

Plant-pollinator networks worldwide are composed by the same specific building blocks

Jose B. Lanuza^{1,2} | Alfonso Allen-Perkins^{2,3} | Romina Rader¹ | Ignasi Bartomeus²

1 School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2350, Australia.

2 Estación Biológica de Doñana (EBD-CSIC), E-41092 Seville, Spain.

3 Departamento de Ingeniería Eléctrica, Electrónica, Automática y Física Aplicada, ETSIDI, Universidad Politécnica de Madrid, 28040 Madrid, Spain.

Abstract

Ecological processes leave distinct structural imprints on species interactions shaping the topology of mutualistic networks. Detecting those relationships is not trivial since they go beyond pair-wise interactions, but may get blurred when considering full network descriptors. However, recent work has shown the network meso-scale can capture this important information. The meso-scale describes network subgraphs representing patterns of interactions between a small number of species (i.e. motifs) and those constitute the building blocks of the whole network. Here, we have compiled 60 networks from 18 different studies and show that some motifs are consistently under- and over-represented worldwide, suggesting that the building blocks of plant-pollinator networks are not random. Interestingly, different functional groups are associated to specific motif position across both trophic levels. HERE I WOULD PUT AN EXAMPLE OF E.G. FAN POSITIONS, DEFINED BY, TEND TO BE OCCUPIED BY BEES AND WHATEVER TYPE OF PLANTS, RESPECTIVELY. This findings open the door to predict how novel interactions will be formed within a community context.

INTRODUCTION

Ecological communities are formed by a plethora of interacting species that form networks of interactions. Because of the tremendous complexity of these ecological networks, species interactions are generally condensed in metrics that summarize this information (Guimarães 2020). Plant-pollinator interactions are no exception, and they are often studied with indices that aggregate the information at the species (micro-scale) or network level (macro-scale). For example, plant-pollinator network level approaches have identified common invariant structural properties across networks, including a degree distribution that decays as a power law (Jordano 1987), nestedness (Bascompte et al. 2003), or modularity (Olesen et al. 2007). In addition, the local species position within the network can define its degree of specialization (Blüthgen, Menzel, and Blüthgen 2006) or its role connecting the rest of the community (Olesen et al. 2007). Despite the unquestionable progress with the use of these metrics at both species and network level, condensing the information into a single metric

implies the loss of relevant ecological information that obscures the understanding of the ecological processes (Cirtwill et al. 2018; Simmons, Cirtwill, et al. 2019).

Traditionally, plant-pollinator research has focused on direct interactions but overlooked indirect interactions (i.e., the mediated effect between two species by a third species), such as facilitative or competitive interactions between plants for pollinators (Moeller 2004; Sargent and Ackerly 2008). Despite the widespread nature of indirect interactions in ecological communities (Strauss 1991), plant-pollinator research often fails to finely capture those indirect interactions with the conventional analytical tools that condense the information either by species (e.g., interaction frequency) or in global topological indices (e.g., nestedness). Nonetheless, the emerging framework of network motifs (small sub-networks recorded within a given network) in plant-pollinator research allows to consider both direct and indirect interactions (Simmons, Cirtwill, et al. 2019). Motifs are often referred to as the building blocks of a network that depict subsets of interactions (Milo et al. 2002). The analysis of network motifs in plant-pollinator networks have revealed that the different ecological processes that govern species interactions (e.g., species abundances versus trait-matching) can lead to different patterns of indirect interactions (Simmons et al. 2020). Yet, the global patterns of indirect interactions in real plant-pollinator networks are still unknown (e.g., over- and under- represented motifs).

Despite motifs are abstract representations often decoupled from species ecology, linking the structural properties of the meso-scale with the species ecology can help the understanding of ecological processes. For instance, different motifs can have different ecological meanings (Simmons, Cirtwill, et al. 2019) and the position within a motif can determine the species functional role (Stouffer et al. 2012; Baker et al. 2015). However, it is unclear how the species ecology and life history traits determines the species' functional role within the network of interactions (Coux et al. 2016). For example, large pollinators can forage larger distances (Greenleaf et al. 2007), deposit greater pollen quantities (Földesi et al. 2021) and handle complex zygomorphic flowers in comparison with small pollinators that are restricted to lower floral complexity (Gong and Huang 2009). How this different pollinator behavior translates into their interaction topology is unknown. Similarly, recent empirical findings indicate that the meso-scale is the best descriptor of plant reproductive success (Allen-Perkins et al. 2021), but little is known about how plants reproductive strategies shape their position within the network of interactions. Although some studies have evaluated plant reproductive strategies in plant-pollinator networks (Tur, Castro-Urgal, and Traveset 2013; Lázaro et al. 2020), this is an often overlooked aspect in a community context (Devaux, Lepers, and Porcher 2014) and rarely incorporated into plant-pollinator network studies. Hence, exploring how the main plant reproductive strategies integrate with the emergent motif framework can shed light on key aspects of ecosystem functioning.

Here, we used 60 plant-pollinator networks from 18 different studies and 14 countries, alongside functional

groups of plants and floral visitors based on functional traits and taxonomic rank, respectively. To obtain plant functional groups, we used a comprehensive dataset that included floral, reproductive and vegetative traits compiled in a larger set of networks. Floral visitor functional groups were divided by the main taxonomic groups that differed in life form and behaviour. Once we split the different plant-pollinator networks into their motif elements, we explored: (i) if there is a common invariant structural property in the overall motif networks (i.e. over and under-represented motifs); (ii) which functional groups are over and under-represented in the different motif positions; and, (iii) if there are over and under-represented functional group combinations within a motif.

METHODS

Plant-pollinator studies

We have compiled 60 plant-pollinator networks from 18 different studies (**Table S1**). All studies sampled plant-pollinator interactions in natural systems and were selected based on wide geographical coverage and presence of interaction frequency as a measure of interaction strength. In total, there were 503 plant species, 1,111 floral visitors species and 6,248 pairwise interactions registered. For ease of data manipulation, plant and floral visitors species names were standardize with the help of the R package *taxize* version *0.9.99* (Chamberlain et al. 2020). All analyses and data manipulation were conducted in R *version 4.0.5* (R Core Team 2021).

Plant and floral visitors functional groups

First, plant species were grouped through hierarchical clustering into the optimal number of functional groups that summarized the main plant reproductive strategies. For this, we used the trait dataset collated in Chapter 1 that comprised a total of 1506 species including the 503 species considered in this study (Supplementary material Chapter 1 **Table S1**). This dataset consisted on 8 floral, 4 reproductive and 3 vegetative traits excluding traits with high percentage of missing values (over 30%; **Table S2**). We opted to calculate the plant functional groups on this larger set of species because of the higher accuracy when delimiting functional groups with that many variables and species (Dolnicar et al. 2014). To feed the clustering analysis, we calculated the distance between the different qualitative and scaled quantitative variables with Gower distance (Gower 1971). For this, we used the function *gowdis* with method *ward.D2* from the package *FD* version *1.0-12* (Laliberté et al. 2014). Finally, we conducted hierarchical clustering with the function *hclust* from the R stats package version *4.0.5* and calculated the optimal number of clusters with the function *kgs* from the package *maptree* version *1.4-7* (White and Gramacy 2009).

Second, floral visitors were grouped into functional groups based on taxonomic rank as done similarly in past plant-pollinator studies (Fenster et al. 2004; Ollerton et al. 2009). We opted to divide floral visitors on the taxonomic rank level and not with functional traits because the main orders of floral visitors differed in form and behaviour and had lower superior taxonomical complexity (i.e., floral visitors had 6 orders versus plants that had 38). Thus, this allowed us to group floral visitors into functional groups that represented adequately the main life strategies of the possible pollinators: (i) bees (Hymenoptera-Anthophila), (ii) non-bee Hymenoptera (Hymenoptera-non-Anthophila), (iii) syrphids (Syrphidae-Diptera), (iv) non-syrphids-Diptera, (v) Lepidoptera and (vi) Coleoptera. However, a minor set of species belonged to other functional groups that were considered in analyses but not discussed further because of their low representation in the full set of networks (3.55% of the total interactions recorded). These functional groups were ‘lizards’, ‘birds’ and ‘other insects’. This last group was formed by a mix of uncommon insect taxa on the full set of networks.

Overall motif patterns

Following previous work (Simmons, Cirtwill, et al. 2019; Simmons et al. 2020), we broke down the plant-pollinator networks into their constituent motifs. Prior to analyses, we turned the quantitative networks into qualitative (or binary) ones, where interactions are present or absent. To test the effect of singletons, we ran all analysis to the same set of networks, but considering only interactions whose frequency was greater than one (64.98% of interactions). Results are qualitatively consistent and are not further discussed in the main text (**Figures S1 and S2**).

We calculated the frequency of all motifs up to five nodes (see **Figure 1**) for each empirical network, by using the *bmotif* package version 2.0.2 (Simmons, Sweering, et al. 2019). To control for variation in network size, motif frequencies were normalised as a proportion of the total number of motifs within each motif class (i.e., the number of nodes a motif contains). Like Simmons et al. (2020), we just used five-node motifs in our analyses for visualisation, interpretation and computational reasons. In addition, we also excluded two-node motifs (or links) from our analyses because their normalised frequencies would always equal one.

To assess the significance of the observed frequencies, we created 1,000 simulated networks for each binary network using the *nullmodel* function and the *vaznull* model in the *bipartite* package version xxx (Dormann et al. 2009). Generated networks had the same number of plants and floral visitors, as well as the same connectance of their corresponding empirical networks. After extracting the motif frequencies from the simulated networks, for each motif type and empirical network, we calculated the percentage of simulated networks whose frequencies were smaller than the ones observed, that is, we estimated the percentile of the observed motif frequencies. Motifs whose percentile is close to 0 or 100 are under- or over-represented in



Figure 1. Adapted figure of Simmons et al., 2019 with all the possible motifs from two to five species in bipartite networks. There is a total of 17 possible motifs with 46 different positions denoted within each node.

the empirical networks, respectively, and thus they cannot be predicted by connectance and the number of species alone. To summarize general patterns across networks, we used an intercept-only linear mixed model (LMM) per motif with the help of the package lmer version 1.1-21 (Bates et al. 2015), where the response variable was the observed motif percentile per network. In these models, we used the study identifiers in **Table S1** as a random intercept. By doing so, we obtained estimates of the average motif frequency, but controlling the variation at the study level.

Over and under-represented functional groups on motif positions

We calculated which functional groups were over or under-represented in different motif positions by comparing position frequencies of empirical networks with those of their corresponding simulated counterparts. To estimate the position frequencies of each functional group in a given network, we added the frequencies of those species that belong to the group, and then, we normalised the resulting frequencies by dividing the position measure for each group by the total number of times that a group appears in any position within the same motif size class. Then, we calculated the percentile of the observed position frequencies for each group and network, just like we did with motif frequencies. To outline the general patterns of position frequencies across networks and functional groups, we fit a LMM per motif position, where the response variable was the observed position percentile per network. We used the functional group identifier as an explanatory variable

and the study identifiers as a random intercept. By adding the functional group estimates to the model intercept, we assessed the average motif frequency, after controlling the variation at the study level. Finally, we visualized with the help of the package *ComplexHeatmap* version 2.6.2 (Gu, Eils, and Schlesner 2016) over- and under- representation of plant and pollinator functional groups on the different motif positions.

Over- and under-represented functional group combinations of motifs

Finally, we studied which combinations of functional groups tend to appear together within the same motifs (up to five nodes). That is which combinations are over or under-represented. This analysis use 57 out of the 60 networks available due to computational limitations to identify all the nodes in the motifs of the three networks with the highest number of links. To do so, for each of the 53,250 possible motif combinations, we estimated the observed and the expected probability of finding those combination in empirical networks, respectively. Then, we determined whether the observed probabilities are likely to come from the expected probabilities or not. To calculate the observed probability (p_i^O) of functional groups within a motif i (e.g., motif 3, ‘bee’ + ‘bee’ + ‘selfing herbs’), we divided the number of times that i appears in our set of empirical networks (n_i^O) by the sum of the number of times that each possible combination appeared: $p_i^O = n_i^O / \sum_{k=1}^{53,250} n_k^O$. To estimate the expected probability of a given motif combination i , p_i^E , firstly, we calculated the probability of finding a given functional group x in the position α of i , $p_i(x, \alpha)$. Then, by assuming the independency of $p_i(x, \alpha)$, we computed the expected probability of the combination i as the product of the probability of its pairs (x, α) , that is, $p_i^E = \prod_{(x, \alpha) \in i} p_i(x, \alpha)$. To obtain $p_i(x, \alpha)$, we proceeded as follows. First, we calculated the number of times that the functional group x appears in the position α in each empirical network η , denoted as $n_i^\eta(x, \alpha)$. Then, by controlling the variation at the network level, we assessed the average value of the absolute frequency of functional group x appears in the position α , $n_i(x, \alpha) = E[n_i^\eta(x, \alpha)]$, and estimated $p_i(x, \alpha)$ as $p_i(x, \alpha) = n_i(x, \alpha) / \sum_k n_i(k, \alpha)$. To obtain $n_i(x, \alpha)$, we fitted a LMM per motif position, where the response variable was the number of times that a given position was observed per network, the explanatory variable was functional group identifier, and the random intercept was given by network identifiers nested within the study identifiers.

Once we obtained p_i^O and p_i^E , we used a simulation approach to determine whether the former is likely to come from the latter or not. This approach was preferred since the large number of possible motif combinations and the small probabilities for some of them advise against using an exact test of goodness-of-fit or a Chi-square one. Specifically, we created 1,000 random samples with repetition of possible motif combinations, where each sample contained 10 million elements and, for each combination, the probability of being selected was equal to its expected probability. From those random samples, we extracted the mean and the standard deviation of the expected probability of i , denoted as $E[p_i^E]$ and $\sigma[p_i^E]$, respectively, and calculated the z-scores

of p_i^O as $z_i^O = (p_i^O - E[p_i^E]) / \sigma[p_i^E]$, for those motif combinations with $p_i^O > 0$. According to the usual interpretation of z-scores, combinations with $z_i^O > 1.96$ are over-represented, whereas those with $z_i^O < -1.96$ are under-represented, at the 95% confidence level. Notice that we focused on combinations with $p_i^O > 10^{-7}$ because, beyond the interest that can arouse the combinations that appear at most once, we do not have enough numerical resolution to accurately detect whether or not those combinations are under-represented (due to the limited size of our random samples).

RESULTS

Plant and floral visitor functional groups

The hierarchical cluster analysis divided the dataset into five different groups with different and overlapping characteristics (**Figure S3** and **Figure S4**). The first group referred to as ‘selfing herbs’ consisted on herbs with hermaphroditic flowers with high levels of autonomous selfing. The second group named ‘small outcrossing perennials’ had small perennial species with a mixed of life forms (i.e., trees shrubs and herbs) with outcrossing hermaphroditic flowers. The third group referred to as ‘self-incompatible perennials with large flowers’ comprised perennial species with a mixed of life forms and large self-incompatible hermaphroditic flowers with high number of ovules. The fourth group named ‘tall plants with small unisexual flowers’ had the tallest species, highest proportion of shrub and tree life forms, dioecious and monoecious breeding systems, small flowers and the highest numbers of flowers and inflorescences per plant. Finally, the fifth group named ‘short-lived outcrossers with long zygomorphic flowers’ consisted on small perennial and short-lived herbs with long self-compatible zygomorphic flowers that were unable to self-pollinate.

In total, there were 1,126 species of floral visitor with 6,325 interactions recorded with plants. Most plants interacted with bees (2,256 interactions) and non-syrphid Diptera (1,768) followed by syrphids (845), Lepidoptera (437), Coleoptera (432) and non-bee Hymenoptera (362).

Overall motif patterns

Most motifs were under- and over-represented (close to the 1st and 99th percentile, respectively) in the comparison between empirical and simulated networks (**Figure 2**). Motifs 3, 5, 9, 10 and 14 were under-represented in empirical networks, that is, all were close to the 1st percentile and under the 25th percentile. Interestingly, four out of five of these motifs belonged to the largest path length classification (i.e., core-peripheral). In addition, motifs 2, 6, 7, 16 and 17 were over-represented, all over the 75th percentile and thus the closest to the 99th percentile. In contrast to the under-represented motifs, over-represented motifs belonged to the two shortest path length groups (i.e., complete and fan). The remaining motifs (i.e., 4, 8, 11,

12, 13, and 15) were between the 25th and 75th percentile. Notably, the exclusion of singletons from this analysis did not change the observed results (**Figure S1**).

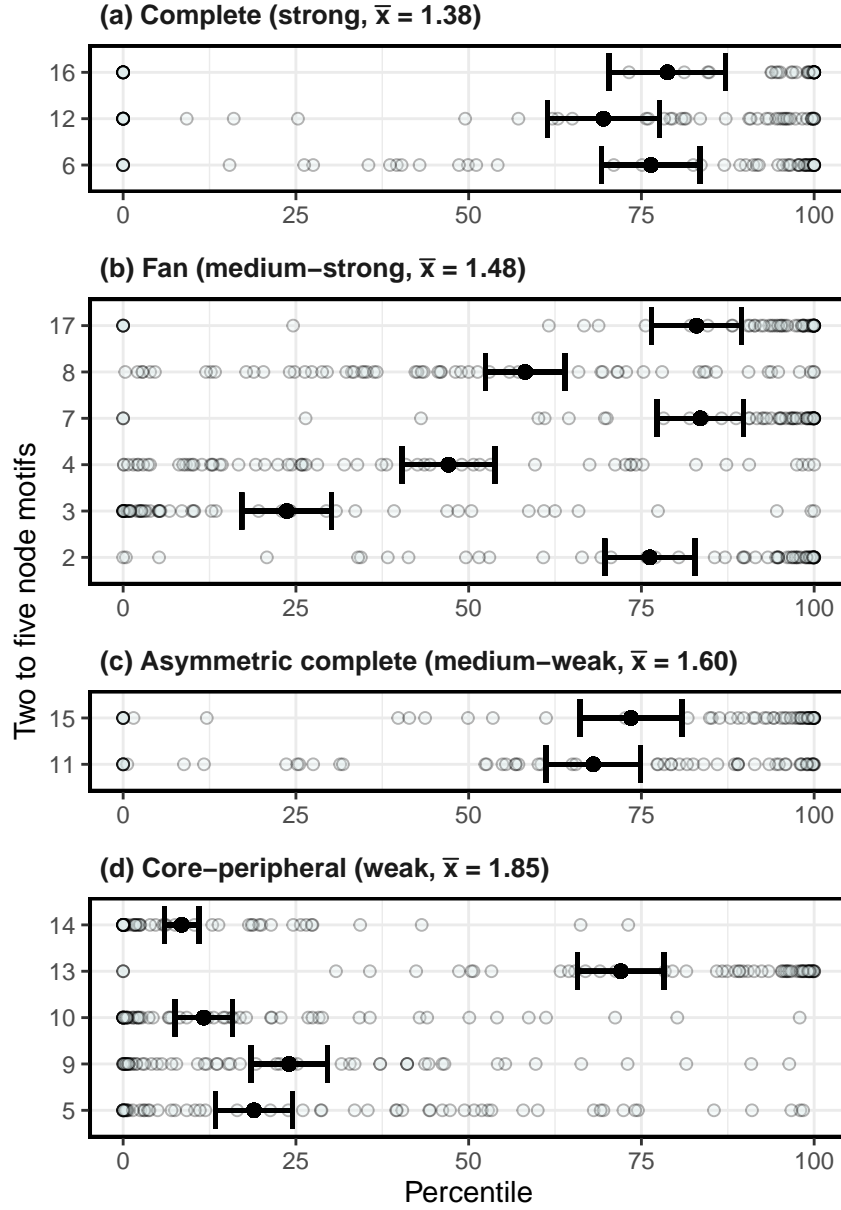


Figure 2. Comparison of motif frequencies between empirical and simulated networks grouped by average path length (plots a, b, c and d) as determined in Simmons et al. (2020). This is shown with the mean percentage of motif frequencies in empirical networks that were over the motif frequencies of the simulated ones (percentiles). This was done by network (light blue dots) and then averaged for all networks (black dots with error bars that correspond to the standard deviation).

Over and under-represented functional groups on motif positions

The comparison of the functional group frequencies per motif position between empirical and simulated networks showed a 6.36% and 25.38% of over- ($>75^{th}$) and under- ($<25^{th}$) represented functional groups in

the different positions, respectively (**Figure 3**). Floral visitors showed a total of 4.55% and 32.58% of over- and under-represented functional groups in the different positions and plants 8.18% and 18.18% of over and under-represented groups, respectively. Notably, the differences across functional groups were more marked for floral visitors than for plant functional groups (the differences between min and max percentiles per position were generally two-three times larger for floral visitors). From most over- to under-represented floral visitors functional groups on the different motif positions (indicated with the dendrogram order in **Figure 3**), we found: bees, non-syrphids Diptera, syrphids, Coleoptera, non-bee Hymenoptera and Lepidoptera. Although plant functional groups showed less differences between them, there were also more represented functional groups than others, thus from most over- to under-represented groups on the different motif positions we found: self incompatible perennials with large flowers, small outcrossing perennials, tall plants with unisexual flowers, selfing herbs and short lived outcrossers with long zygomorphic flowers.

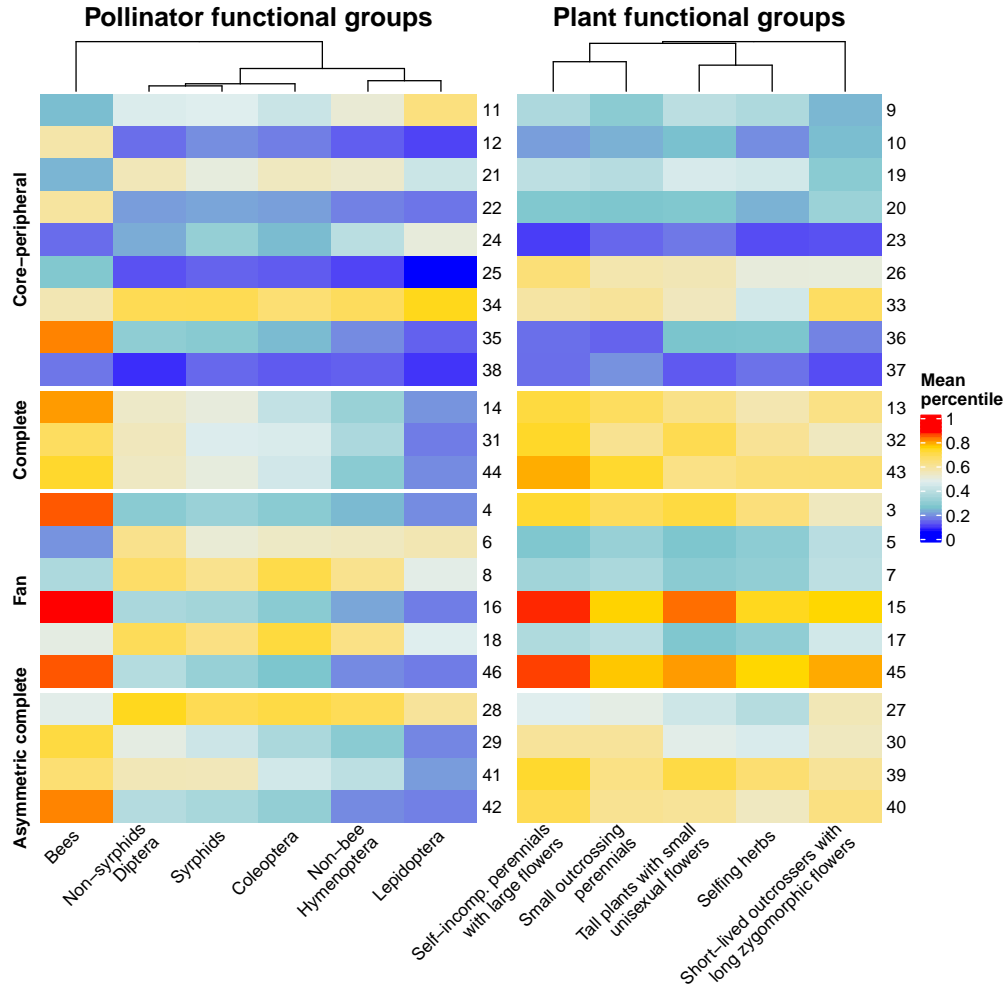


Figure 3. Heatmap indicating under- and over- representation of pollinator and plant functional groups in the different motif positions. The different motif positions are divided by the average path length clasification determined by Simmons et al. (2020). The superior dendrogram indicates the differences across groups with the more separated groups showing larger differences.

Over- and under-represented functional group combinations of motifs

The different observed frequencies on the different motif positions showed a hierarchical order of probabilities for floral visitors (**Figure 4**). That is, the functional group of ‘bees’ was the most abundant one on all motif positions followed by non-syrphid Diptera which was after bees, the second most frequent functional group on all positions. Then, the group of syrphids was the most abundant in all positions but two (positions 28 and 31 from motifs 11 and 12, respectively). After syrphids, Coleoptera was the most frequent group on the different positions followed by non-bee Hymenoptera and finally, Lepidoptera which was the less frequent group on all positions but two (positions 12 and 22 from motifs 5 and 9, respectively). Remarkably, plant functional groups were more variable on the different motif positions. However, there were also predominant functional groups found across the different motif positions. The three most frequent groups were ‘tall plants with small unisexual flowers’, ‘self-incompatible perennials with large flowers’ and ‘small outcrossing perennials’. On the contrary, the groups of ‘selfing herbs’ and ‘short lived outcrossers with long zygomorphic flowers’ had the lowest probability to be present on the different motifs plant positions.

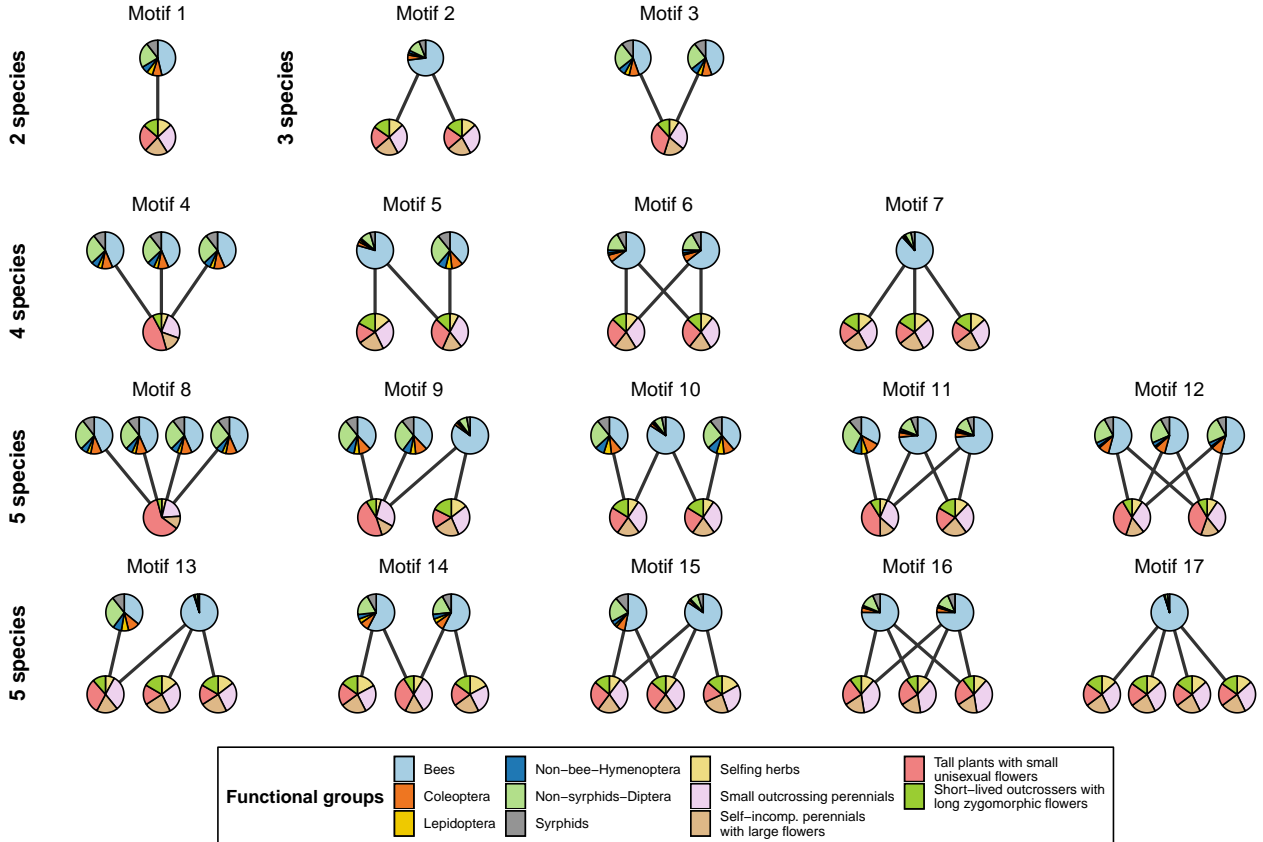


Figure 4. Graphical representation of the probability of finding a given functional group x in the position α of motif i , $p_i(x, \alpha)$, for all the possible motifs from two to five species in bipartite networks. The slices in the nodes for a given functional group x are proportional to the corresponding value of $p_i(x, \alpha)$.

The statistical comparison with z-scores between the observed and expected functional group combinations showed that we can not recover the observed combinations from its probability of occurrence. In fact, most combinations are under- or over- represented and follow a modified Gaussian distribution (Johnson's S_U) with 65% of under-represented group combinations, 20% of no statistical difference and 15% of under-represented ones (**Figure 5**). In addition, motifs with small node combinations (i.e., 2 and 3 nodes) only appeared as under-represented. Motifs with 4 and 5-node combinations appeared in the three statistical categories but 4 node motifs had a higher proportion in the under-represented category (10% in comparison with the 1% and the 3% of the no statistical difference and over-represented categories, respectively). Finally, 5-node motifs were in similar proportions in the no statistical difference and over-represented categories (99% and 97%, respectively) but they appeared in lower proportion in the under-represented category (89%). When, we explore the identity on the different positions of the most probable motif combinations we find as expected that the most common functional groups rule these positions on the most probable motif combinations (lower panel **Figure 5**).

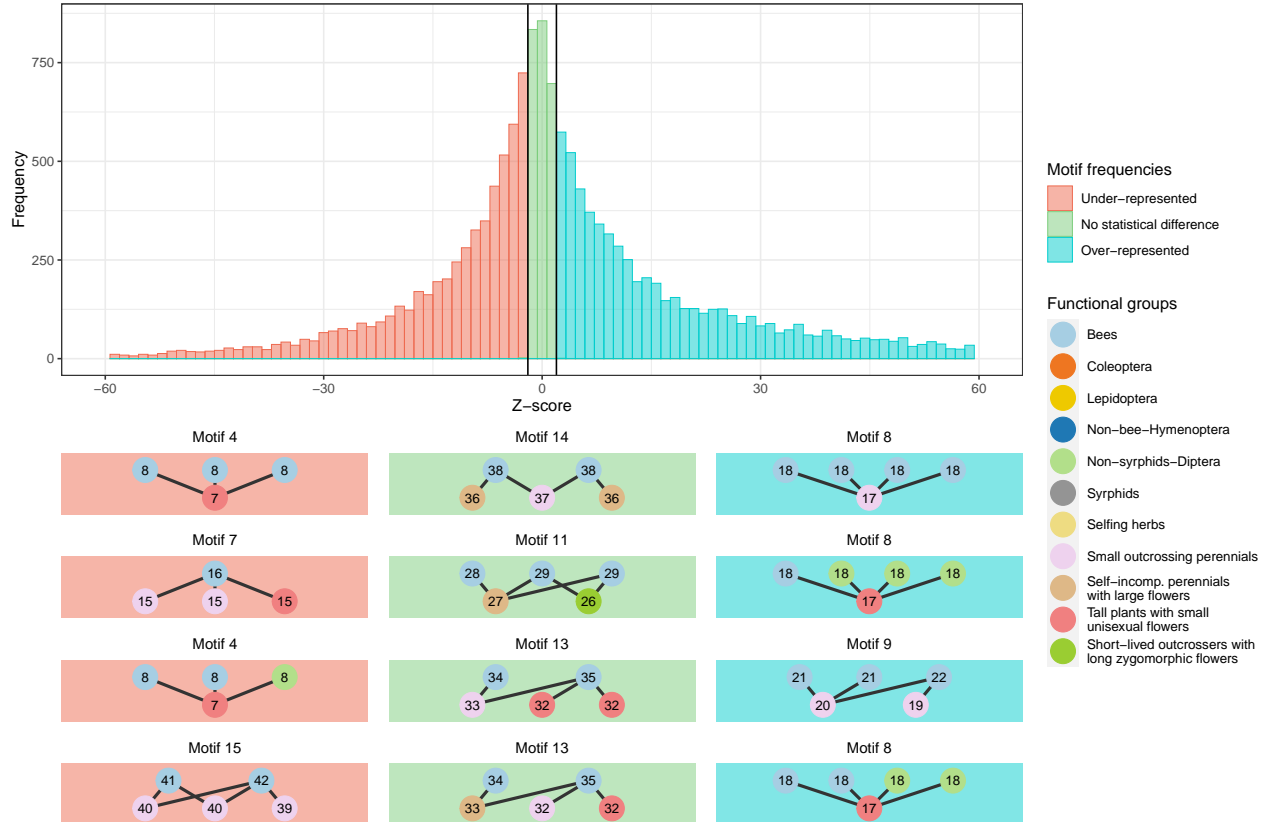


Figure 5. Z-score comparison (95% confidence level) of the observed and expected frequencies of motif combinations with four specific graphical examples of the most probable motifs of being observed for each category delimited by the z-score critical values: under-represented, no statistical difference and over-represented.

DISCUSSION

Understanding the global patterns of network structure is key to unravel the processes that govern community dynamics (Bascompte and Jordano 2007; Bastolla et al. 2009; Guimarães 2020). However, common analytical approaches are unable to capture the full complexity of ecological systems (Simmons, Cirtwill, et al. 2019). Because network motifs can capture both direct and indirect interactions, by analysing over- and under-representation of motifs we are able to explore in greater detail ecological interactions. We have explored for the first time the overall structure and representation of network motifs in real plant-pollinator networks and shown that the different motifs up to five nodes are consistently over- and under-represented in comparison with simulated networks indicating the presence of ecological processes shaping their structure. Despite the presence of multiple ecological processes likely operating at the same time (e.g., neutral effects, morphological matching or phenological overlap), we found a tendency for over-represented structures with more densely connected motifs (i.e., complete, fan and asymmetric complete) suggesting that neutral effects are likely the predominant processes governing the structure of these network motifs (Simmons et al. 2020). Thus, as indicated for other network properties like nestedness (Krishna et al. 2008; Suweis et al. 2013), species relative abundances (i.e., neutral effects) are possibly one of the main determinants of indirect interactions in real plant-pollinator networks.

In accordance with the findings of network motif patterns, we found higher representation of plant and pollinator functional groups in complete, fan and asymmetric complete motifs (more densely connected) in contrast to core-peripheral motifs (less densely connected) that were under-represented. As expected, the functional group of bees (the one with the highest number of interactions) was the most abundant in the different network motif positions. This is likely to be the case for most plant-pollinator systems as bees are generally the main functional group visiting flowers in plant-pollinator communities (Ollerton 2017). Interestingly, bees were highly over-represented in fan motif positions 4, 16 and 46 (**Figure 3**) which are associated with the highest levels of generalism. Further, this generalist tendency was also observed at motif level (e.g., **Figure 2** comparison of the percentiles between mirror image motifs in fan positions that involve pollinator specialization and generalism). Despite this in good agreement with the current view of the generalist nature of pollinators (Waser et al. 1996; Olesen and Jordano 2002), we found that this over-representation of the generalist network motifs was mainly driven by bees while the rest of functional groups tended to be under-represented in these generalist motifs. Further, unlike floral visitors, plant functional groups showed little differences across them on the different motif positions. However, the group of self incompatible perennials with large flowers, one of the groups that is expected to have higher levels of outcrossing rate (Goodwillie et al. 2010), also showed over-representation on the same generalist fan motif of

bees (**Figure 3**).

showed also high over-representation on the same fan generalist motifs where the bees were over-represented (**Figure 3** positions x and x).

- 3) Ecology is complex, combinations not recovered by positions. This is a very cool result, because imply that knowing the probabilities of each position is not enough to predict network realizations.
- 4) Limitations (but keep it short and simple): FG are hard to describe, bias in sampling worldwide, ...
 - FG hard to describe: <https://doi.org/10.1093/aob/mcp031> and the fenster one
 - Paper of Poisot 2020
- 5) Future direction and conclusions (also short). This opens the door to help predicting novel interactions beyond pairwise interactions. We not only know now how the building blocks are structures, but also that who conform this building blocks is not random.

REFERENCES

- Allen-Perkins, Alfonso, Maria Hurtado, David Garcia-Callejas, Oscar Godoy, and Ignasi Bartomeus. 2021. "Community Level Individual-Based Plant-Pollinator Networks Unveil Pollen Flow Dynamics and Plant Reproductive Success." *bioRxiv*. Cold Spring Harbor Laboratory.
- Baker, Nick J, Riikka Kaartinen, Tomas Roslin, and Daniel B Stouffer. 2015. "Species' Roles in Food Webs Show Fidelity Across a Highly Variable Oak Forest." *Ecography* 38 (2). Wiley Online Library: 130–39.
- Bascompte, Jordi, and Pedro Jordano. 2007. "Plant-Animal Mutualistic Networks: The Architecture of Biodiversity." *Annu. Rev. Ecol. Evol. Syst.* 38. Annual Reviews: 567–93.
- Bascompte, Jordi, Pedro Jordano, Carlos J Melián, and Jens M Olesen. 2003. "The Nested Assembly of Plant–animal Mutualistic Networks." *Proceedings of the National Academy of Sciences* 100 (16). National Acad Sciences: 9383–7.
- Bastolla, Ugo, Miguel A Fortuna, Alberto Pascual-García, Antonio Ferrera, Bartolo Luque, and Jordi Bascompte. 2009. "The Architecture of Mutualistic Networks Minimizes Competition and Increases Biodiversity." *Nature* 458 (7241). Nature Publishing Group: 1018–20.
- Bates, Douglas, Martin Mächler, Ben Bolker, and Steve Walker. 2015. "Fitting Linear Mixed-Effects Models

- Using lme4.” *Journal of Statistical Software* 67 (1): 1–48. doi:10.18637/jss.v067.i01.
- Blüthgen, Nico, Florian Menzel, and Nils Blüthgen. 2006. “Measuring Specialization in Species Interaction Networks.” *BMC Ecology* 6 (1): 9. doi:10.1186/1472-6785-6-9.
- Chamberlain, Scott, Eduard Szoecs, Zachary Foster, Zebulun Arendsee, Carl Boettiger, Karthik Ram, Ignasi Bartomeus, et al. 2020. *Taxize: Taxonomic Information from Around the Web*.
- Cirtwill, Alyssa R, Tomas Roslin, Claus Rasmussen, Jens Mogens Olesen, and Daniel B Stouffer. 2018. “Between-Year Changes in Community Composition Shape Species’ Roles in an Arctic Plant–pollinator Network.” *Oikos* 127 (8). Wiley Online Library: 1163–76.
- Coux, Camille, Romina Rader, Ignasi Bartomeus, and Jason M Tylianakis. 2016. “Linking Species Functional Roles to Their Network Roles.” *Ecology Letters* 19 (7). Wiley Online Library: 762–70.
- Devaux, Celine, Clotilde Lepers, and Emmanuelle Porcher. 2014. “Constraints Imposed by Pollinator Behaviour on the Ecology and Evolution of Plant Mating Systems.” *Journal of Evolutionary Biology* 27 (7). Wiley Online Library: 1413–30.
- Dolnicar, Sara, Bettina Grün, Friedrich Leisch, and Kathrin Schmidt. 2014. “Required Sample Sizes for Data-Driven Market Segmentation Analyses in Tourism.” *Journal of Travel Research* 53 (3). Sage Publications Sage CA: Los Angeles, CA: 296–306.
- Dormann, C. F., J. Frueund, N. Bluethgen, and B. Gruber. 2009. “Indices, Graphs and Null Models: Analyzing Bipartite Ecological Networks.” *The Open Ecology Journal* 2: 7–24.
- Fenster, Charles B., W. Scott Armbruster, Paul Wilson, Michele R. Dudash, and James D. Thomson. 2004. “Pollination Syndromes and Floral Specialization.” *Annual Review of Ecology, Evolution, and Systematics* 35 (1): 375–403. doi:10.1146/annurev.ecolsys.34.011802.132347.
- Földesi, Rita, Brad G Howlett, Ingo Grass, and Péter Batáry. 2021. “Larger Pollinators Deposit More Pollen on Stigmas Across Multiple Plant Species—A Meta-Analysis.” *Journal of Applied Ecology* 58 (4). Wiley Online Library: 699–707.
- Gong, Yan-Bing, and Shuang-Quan Huang. 2009. “Floral Symmetry: Pollinator-Mediated Stabilizing Selection on Flower Size in Bilateral Species.” *Proceedings of the Royal Society B: Biological Sciences* 276 (1675). The Royal Society: 4013–20.
- Goodwillie, Carol, Risa D. Sargent, Christopher G. Eckert, Elizabeth Elle, Monica A. Geber, Mark O. Johnston, Susan Kalisz, et al. 2010. “Correlated Evolution of Mating System and Floral Display Traits in

- Flowering Plants and Its Implications for the Distribution of Mating System Variation.” *The New Phytologist* 185 (1): 311–21. doi:10.1111/j.1469-8137.2009.03043.x.
- Gower, John C. 1971. “A General Coefficient of Similarity and Some of Its Properties.” *Biometrics*. JSTOR, 857–71.
- Greenleaf, Sarah S, Neal M Williams, Rachael Winfree, and Claire Kremen. 2007. “Bee Foraging Ranges and Their Relationship to Body Size.” *Oecologia* 153 (3). Springer: 589–96.
- Gu, Zuguang, Roland Eils, and Matthias Schlesner. 2016. “Complex Heatmaps Reveal Patterns and Correlations in Multidimensional Genomic Data.” *Bioinformatics* 32 (18). Oxford University Press: 2847–9.
- Guimarães, Paulo R Jr. 2020. “The Structure of Ecological Networks Across Levels of Organization.” *Annual Review of Ecology, Evolution, and Systematics* 51. Annual Reviews: 433–60.
- Jordano, Pedro. 1987. “Patterns of Mutualistic Interactions in Pollination and Seed Dispersal: Connectance, Dependence Asymmetries, and Coevolution.” *The American Naturalist* 129 (5). University of Chicago Press: 657–77.
- Krishna, Abhay, Paulo R Guimaraes Jr, Pedro Jordano, and Jordi Bascompte. 2008. “A Neutral-Niche Theory of Nestedness in Mutualistic Networks.” *Oikos* 117 (11). Wiley Online Library: 1609–18.
- Laliberté, Etienne, Pierre Legendre, Bill Shipley, and Maintainer Etienne Laliberté. 2014. “Package ‘Fd’.” *Measuring Functional Diversity from Multiple Traits, and Other Tools for Functional Ecology*.
- Lázaro, Amparo, Carmelo Gómez-Martínez, David Alomar, Miguel A González-Estévez, and Anna Traveset. 2020. “Linking Species-Level Network Metrics to Flower Traits and Plant Fitness.” *Journal of Ecology* 108 (4). Wiley Online Library: 1287–98.
- Milo, Ron, Shai Shen-Orr, Shalev Itzkovitz, Nadav Kashtan, Dmitri Chklovskii, and Uri Alon. 2002. “Network Motifs: Simple Building Blocks of Complex Networks.” *Science* 298 (5594). American Association for the Advancement of Science: 824–27.
- Moeller, David A. 2004. “Facilitative Interactions Among Plants via Shared Pollinators.” *Ecology* 85 (12). Wiley Online Library: 3289–3301.
- Olesen, Jens M, Jordi Bascompte, Yoko L Dupont, and Pedro Jordano. 2007. “The Modularity of Pollination Networks.” *Proceedings of the National Academy of Sciences* 104 (50). National Acad Sciences: 19891–6.
- Olesen, Jens M., and Pedro Jordano. 2002. “Geographic Patterns in PlantPollinator Mutualistic Networks.”

Ecology 83 (9): 2416–24. doi:10.1890/0012-9658(2002)083[2416:GPIPPM]2.0.CO;2.

Ollerton, Jeff. 2017. “Pollinator Diversity: Distribution, Ecological Function, and Conservation.” *Annual Review of Ecology, Evolution, and Systematics* 48. Annual Reviews: 353–76.

Ollerton, Jeff, Ruben Alarcón, Nickolas M Waser, Mary V Price, Stella Watts, Louise Cranmer, Andrew Hingston, Craig I Peter, and John Rotenberry. 2009. “A Global Test of the Pollination Syndrome Hypothesis.” *Annals of Botany* 103 (9). Oxford University Press: 1471–80.

R Core Team. 2021. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>.

Sargent, Risa D, and David D Ackerly. 2008. “Plant–pollinator Interactions and the Assembly of Plant Communities.” *Trends in Ecology & Evolution* 23 (3). Elsevier: 123–30.

Simmons, Benno I, Andrew P Beckerman, Katrine Hansen, Pietro K Maruyama, Constantinos Televantos, Jeferson Vizentin-Bugoni, and Bo Dalsgaard. 2020. “Niche and Neutral Processes Leave Distinct Structural Imprints on Indirect Interactions in Mutualistic Networks.” *Functional Ecology*. Wiley Online Library.

Simmons, Benno I, Alyssa R Cirtwill, Nick J Baker, Hannah S Wauchope, Lynn V Dicks, Daniel B Stouffer, and William J Sutherland. 2019. “Motifs in Bipartite Ecological Networks: Uncovering Indirect Interactions.” *Oikos* 128 (2). Wiley Online Library: 154–70.

Simmons, Benno I, Michelle JM Sweering, Maybritt Schillinger, Lynn V Dicks, William J Sutherland, and Riccardo Di Clemente. 2019. “Bmotif: A Package for Motif Analyses of Bipartite Networks.” *Methods in Ecology and Evolution* 10 (5). Wiley Online Library: 695–701.

Stouffer, Daniel B, Marta Sales-Pardo, M Irmak Sirer, and Jordi Bascompte. 2012. “Evolutionary Conservation of Species’ Roles in Food Webs.” *Science* 335 (6075). American Association for the Advancement of Science: 1489–92.

Strauss, Sharon Y. 1991. “Indirect Effects in Community Ecology: Their Definition, Study and Importance.” *Trends in Ecology & Evolution* 6 (7). Elsevier: 206–10.

Suweis, Samir, Filippo Simini, Jayanth R Banavar, and Amos Maritan. 2013. “Emergence of Structural and Dynamical Properties of Ecological Mutualistic Networks.” *Nature* 500 (7463). Nature Publishing Group: 449–52.

Tur, Cristina, Rocío Castro-Urgal, and Anna Traveset. 2013. “Linking Plant Specialization to Dependence in

Interactions for Seed Set in Pollination Networks.” *PloS One* 8 (10). Public Library of Science: e78294.

Waser, Nikolas M., Lars Chittka, Mary V. Price, Neal M. Williams, and Jeff Ollerton. 1996. “Generalization in Pollination Systems, and Why It Matters.” <https://esajournals.onlinelibrary.wiley.com/doi/abs/10.2307/2265575>.

White, D, and RB Gramacy. 2009. “Maptree: Mapping, Pruning, and Graphing Tree Models. R Package Version 1.4-5.”

SUPPLEMENTARY MATERIAL

TABLES

Table S1 List of plant-pollinator network studies.

Table S2 Type of plant traits used in this study.

FIGURES

Figure S1 Over and under-estimated motif frequencies.

Figure S2 Over and under-estimated functional groups on motif positions.

Figure S3 Plant trait composition of functional groups.

Figure S4 Plant dendrogram with functional groups.

Table S1. List of studies ordered by author with the year of publication, number of contributed networks and digital object identifier

| First author | Year | Number of networks | Country | DOI |
|----------------|------|--------------------|---------------------------------|---|
| Arroyo-Correa | 2019 | 3 | New Zealand | https://doi.org/10.1111/1365-2745.13332 |
| Bartomeus | 2008 | 6 | Spain | https://doi.org/10.1007/s00442-007-0946-1 |
| Bartomeus | 2015 | 16 | Spain | https://github.com/ibartomeus/BeeFunData |
| Bundgaard | 2003 | 1 | Denmark | Unpublished, Master thesis |
| Burkle | 2013 | 1 | United States | https://doi.org/10.1126/science.1232728 |
| Dicks | 2002 | 2 | England | https://doi.org/10.1046/j.0021-8790.2001.00572.x |
| Dupont | 2003 | 3 | Denmark | https://doi.org/10.1111/j.1365-2656.2008.01501.x |
| Elberling | 1999 | 1 | Sweden | https://doi.org/10.1111/j.1600-0587.1999.tb00507.x |
| Fang | 2008 | 1 | China | https://doi.org/10.1111/1749-4877.12190 |
| Inouye | 1988 | 1 | United States | https://doi.org/10.1111/j.1442-9993.1988.tb00968.x |
| Kaiser-Bunbury | 2017 | 8 | Seychelles | https://doi.org/10.1038/nature21071 |
| Kaiser-Bunbury | 2011 | 6 | Seychelles | https://doi.org/10.1111/j.1365-2745.2010.01732.x |
| Kaiser-Bunbury | 2010 | 2 | Mauritius | https://doi.org/10.1016/j.ppees.2009.04.001 |
| Lundgren | 2005 | 1 | Denmark (Greenland) | https://doi.org/10.1657/1523-0430(2005)037[0514:TDAHCW]2.0.CO;2 |
| Olesen | 2002 | 2 | Mauritius and Portugal (Azores) | https://doi.org/10.1046/j.1472-4642.2002.00148.x |
| Peralta | 2006 | 4 | Argentina | https://doi.org/10.1111/ele.13510 |
| Small | 1976 | 1 | Japan | /13960/t4km08d21 |

Table S1. List of studies ordered by author with the year of publication, number of contributed networks and digital object identifier (*continued*)

| First author | Year | Number of networks | Country | DOI |
|--------------|------|--------------------|---------|---|
| Souza | 2017 | 1 | Brazil | https://doi.org/10.1111/1365-2745.12978 |

Table S2. Traits used to delimit the different plant functional groups divided in quantitative and categorical traits.

| Quantitative traits | | Categorical traits | |
|---------------------|--------------------------------|--------------------|-----------------------------|
| Type | Traits | Type | Traits |
| Vegetative | Plant height (m) | Vegetative | Lifepan |
| Floral | Flower width (mm) | Vegetative | Life form |
| Floral | Flower length (mm) | Floral | Flower shape |
| Floral | Inflorescence width (mm) | Floral | Flower symmetry |
| Floral | Style length (mm) | Reproductive | Autonomous selfing |
| Floral | Ovules per flower | Reproductive | Compatibility system |
| Floral | Flowers per plant | Reproductive | Breeding system |
| Reproductive | Autonomous selfing (fruit set) | | |

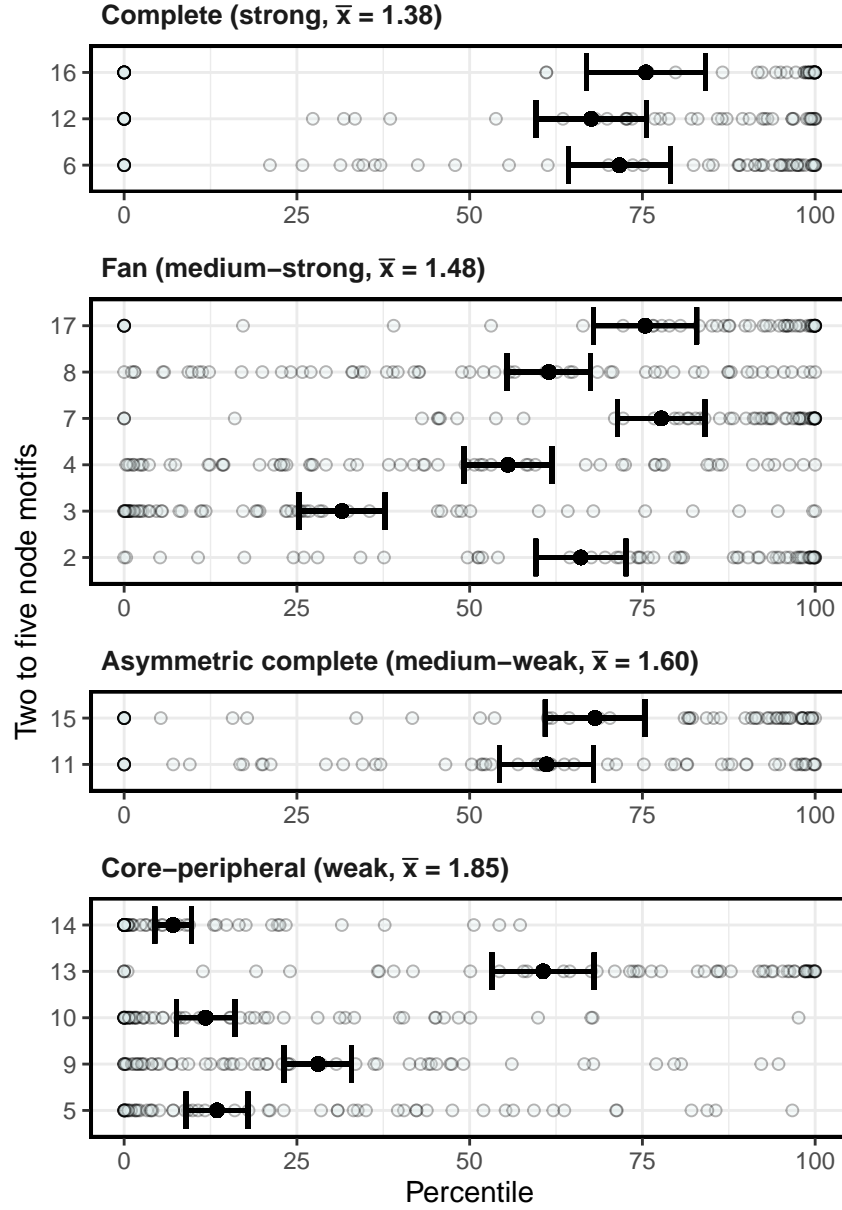


Figure S1. Comparison of motif frequencies between empirical and simulated networks grouped by average path length (plots a, b, c and d) as determined in Simmons et al. (2020) without considering singletons. This is shown with the mean percentage of motif frequencies in empirical networks that were over the motif frequencies of the simulated ones (percentiles). This was done by network (light blue dots) and then averaged for all networks (black dots with error bars that correspond to the standard deviation).

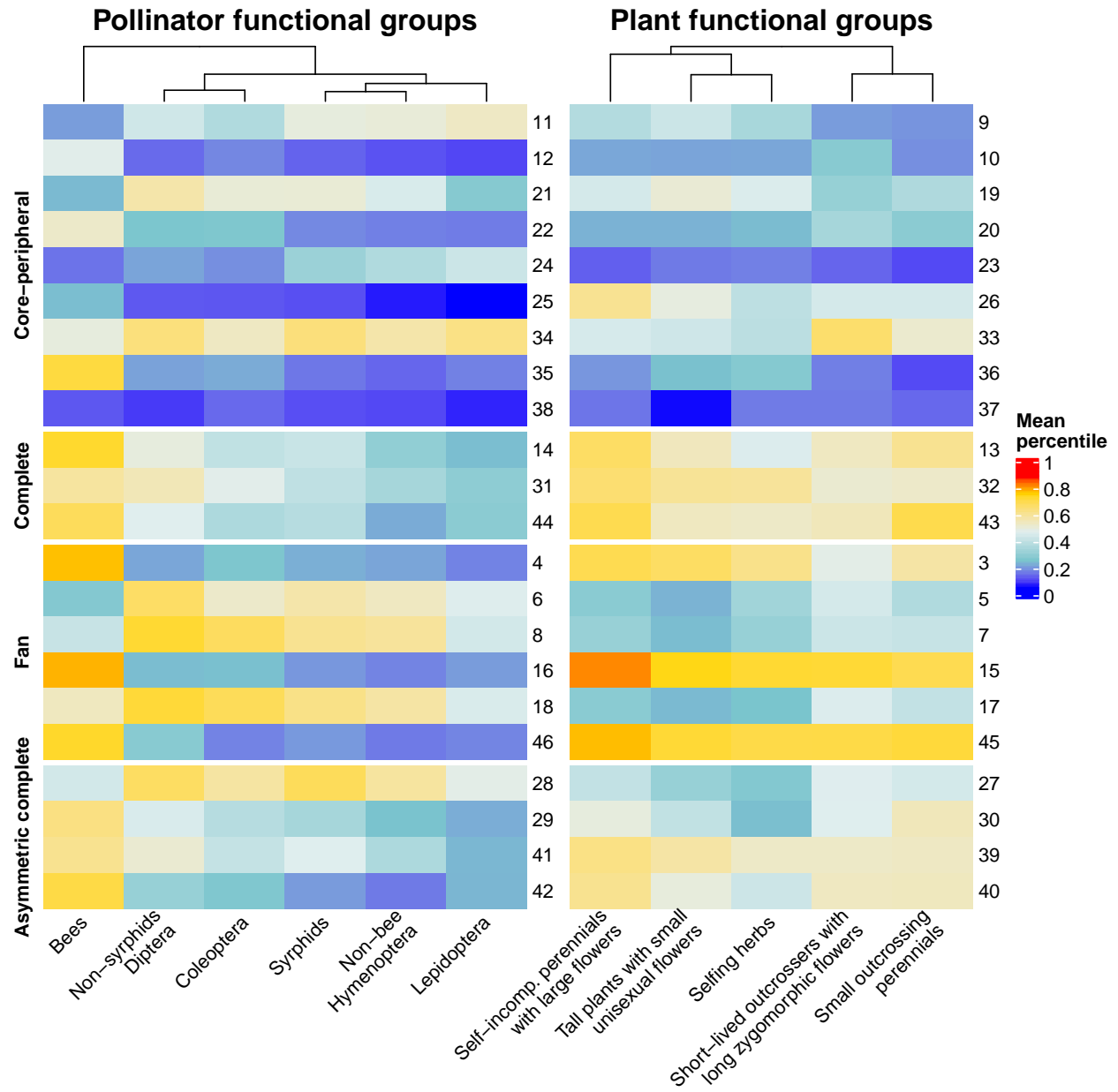


Figure S2. Heatmap indicating under- and over- representation of pollinator and plant functional groups in the different motif positions after removing non-robust links (singletons). The different motif positions are divided by the average path length classification by Simmons et al. (2020). The superior dendrogram indicates the differences across groups with the more separated groups showing larger differences.

Plant functional group composition

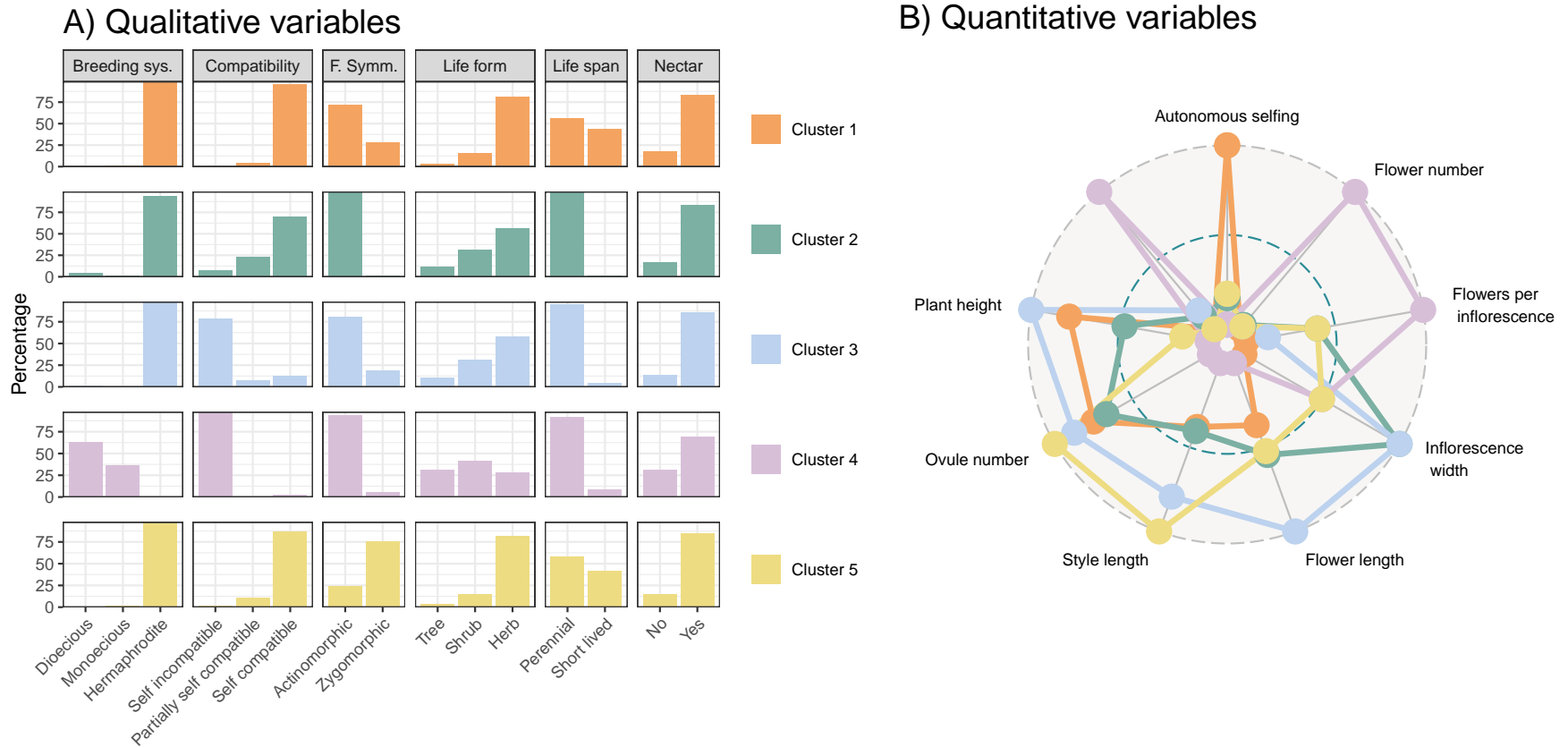


Figure S3. Plant functional group composition separated in qualitative and quantitative variables. Panel A) shows the percentage of the different categories within trait represented with different colours for each functional group. Plot B) shows the radar plot of the different quantitative variables standardized on the same scale also coloured with the same patterns of colours as qualitative variables per cluster.

Plant functional groups

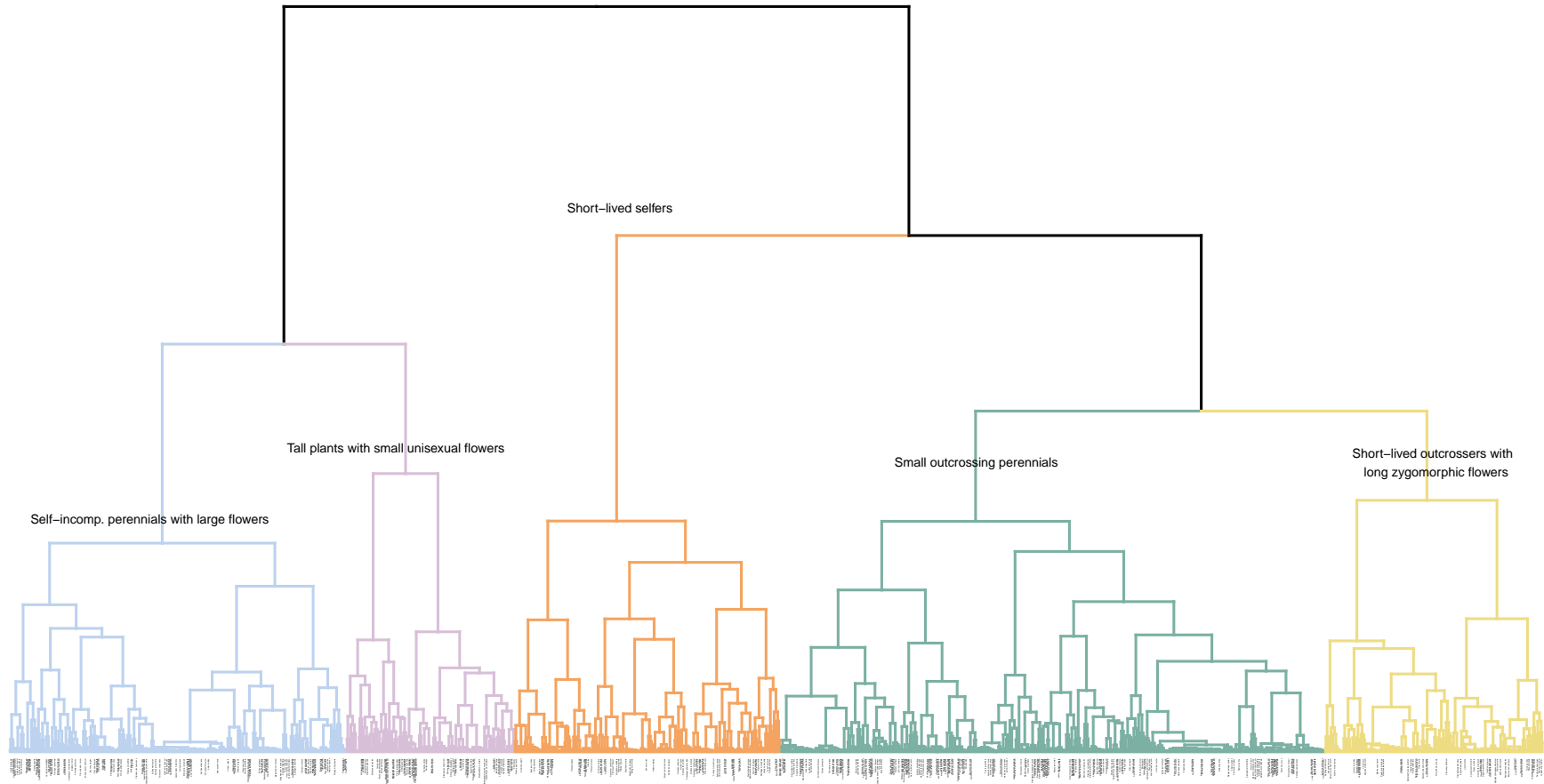


Figure S4. Hierarchical clustering dendrogram with the branches coloured by the optimal number of clusters (5). The labels of the subgroup of species ($N = 524$) used in this study are coloured in black in order to show the evenness of the distribution of the species across clusters. The rest of species labels are omitted for visualization purposes ($N = 982$).