. – R package ver. 2.4. <<https://cran.r-project.org/web/packages/sna/>>.
- Chamberlain, S. A. et al. 2014. How context dependent are species interactions? – *Ecol. Lett.* 17: 881–890.
- Chittka, L. et al. 1999. Flower constancy, insect psychology and plant evolution. – *Naturwissenschaften* 86: 361–377.
- Coux, C. et al. 2016. Linking species functional roles to their network roles. – *Ecol. Lett.* 19: 762–770.
- Cuartas-Hernández, S. and Medel, R. 2015. Topology of plant–flower-visitor networks in a tropical mountain forest: insights on the role of altitudinal and temporal variation. – *PLoS One* 10: e0141804.

- Dalsgaard, B. et al. 2017. Opposed latitudinal patterns of network-derived and dietary specialization in avian plant–frugivore interaction systems. – *Ecography* 40: 1395–1401.
- Dattilo, W. et al. 2016. Unravelling Darwin's entangled bank: architecture and robustness of mutualistic networks with multiple interaction types. – *Proc. R. Soc. B* 283: 20161564.
- Dormann, C. F. et al. 2008. Introducing the bipartite package: analysing ecological networks. – *R News* 8: 8–11.
- Dormann, C. F. and Strauss, R. 2014. A method for detecting modules in quantitative bipartite networks. – *Methods Ecol. Evol.* 5: 90–98.
- Dupont, Y. L. et al. 2014. Spatial structure of an individual-based plant–pollinator network. – *Oikos* 123: 1301–1310.
- Forup, M. L. and Memmott, J. 2005. The restoration of plant–pollinator interactions in hay meadows. – *Restor. Ecol.* 13: 265–274.
- Forup, M. L. et al. 2008. The restoration of ecological interactions: plant–pollinator networks on ancient and restored heathlands. – *J. Appl. Ecol.* 45: 742–752.
- Genini, J. et al. 2010. Cheaters in mutualism networks. – *Biol. Lett.* 6: 494–497.
- Genrich, C. M. et al. 2017. Duality of interaction outcomes in a plant–frugivore multilayer network. – *Oikos* 126: 361–368.
- Gibson, R. H. et al. 2006. Pollinator webs, plant communities and the conservation of rare plants: arable weeds as a case study. – *J. Appl. Ecol.* 43: 246–257.
- Guimera, R. and Amaral, L. A. N. 2005. Functional cartography of complex metabolic networks. – *Nature* 433: 895–900.
- Guimarães, P. R. and Guimarães, P. 2006. Improving the analyses of nestedness for large sets of matrices. – *Environ. Model. Softw.* 21: 1512–1513.
- Hargreaves, A. L. et al. 2009. Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. – *Biol. Rev.* 84: 259–276.
- Hothorn, T. et al. 2008. Simultaneous inference in general parametric models. – *Biometrical J.* 50: 346–363.
- Inouye, D. W. 1980. The terminology of floral larceny. – *Ecology* 61: 1251–1253.
- Irwin, R. E. et al. 2010. Nectar robbing: ecological and evolutionary perspectives. – *Annu. Rev. Ecol. Evol. Syst.* 41: 271–292.
- Jędrzejewska-Szmek, K. and Zych, M. 2013. Flower-visitor and pollen transport networks in a large city: structure and properties. – *Arthropod Plant Interact.* 7: 503–516.
- Jones, E. I. et al. 2015. Cheaters must prosper: reconciling theoretical and empirical perspectives on cheating in mutualism. – *Ecol. Lett.* 18: 1270–1284.
- Jones, G. D. and Bryant, V. M. Jr. 2007. A comparison of pollen counts: light vs. scanning electron microscopy. – *Grana* 46: 20–33.
- King, C. et al. 2013. Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. – *Methods Ecol. Evol.* 4: 811–818.
- Larson, B. M. H. et al. 2001. Flies and flowers: taxonomic diversity of anthophiles and pollinators. – *Can. Entomol.* 133: 439–465.
- Lopezaraiza-Mikel, M. E. et al. 2007. The impact of an alien plant on a native plant–pollinator network: an experimental approach. – *Ecol. Lett.* 10: 539–550.
- Maruyama, P. K. et al. 2015. Nectar robbery by a hermit hummingbird: association to floral phenotype and its influence on flowers and network structure. – *Oecologia* 178: 783–793.
- Mayfield, M. M. et al. 2001. Exploring the 'most effective pollinator principle' with complex flowers: bumblebees and *Ipomopsis aggregata*. – *Ann. Bot.* 88: 591–596.
- Memmott, J. 1999. The structure of a plant–pollinator food web. – *Ecol. Lett.* 2: 276–280.
- Michener, C. D. 2007. *The bees of the world*. – The Johns Hopkins Univ. Press.
- Olesen, J. M. et al. 2011. Missing and forbidden links in mutualistic networks. – *Proc. R. Soc. B.* 278: 725–732.
- Olesen, J. M. et al. 2007. The modularity of pollination networks. – *Proc. Natl Acad. Sci. USA* 104: 19891–19896.
- Ollerton, J. et al. 2007. Multiple meanings and modes: on the many ways to be a generalist flower. – *Taxon* 56: 717–728.
- Patefield, W. M. 1981. An efficient method of generating random RxC tables with given row and column totals. – *Appl. Stat.* 30: 91–97.
- Pocock, M. J. O. et al. 2012. The robustness and restoration of a network of ecological networks. – *Science* 335: 973–977.
- Popic, T. J. et al. 2013. Flower–visitor networks only partially predict the function of pollen transport by bees. – *Austral Ecol.* 38: 76–86.
- Rader, R. et al. 2011. Pollen transport differs among bees and flies in a human-modified landscape. – *Divers. Distrib.* 17: 519–529.
- Ramírez-Burbano, M. B. et al. 2017. The role of the endemic and critically endangered colorful puffleg *Eriocnemis mirabilis* in plant–hummingbird networks of the Colombian Andes. – *Biotropica* 49: 555–564.
- Saavedra, S. et al. 2011. Strong contributors to network persistence are the most vulnerable to extinction. – *Nature* 478: 233–235.
- Schemske, D. W. and Horvitz, C. C. 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialization. – *Science* 225: 519–521.
- Schleuning, M. et al. 2012. Specialization of mutualistic interaction networks decreases towards tropical latitudes. – *Curr. Biol.* 22: 1925–1931.
- Schleuning, M. et al. 2014. Ecological, historical and evolutionary determinants of modularity in weighted seed-dispersal networks. – *Ecol. Lett.* 17: 454–463.
- Sebastián-González, E. et al. 2015. Macroecological trends in nestedness and modularity of seed-dispersal networks: human impact matters. – *Global Ecol. Biogeogr.* 24: 293–303.
- Thébault, E. and Fontaine, C. 2010. Stability of ecological communities and the architecture of mutualistic and trophic networks. – *Science* 329: 853–856.
- Traveset, A. et al. 2015. Global patterns of mainland and insular pollination networks. – *Global Ecol. Biogeogr.* 25: 880–890.
- Trøjelsgaard, K. and Olesen, J. M. 2013. Macroecology of pollination networks. – *Global Ecol. Biogeogr.* 22: 149–162.
- Tur, C. et al. 2015. Increasing modularity when downscaling networks from species to individuals. – *Oikos* 124: 581–592.
- Tylianakis, J. M. and Morris, R. J. 2017. Ecological networks across environmental gradients. – *Annu. Rev. Ecol. Evol. Syst.* 48: 25–48.
- Vizentin-Bugoni, J. et al. 2016. Influences of sampling effort on detected patterns and structuring processes of a Neotropical plant–hummingbird network. – *J. Anim. Ecol.* 85: 262–272.

- Waser, N. M. et al. 1996. Generalization in pollination systems, and why it matters. – *Ecology* 77: 1043–1060.
- Wilson, P. and Thomson, J. D. 1991. Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. – *Ecology* 72: 1503–1507.
- Zanata T. B. et al. 2017. Global patterns of interaction specialization in bird–flower networks. – *J. Biogeogr.* 44: 1891–1910.
- Zhao, Y. H. et al. 2016. Floral traits influence pollen vectors' choices in higher elevation communities in the Himalaya–Hengduan Mountains. – *BMC Ecol.* 16: 26.
- Zhao, Y. H. et al. 2018. Data from: the topological differences between visitation and pollen transport networks: a comparison in species rich communities of the Himalaya–Hengduan Mountains. – Dryad Digital Repository, <<http://dx.doi.org/10.5061/dryad.7ms1bb5>>.
- Zuur, A. F. et al. 2009. Mixed effects models and extensions in ecology with R. – Springer.
- Zych, M. 2006. On flower visitors and true pollinators: the case of protandrous *Heracleum sphondylium* L. (Apiaceae). – *Plant Syst. Evol.* 263: 159–179.