

1 Niche and neutral processes leave distinct structural imprints on 2 indirect interactions in mutualistic networks

3 Benno I. Simmons^{1,2*}, Andrew P. Beckerman¹, Katrine Hansen³, Pietro K. Maruyama⁴,
4 Constantinos Televantos⁵, Jeferson Vizentin-Bugoni⁶ and Bo Dalsgaard³

5 ¹ Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK

6 ² Centre for Ecology and Conservation, College of Life and Environmental Sciences,
7 University of Exeter, Cornwall Campus, Penryn, TR10 9FE, UK

8 ³ Center for Macroecology, Evolution and Climate, GLOBE Institute, University of
9 Copenhagen, Universitetsparken 15, 2100 Copenhagen Ø, Denmark

10 ⁴ Centro de Síntese Ecológica e Conservação, Departamento de Genética, Ecologia e
11 Evolução, ICB, Universidade Federal de Minas Gerais – MG, Brazil

12 ⁵ Molecular Immunity Unit, Department of Medicine, University of Cambridge, MRC
13 Laboratory of Molecular Biology, Cambridge CB2 0QH, UK

14 ⁶ Department of Natural Resources and Environmental Sciences, University of Illinois at
15 Urbana-Champaign, 1102 S. Goodwin Ave, Urbana, IL, 61801, USA

16 * **Corresponding author:** Benno I. Simmons. Centre for Ecology and Conservation, College
17 of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn, UK.
18 Email: benno.simmons@gmail.com

19

20 Author contributions

21 BIS conceived the idea, conducted the analyses and wrote the first draft of the manuscript. CT
22 assisted with analysis and figures. PKM, JVB and BD compiled data. All authors contributed
23 critically to subsequent drafts.

24 Data availability

25 All data used in this analysis are publicly available from
26 <https://doi.org/10.5061/dryad.dncjsxkw2> (Sonne et al., 2020a, 2020b)

27 Acknowledgements

28 BIS and APB are supported by the Natural Environment Research Council [NE/S001395/1].
29 BIS is also supported by a Royal Commission for the Exhibition of 1851 Research Fellowship.
30 BD thanks the Danish National Research Foundation for its support of the Centre for
31 Macroecology, Evolution and Climate (grant no. DNRF96).

32

33 Abstract

34 Indirect interactions are central to ecological and evolutionary dynamics in pollination
35 communities, yet we have little understanding about the processes determining patterns of
36 indirect interactions, such as those between pollinators through shared flowering plants.
37 Instead, research has concentrated on the processes responsible for direct interactions and
38 whole-network structures. This is partly due to a lack of appropriate tools for characterising
39 indirect interaction structures, because traditional network metrics discard much of this
40 information. The recent development of tools for counting motifs (subnetworks depicting
41 interactions between a small number of species) in bipartite networks enable detailed analysis
42 of indirect interaction patterns. Here we generate plant-hummingbird pollination networks
43 based on three major assembly processes – neutral effects (species interacting in proportion to
44 abundance), morphological matching and phenological overlap – and evaluate the motifs
45 associated with each one. We find that different processes produce networks with significantly
46 different patterns of indirect interactions. Neutral effects tend to produce densely-connected
47 motifs, with short indirect interaction chains, and motifs where many specialists interact
48 indirectly through a single generalist. Conversely, niche-based processes (morphology and
49 phenology) produced motifs with a core of interacting generalists, supported by peripheral
50 specialists. These results have important implications for understanding the processes
51 determining indirect interaction structures.

52 **Keywords:** motifs, hummingbird, pollination, plant-pollinator network

53

54 Introduction

55 Species in a community are often influenced by other species they do not interact with directly
56 (Strauss, 1991; Wootton, 1994, 2002). Such indirect interactions are a fundamental component
57 of communities, governing ecological and evolutionary processes as much as, or more than,
58 direct effects (Bailey & Whitham, 2007; Guimarães, Pires, Jordano, Bascompte, & Thompson,
59 2017; Martínez, García, & Herrera, 2014; Strauss, 1991; Vandermeer, Hazlett, & Rathcke,
60 1985). For example, in plant-pollinator communities, indirect interactions between plants can
61 be mediated by shared pollinator species. These can be facilitative, where one plant attracts
62 pollinators that also visit co-occurring plant species, or competitive, where one plant attracts
63 pollinators away from another plant, through being more abundant or more attractive to the
64 pollinator than the competing plant (Carvalho et al., 2014; Mitchell, Flanagan, Brown,
65 Waser, & Karron, 2009; Morales & Traveset, 2009). These indirect interactions can have
66 important implications for community persistence and stability. For example, in communities
67 dominated by apparent competition, the sharing of interaction partners is restricted, and thus
68 perturbations are limited in how much they can propagate through the community (Thébaud &
69 Fontaine, 2010). Conversely, communities dominated by apparent facilitation favour
70 connected, nested structures with enhanced species coexistence (Bastolla et al., 2009; Thébaud
71 & Fontaine, 2010).

72 Despite the importance of indirect interactions for ecological and evolutionary dynamics, we
73 have little understanding of the processes that lead to their formation and maintenance in
74 mutualistic networks. Instead, research has focused on determining processes that give rise to
75 whole-network patterns or individual direct interactions, leaving the determinants of local-
76 scale patterns of indirect interactions largely unexplored (Maruyama, Vizentin-Bugoni,
77 Oliveira, Oliveira, & Dalsgaard, 2014; Olito & Fox, 2015; Simmons, Vizentin-Bugoni, et al.,
78 2019; Vázquez, Chacoff, & Cagnolo, 2009).

79 Knowledge of the processes responsible for indirect interactions is not only important in terms
80 of understanding the assembly and maintenance of community structure, but could also have
81 implications for conservation. Three distinct processes have been used to explain mutualistic
82 network structure: morphological matching (similarity in size and shape of a flower's corolla
83 and a pollinators feeding apparatus [Sonne et al., 2020b]); phenological overlap (co-occurrence
84 in time of a flower and pollinator), and neutral effects (assembly based on species interacting
85 randomly in proportion to their abundance). If, for example, indirect interaction structures are
86 a result of neutral effects, then conservation might focus on preserving species' abundance
87 distributions. If network structure is primarily determined by morphological matches between
88 species, then conservation might focus on ensuring the presence and persistence of species with
89 complementary sets of morphological traits. If phenological overlap between species is the
90 main process that governs network structure, then conservation might need to ensure the
91 community comprises sets of species with synchronous timings, so that interactions remain
92 established under climate change. Moreover, if it can be established that different processes
93 form different indirect interaction structures – that is, if different processes leave distinct
94 structural imprints – it may be possible to infer the processes operating in a community from
95 network topology alone, without having to expend valuable time and money collecting the
96 extra data required to measure processes explicitly.

97 Here we aim to understand the determinants of different indirect interaction structures by
98 comparing the indirect interaction structures produced by three distinct assembly processes:
99 morphological matching and phenological overlap (collectively known as niche-based
100 processes), and abundance (neutral effects). We use 24 empirical datasets on species
101 abundance, morphology and phenology from plant-hummingbird pollination communities
102 across the Americas to understand network structures that result from different processes. We
103 find that different processes leave distinct imprints on the structure of indirect interactions in
104 mutualistic networks, and conclude that this could have important implications for
105 conservation in the future.

106

107 Materials and Methods

108 We created simulated networks under three processes (morphological matching, phenological
109 overlap and neutral effects) from a dataset of 24 plant-hummingbird pollination networks
110 (Sonne et al., 2020b, 2020a), which contained corresponding information on plant and
111 hummingbird abundance, morphology (hummingbird bill length and floral corolla depth) and
112 phenology. Communities sampled span from Mexico to Brazil. Full details of the data are given
113 in Sonne et al. (2020b).

114 For each of these sets of abundance, morphology and phenology data, we generated matrices
115 giving the probabilities of species interactions under three different processes, following
116 Vázquez *et al.* (2009): neutral effects, morphological matching and phenological overlap.
117 Neutrality was simulated using an abundance matrix, \mathbf{A} . Elements of \mathbf{A} were the product of
118 each species' relative abundance. Thus, element a_{ij} represents the interaction probability
119 between plant species i and hummingbird species j and is equal to the product of the relative
120 abundances of i and j . This matrix therefore represents neutrality: the likelihood of species
121 interacting randomly in proportion to their abundance.

122 We create two morphological match matrices, corresponding to two different methods in the
123 literature. In the first matrix, \mathbf{M}_F , hummingbird bill lengths were first multiplied by 4/3 to
124 account for the extension of the tongue beyond the length of the bill (J. Vizentin-Bugoni,

125 Maruyama, & Sazima, 2014). Matrix elements were then set to 1 if the bill length (plus the
126 extension of the tongue) equalled or exceeded the floral corolla depth, and 0 otherwise (J.
127 Vizentin-Bugoni et al., 2014). This follows the ‘forbidden link’ concept where species are only
128 able to interact if there is a morphological match (i.e. if the hummingbird can reach the nectar
129 in the floral corolla). Matrix elements were then divided by the sum of the matrix to convert
130 the elements to probabilities (J. Vizentin-Bugoni et al., 2014). In the second matrix, \mathbf{M}_D ,
131 probabilities were inversely proportional to the difference between floral corolla depth and
132 hummingbird bill length (Weinstein & Graham, 2017). This approach relaxes the assumption
133 that a hummingbird is equally likely to interact with all flowers that have a floral corolla equal
134 to or shorter than its bill, and makes morphological match a continuous, rather than binary,
135 quantity. If the difference between floral corolla depth and hummingbird bill length was 0, the
136 difference was set to the minimum non-zero difference between corolla depth and bill length
137 in the web to prevent errors when dividing by zero values.

138 Elements of the phenological overlap matrix, \mathbf{P} , were calculated using matrix multiplication
139 (Vázquez et al., 2009). Plant and hummingbird phenology data, \mathbf{O}_P and \mathbf{O}_H respectively, had
140 species as rows and dates as columns, with cells set to 1 for presence and 0 for absence of
141 hummingbirds/flowers. Phenological overlap was then quantified as $\mathbf{P} = \mathbf{O}_P \mathbf{O}_H'$, where '
142 indicates the matrix was transposed (Vázquez et al., 2009). Thus, element p_{ij} of \mathbf{P} represents
143 the number of time slices in which plant species i and hummingbird species j co-occur.

144 For each assembly process, and for each dataset, we generated 1000 binary interaction matrices
145 from the probability matrix using the ‘mgen’ function in the ‘bipartite’ R package (Dormann,
146 Frund, Bluthgen, & Gruber, 2009). In total there were 96,000 binary matrices (1000 generated
147 matrices \times four assembly processes \times 24 sets of abundance, morphology and phenology data).
148 Generated matrices had the same connectance as their corresponding empirical matrices.

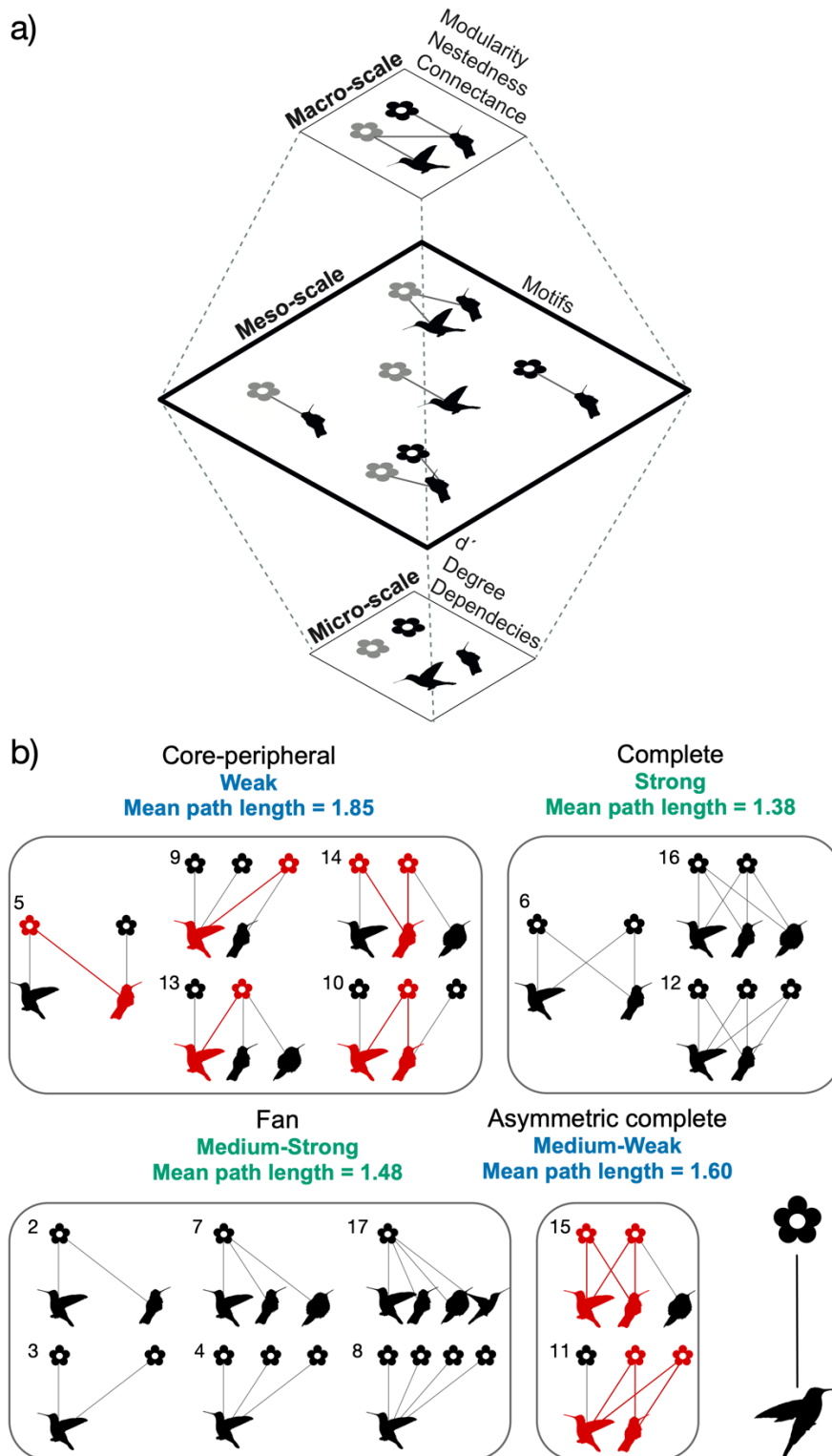
149 *Characterising indirect interactions using motifs*

150 We next characterised the different patterns of indirect interactions for each network and
151 assembly process. Mutualistic networks are generally characterised using metrics that capture
152 a particular facet of whole-network structure in a single number, such as levels of connectance,
153 nestedness and modularity (Dalsgaard et al., 2013; Olesen & Jordano, 2002). While these
154 metrics are undoubtedly useful, they are not always appropriate for considering indirect
155 interactions in detail because compressing a network into a single number necessarily discards
156 a substantial amount of topological information about indirect interactions (Simmons, Cirtwill,
157 et al., 2019).

158 Here we instead characterise network structure using motifs, which have recently been
159 proposed as a way to capture indirect interactions in bipartite networks in much greater detail
160 than traditional metrics like nestedness and modularity (Simmons, Cirtwill, et al., 2019;
161 Simmons, Sweering, et al., 2019). As motifs are a relatively new technique in the study of
162 mutualistic networks, below we provide a brief introduction to the approach and outline a motif
163 typology to aid their interpretation.

164 Just as LEGO sets are complex structures made from many small, distinct parts (Jordano,
165 2016a), networks can be thought of as being composed of many small subnetworks, or
166 ‘building blocks’, known as motifs. Motifs take the form of small groups of species interacting
167 with each other in particular ways. While network-level metrics like connectance, nestedness
168 and modularity characterise network structure at the ‘macro-scale’, and species-level metrics
169 like degree, d' , and centrality measures characterise the role of individual nodes at the ‘micro-

170 scale', motifs sit between these two extremes and capture 'meso-scale' network structure: local
 171 patterns of indirect interactions (Figure 1a).



173 **Figure 1:** (a) Schematic showing how a small four-species network (top) can be characterised at three scales.
 174 Macro-scale metrics, such as modularity, nestedness and connectance, summarise the structure of the whole
 175 network. Micro-scale metrics, such as d' , degree or dependencies, characterise the structure of a single node.
 176 Motifs sit between these two extremes, at the meso-scale, capturing local-scale patterns of indirect interactions
 177 between species. The 'meso-scale' level shows the five types of motif that make up the macro-scale network.
 178 Note that the network itself is a four-species motif and so, for this example, we only consider motifs with fewer
 179 than four species (two- and three-species motifs). Importantly, motifs do not discard information about macro-

180 scale structure. (b) A possible grouping of three- to five-node motifs by the broad indirect interaction structures
181 they represent. Nodes in the bottom level of motifs are hummingbirds and nodes in the top level of motifs are
182 plants. Each node represents a different species of animal or plant. Small numbers next to each motif are the ID
183 of that motif, following Simmons, Cirtwill, et al. (2019) ‘Core-peripheral’ motifs contain a core of interacting
184 generalist plants and pollinators (highlighted in red), connected to two or more peripheral specialist species.
185 ‘Complete’ motifs are where generalists interact with generalists and all possible interactions are realised. ‘Fan’
186 motifs feature two or more specialists interacting with a single generalist. ‘Asymmetric complete’ motifs are the
187 same as ‘complete’ motifs but linked to a single specialist. Thus, they are a particular type of ‘core-peripheral’
188 motif. Again, the core of interacting generalists is highlighted in red. For each motif, we calculated the mean path
189 length (mean number of links between all pairs of nodes), and report the mean of these values across all motifs in
190 each group below the group name. ‘Weak’, ‘Strong’, ‘Medium-Strong’, and ‘Medium-Weak’ indicate the possible
191 strength of indirect interactions within motifs of each group. The strength of the indirect effect between two nodes
192 tends to decay with increase path length between the nodes, such that nodes that are close to each other,
193 topologically, likely have stronger indirect effects between them than nodes that are far apart. Thus, for example,
194 nodes in ‘core-peripheral’ motifs with a high average path length likely experience weaker indirect effects than
195 ‘complete’ motifs, which have short average path lengths.

196 As there is only a finite number of ways to arrange interactions between a given number of
197 species, there is also only a finite number of motifs with a given number of nodes. In other
198 words, all networks are made up of a limited number of different types of building block. For
199 example, there are only 17 possible ways to arrange interactions between up to five species,
200 and hence there exist 17 different motifs containing between two and five nodes (Figure 1b;
201 note here that only 16 motifs are shown because we omit the simple two-node motif comprising
202 a single link between one plant and one pollinator because it represents a direct interaction only
203 without indirect effects).

204 In Figure 1b we propose a classification of motifs into four groups based on their indirect
205 interaction structure. ‘Core-peripheral’ motifs are motifs comprising a core of interconnected
206 generalist species, attached to two or more peripheral specialists. The mean path length
207 between species in these motifs is high. *Path length* is defined as the number of links between
208 two nodes. For example, in ‘core-peripheral’ motif 5, the path length between the black
209 hummingbird and the black plant is 3 because the shortest (and in this case, only) path between
210 these species involves 3 links (Figure 1b). The *mean path length* of a network is the average of
211 the shortest paths between all pairs of species. The relatively high mean path length of 1.85 in
212 core-peripheral motifs means that, on average, nodes are further apart. In turn, we expect
213 indirect interactions in these motifs to be weaker, because indirect effects are expected to decay
214 with increasing path length. For example, a change in the abundance of species *A* is likely to
215 more strongly affect the abundance of species *B* if there is only one intermediary species ($A \rightarrow$
216 $C \rightarrow B$) than if there are four intermediary species ($A \rightarrow C \rightarrow D \rightarrow E \rightarrow F \rightarrow B$). As well as
217 pathways between non-interacting species being longer, core-peripheral motifs also have fewer
218 pathways between non-interacting species, because these motifs contain few links. Again, this
219 reduces indirect effects (Guimarães et al., 2017) which, in turn, could help stop perturbations
220 spreading through a network (Thébault & Fontaine, 2010).

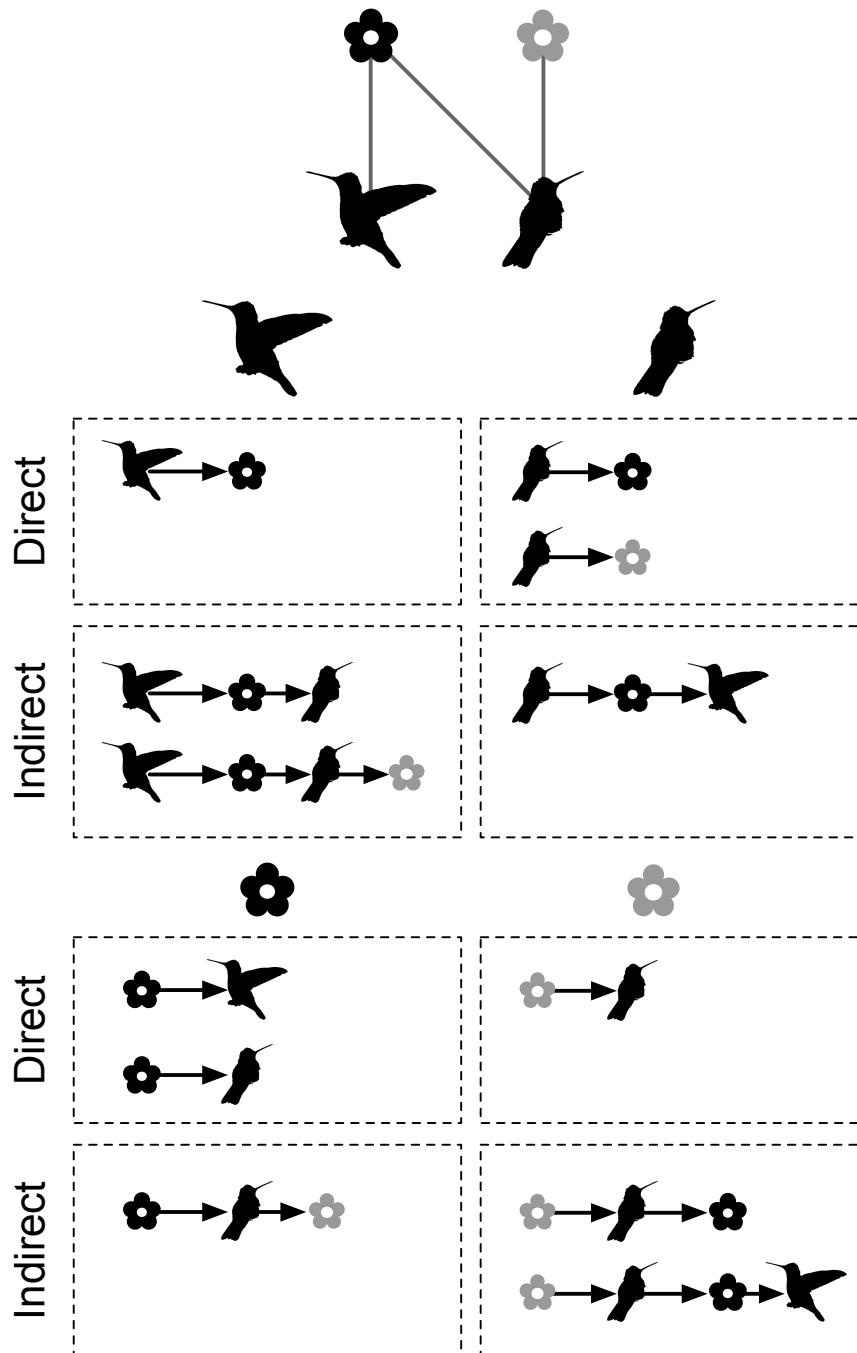
221 ‘Complete’ motifs stand in stark contrast to core-peripheral motifs. In complete motifs, all
222 species interact with each other, creating many pathways between non-interacting species, and
223 thus many ways for indirect effects to be transmitted. The mean path length is short, so the
224 many indirect effects are also likely to be stronger. Predicting dynamics in complete motifs is
225 likely to be harder, due to the multitude of possible pathways.

226 ‘Asymmetric complete’ motifs are similar to complete motifs, except that one of the species in
227 the motif is a specialist. Thus, asymmetric complete motifs contain a core where all species
228 interact with each other, attached to a lone specialist species. Asymmetric complete motifs are
229 a special case of ‘core-peripheral’ motif, with lower mean path length and higher number of

230 pathways than the main set of core-peripheral motifs, and thus slightly stronger indirect effects.
231 As the generalists in these motifs might be able to buffer changes in each other's abundances,
232 it is likely that the generalists have a stronger effect on the specialist, than *vice versa* (Simmons,
233 Cirtwill, et al., 2019). The specialist species' generalist partner has high levels of redundancy
234 in its interactions and thus may be a reliable partner for the specialist. However, asymmetric
235 complete motifs are likely less effective than core-peripheral motifs at curbing the spread of
236 perturbations through the network as a whole, as most of their constituent species are involved
237 in the hyper-connected core (Vieira & Almeida-Neto, 2015).

238 'Fan' motifs are the final group, comprising two or more specialists indirectly interacting via
239 a shared generalist. These motifs extend the classic apparent competition and exploitative
240 competition motifs from food webs to having any number of specialists interacting with a
241 single generalist. Consider a motif where two plant species interact indirectly through a single
242 pollinator species (the 'fan' motif 3 in Figure 1b). This could represent indirect facilitation,
243 where an increase in the abundance of the first plant, increases the abundance of the shared
244 pollinator which, in turn, increases the abundance of the second plant (Moeller, 2004;
245 Sotomayor & Lortie, 2015). Alternatively, such a motif could represent exploitative
246 competition for the pollinator or interference competition through heterospecific pollen
247 deposition (Chittka & Schürkens, 2001; Flanagan, Mitchell, & Karron, 2010; Hochkirch,
248 Mertes, & Rautenberg, 2012; Mitchell et al., 2009; Moeller, 2004; Simmons, Cirtwill, et al.,
249 2019; Ye et al., 2014). Indirect interactions in fan motifs are relatively strong, with a medium-
250 short path length.

251



252

253 **Figure 2:** Motifs capture indirect interaction chains, defined as chains of species where a change in the abundance
 254 of one species alters the abundance of another species, through altering the abundance of one or more intermediary
 255 species (Simmons, Cirtwill, et al., 2019; Wootton, 1994). Here we consider a simple four-species motif and its
 256 constituent direct interactions and indirect interaction chains. It is clear how even a small motif contains rich detail
 257 on indirect interactions. The arrow shows the direction of the effect. For example, the left hummingbird has a
 258 direct interaction with the black plant, but also indirect interactions with the right hummingbird (via changing the
 259 abundance of the black plant) and the grey plant (via changing the abundance of the black plant and the right
 260 hummingbird).

261 By breaking down a network into its constituent motifs, it is possible to explicitly characterise
 262 indirect interaction structures between small groups of species, without losing any information
 263 about broader network structure. Specifically, motifs capture the topology of interaction chains,
 264 where changes in the abundance of one species influence the abundance of another species,
 265 through altering the abundance of one or more intermediary species (Simmons, Cirtwill, et al.,

266 2019; Wootton, 1994). Even a simple four-species motif contains six different indirect
267 interaction chains with up to two intermediary species (Figure 2). Larger and more complex
268 motifs contain even richer detail on indirect interaction structures. This high level of detail is
269 the advantage of the motif approach, allowing information about indirect interactions to be
270 captured with a level of precision that is not possible when using traditional network metrics.
271 Importantly, this extra information translates into novel and important insights into empirical
272 data. For example, a recent study quantified species roles using a popular specialisation metric,
273 d' , which measures the extent to which species' interactions diverge from what would be
274 expected if available partners were visited randomly (Kaiser-Bunbury et al., 2017). Using this
275 metric, two key pollinator species were found to play similar roles in the community, both
276 being super-generalists (Kaiser-Bunbury et al., 2017). However, when their roles were
277 quantified using motifs, details of their indirect interactions were uncovered, revealing that the
278 species actually played significantly different roles in the community: one was found to interact
279 indirectly with generalist pollinators, while the other interacted indirectly with more specialist
280 pollinators via shared specialist plants (Simmons, Cirtwill, et al., 2019).

281 We therefore used motifs to characterise indirect interactions in our analysis. Specifically, we
282 calculated the mean frequency of all motifs up to five nodes (see motif topologies in Figure
283 1b) for each network and assembly process using the 'bmotif' R package (Simmons, Sweering,
284 et al., 2019). To control for variation in network size, motif frequencies were normalised as a
285 proportion of the total number of motifs within each motif size class (the number of nodes a
286 motif contains) (Baker, Kaartinen, Roslin, & Stouffer, 2015). This was done to control for the
287 fact that smaller motifs can be nested within larger motifs. As there is only one two-node motif
288 (a single link between two nodes), and thus only one motif in the two-node size class, this was
289 excluded from analyses because its normalised frequency would always equal one. Six-node,
290 and larger, motifs were excluded because commonly-studied indirect interactions, like apparent
291 competition, are present in smaller motifs and five-node motifs already contain varied and long
292 interaction chains with up to three intermediary species between two indirectly interacting
293 partners. Limiting to five-node motifs was also beneficial for visualisation, interpretation and
294 computational reasons.

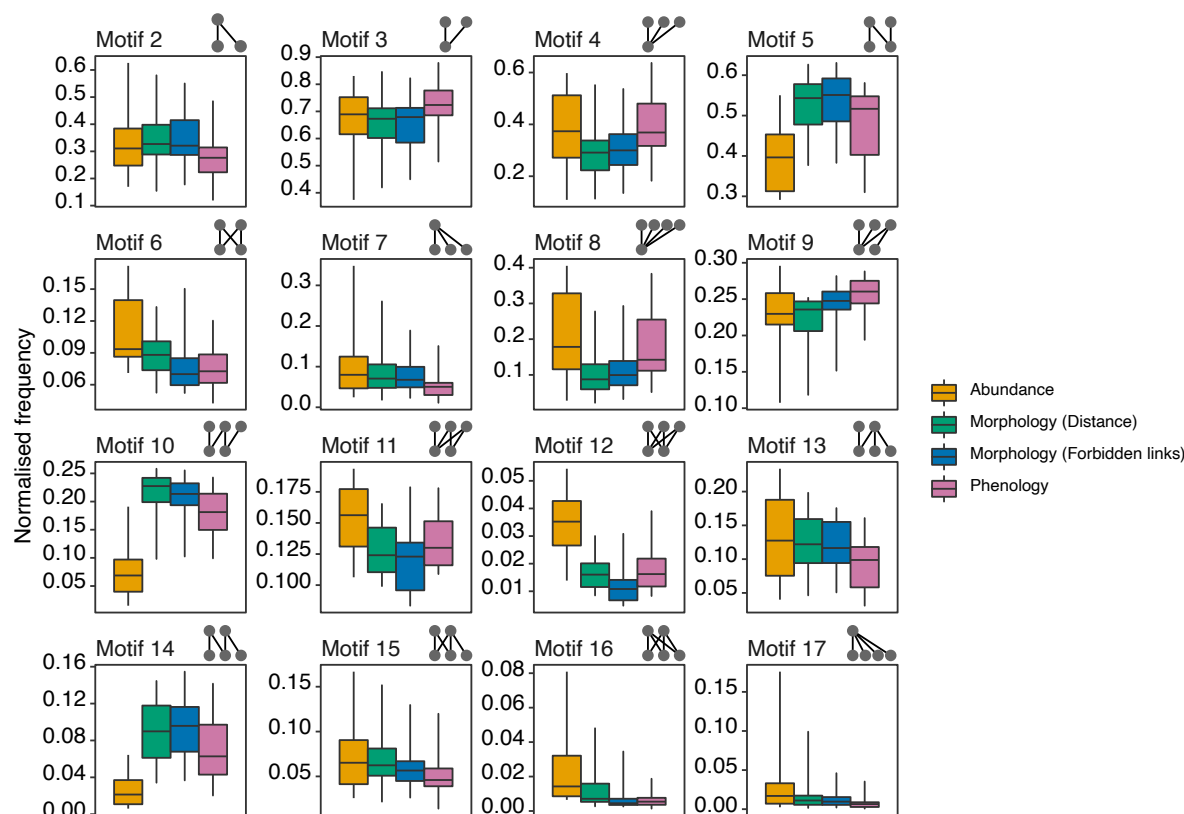
295 *Statistical analysis*

296 We used an ANOVA framework to assess statistical differences between the frequencies of
297 motifs in networks generated using different assembly processes. First, a MANOVA was used
298 with frequencies of all 16 motifs as dependent variables and assembly process as the
299 independent variable to determine whether there was an overall effect of assembly process on
300 motif frequency distribution. Then, to identify how assembly processes affect specific
301 dependent variables, we conducted univariate ANOVAs for each motif. For this, pairwise
302 comparisons between assembly processes were calculated using the 'multcomp' R package
303 (Hothorn, Bretz, & Westfall, 2008). Adjusted p-values were used to account for multiple
304 comparisons, using the 'single-step' method in 'multcomp'.

305

306 Results

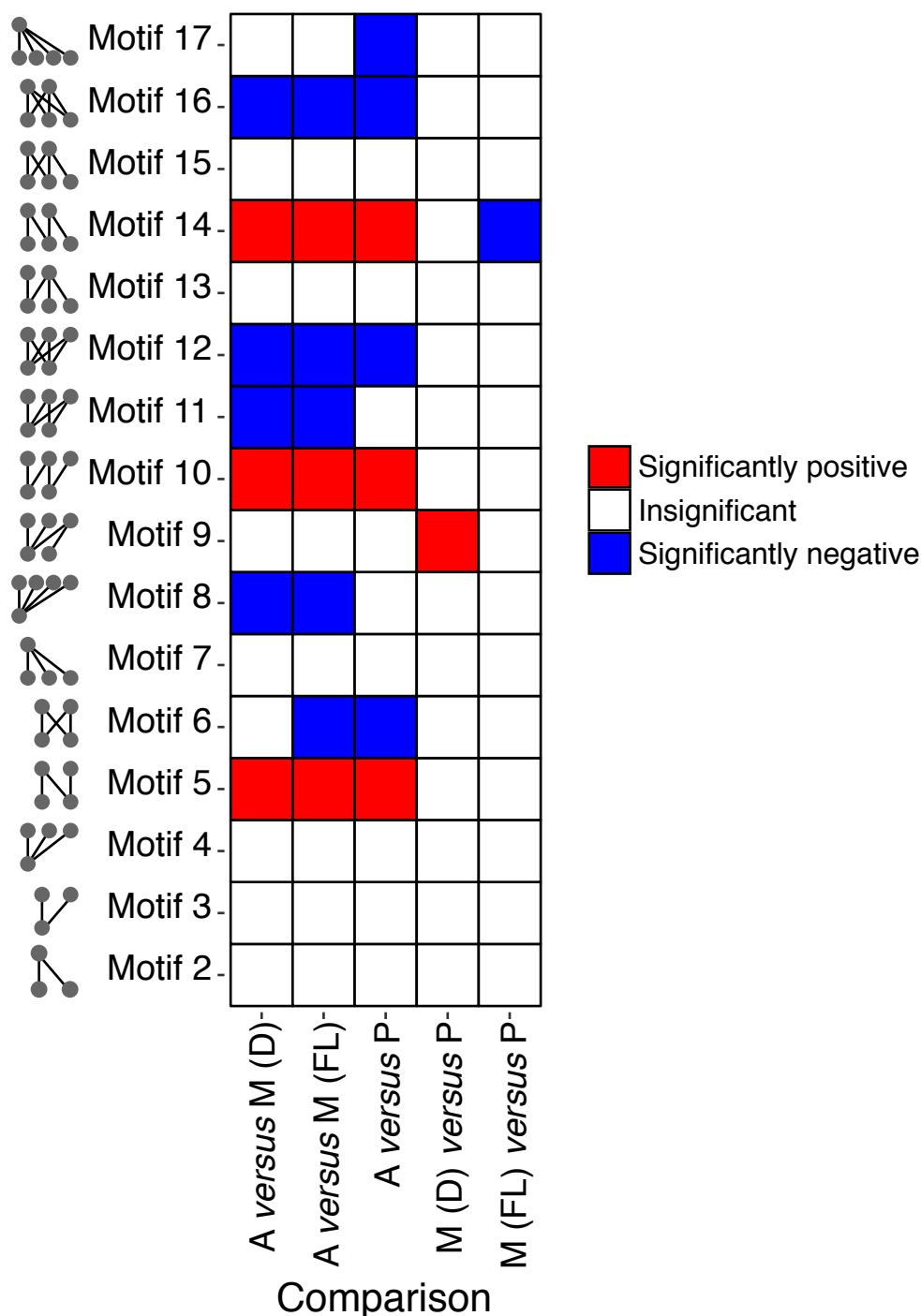
307 Different assembly processes produced significantly different motif distributions (MANOVA:
308 $df = 4$, $F = 2530.5$, $p < 0.001$): neutral processes (abundance) were associated with more
309 occurrences of complete, asymmetric complete and fan motifs (motifs 6, 8, 11, 12, 16 and 17),
310 while niche-based processes (morphological match and phenological overlap) were associated
311 with more occurrences of core-peripheral motifs (motifs 5, 10 and 14) (Figures 1b, 3 and 4).
312 Furthermore, some differences were observed between morphological matching and
313 phenological overlap matrices: phenological overlap matrices had significantly higher
314 frequencies of motif 9 (a core-peripheral motif) than morphological matching, but significantly
315 lower frequencies of motif 14 (another core-peripheral motif; Figures 1b, 3 and 4).



316

317 **Figure 3:** Normalised frequencies of each motif for networks generated using abundance (neutral processes) and
 318 morphological matching and phenological overlap (niche-based processes) for 24 plant-hummingbird interaction
 319 networks sampled across the Americas. Boxplots represent the distribution of mean normalised motif frequencies
 320 for generated networks across the 24 sets of abundance, morphology and phenological data. Upper whiskers
 321 represent 95% quantiles, the upper hinge is the 75% quantile, the middle line is the median, the lower hinge is the
 322 25% quantile and the lower whisker is the 5% quantile. See Figure 4 for significance levels. In the motifs depicted
 323 above each boxplot, nodes in the bottom level of motifs are hummingbirds and nodes in the top level of motifs
 324 are plants.

325



326

327 **Figure 4:** Matrix showing whether there are significant (adjusted $P < 0.05$) differences in normalised motif
 328 frequencies depending on the assembly processes (neutral processes like abundance, or niche-based processes like
 329 morphological matching or phenological overlap). Abbreviations for assembly processes are: 'A' is abundance,
 330 'M (D)' is morphological matching based on distance between corolla depth and bill length, 'M (FL)' is
 331 morphological matching based on the forbidden link concept, and 'P' is phenology. Comparisons are relative to
 332 the first processes expressed. For example, if a cell in the A versus M (D) column is red, this means the motif
 333 frequency was significantly higher in the M (D) matrices than in the A matrices. Conversely, if a cell in the A
 334 versus M (D) column is blue, this means the motif frequency was significantly lower in the M (D) matrices than
 335 in the A matrices.

336

337 Discussion

338 We find that networks generated using different assembly processes have significantly
339 different patterns of indirect interactions. Networks governed by neutral effects (species
340 abundance) tend to have more densely-connected complete, asymmetric complete and fan
341 motifs where either (i) indirect interactions between plants/pollinators are mediated through a
342 single pollinator/plant (fan motifs 8 and 17), or (ii) indirect interactions may be strong because
343 there are multiple routes for indirect effects to travel at the same time (complete and
344 asymmetric complete motifs 6, 11, 12 and 16) (Figures 3 and 4). Conversely, networks
345 produced assuming niche-based processes – those determined by morphology or phenology –
346 contain more core-peripheral motifs, that comprise a core of interacting generalists, supported
347 by peripheral specialists (core-peripheral motifs 5, 10, 14) (Figures 3 and 4).

348 Neutral processes produced two main types of motifs. First, they produced motifs, where
349 specialists affect each other indirectly via a single generalist (fan motifs, such as motifs 8 and
350 17). These fan motifs (Simmons, Cirtwill, et al., 2019) extend the classic apparent competition
351 and exploitative competition structures from food webs (motifs 2 and 3) to having more than
352 two specialists. Importantly, despite being generated by the same process, motifs 8 and 17 are
353 likely to have different levels of competition between the specialist species. In motif 8, many
354 plants compete for a single pollinator (Figure 1b). In this situation, competition is likely to be
355 low between the plants, especially if the pollinator is abundant, as the plants only need one
356 successful visit from a pollinator to disperse their pollen and reproduce. Conversely, in motif
357 17, multiple pollinators are competing for a single plant (Figure 1b). Here, competition is likely
358 to be stronger, as pollinators are relying on the plant as a regular, limited food source.
359 Importantly, however, these networks represent mutualistic interactions between species and
360 thus it is also possible that ‘fan motifs’ represent indirect facilitative, rather than competitive,
361 situations, where specialists indirectly benefit each other through interactions with a single
362 generalist (Moeller, 2004; Sotomayor & Lortie, 2015). For example, the presence of a plant
363 species could increase pollinator visits to one or more coflowering species, or multiple plant
364 species could combine to form a large, shared floral display that increases pollinator visitation
365 to all coflowering plants beyond what would be expected if each of the plants flowered in
366 isolation. Whether indirect interactions are competitive or facilitative can depend on a range of
367 factors, such as the distance between plants and their spatial configuration (Charlebois &
368 Sargent, 2017), however there is evidence that pollinator abundance can have an influence,
369 with facilitation occurring above a threshold abundance, and competition occurring below the
370 threshold (Ye et al., 2014). Thus, combining motif analysis with information on empirical or
371 simulated population dynamics, could give insight into the directionality of indirect effects.

372 The second type of motif produced by neutral processes is complete and asymmetric complete
373 motifs which have many links, providing many possibly pathways through which indirect
374 effects can flow (motifs 6, 11, 12, 16). This likely results from the neutral model’s lack of
375 consideration of ‘forbidden links’ (Canard et al., 2014; Jordano, 2016b): as long as two species
376 are of sufficiently high abundance, they are able to interact, resulting in more pathways
377 (Simmons et al., 2018). This is in contrast to niche-based processes, where poor morphological
378 matches or low temporal co-occurrence would prevent some interactions from being formed.
379 This has important implications for whole-network dynamics, as it suggests that under neutral
380 processes, the average length of the interaction chain between any two species will be lower,
381 increasing the magnitude and number of indirect effects, but decreasing their localisation. In
382 turn this could allow the spread of perturbations through the community (Thébault & Fontaine,
383 2010). In complete motifs 6, 12 and 16, all plants interact with all pollinators. Here we might
384 expect indirect interactions to be strong, as effects can be transmitted through multiple links

385 simultaneously and the indirect interaction chains are shorter, but also less predictable
386 (Simmons, Cirtwill, et al., 2019). For example, in motif 12, if a pollinator decreased in
387 abundance, this would remove the mutualistic benefit to the three plant species, but could also
388 reduce competition between the two pollinators (Simmons, Cirtwill, et al., 2019); further
389 research is necessary to examine the complex dynamics that could occur in these motifs. Motif
390 11 represents a slightly different situation to that in 6, 12 and 16, as motif 11 has a single
391 specialist interacting with a completely connected set of generalists. This is therefore an
392 asymmetric complete motif, where it has been suggested that generalists have a stronger effect
393 on the specialists than the specialists have on the generalists, as the generalists are able to buffer
394 changes in each other's abundances (Simmons, Cirtwill, et al., 2019).

395 Niche-based processes resulted in motifs with a core of interacting generalists, connected to
396 peripheral specialists (core-peripheral motifs 5, 10, 14). The indirect interaction pathways in
397 these motifs can be highly complex. For example, in motif 5, there are four species: two plants
398 in the top left (P_L) and top right (P_R), and two hummingbirds in the bottom left (H_L) and bottom
399 right (H_R). One possible pathway is that P_L can negatively affect H_R indirectly, by providing a
400 mutualistic benefit to H_R 's competitor H_L , and by competing with P_R , reducing the mutualistic
401 benefit to H_R (Simmons, Cirtwill, et al., 2019; Vázquez, Ramos-Jiliberto, Urbani, &
402 Valdovinos, 2015). While a complete study of the dynamics of each motif is beyond the scope
403 of this work, our results do suggest that niche-based processes restrict the sharing of interaction
404 partners, thus forcing indirect pathways between species to be longer. Given that longer
405 pathways likely have weaker indirect effects, niche-based processes likely reduce the
406 magnitude of indirect effects in the community (Guimarães et al., 2017). In turn, this could
407 limit the spread of perturbations through the network (Thébault & Fontaine, 2010).

408 While there were few differences between different niche-based processes, networks based on
409 phenological overlap had significantly higher frequencies of motif 9 (a core-peripheral motif
410 with two generalists interacting) and significantly lower frequencies of motif 14 (a core-
411 peripheral motif with three generalists interacting) than morphological matching models. This
412 could reflect the degree of constraint between these two processes. *A priori*, it is difficult to
413 say whether phenological overlap or morphological matching represents a greater constraint
414 on species interactions. Phenological overlap requires species to co-occur in time to interact,
415 but ignores species morphology, while morphological matching only allows species to interact
416 if the hummingbird bill length and floral corolla depth are sufficiently matching, regardless of
417 temporal co-occurrence. For our data, the phenological overlap model produced significantly
418 more motifs with two generalists, and significantly fewer motifs with three generalists, than
419 the morphological matching model. This suggests that interactions between generalists are
420 rarer under phenological overlap, indicating that lack of phenological overlap may impose
421 more forbidden links than morphological mismatch in plant-hummingbird pollination systems.

422 Here we shed light on the different processes associated with patterns of indirect interactions
423 in mutualistic networks, quantified using motifs. As well as being ecologically interesting in its
424 own right, our results are also useful for interpreting the results of motif analyses and for
425 generating motif-driven hypotheses. For example, networks with a high proportion of invasive
426 species may be expected to have higher frequencies of motifs associated with neutral effects,
427 because invasive species lack the coevolutionary associations of native species (Jeferson
428 Vizentin-Bugoni et al., 2019). Overall, our results link indirect interaction structures to distinct
429 generative processes. The normalised motif profiles we present represent a baseline of what
430 structures would be expected in communities dominated by morphological matching,
431 phenological overlap or neutral effects. By measuring the similarity of motif profiles from
432 empirical networks to those idealised profiles presented here, it may be possible to infer the

433 processes acting in a community from the indirect interaction topology alone, and thus inform
434 the type of conservation actions that are needed. Further research along this line is necessary,
435 alongside empirical validation, but our findings suggest potential for using structure as a proxy
436 for processes in a conservation context.

437

438 References

- 439 Bailey, J. K., & Whitham, T. G. (2007). Biodiversity is related to indirect interactions among
440 species of large effect. In T. Ohgushi, T. P. Craig, & P. W. Price (Eds.), *Ecological*
441 *communities: plant mediation in indirect interaction webs* (pp. 306–328). Cambridge:
442 Cambridge University Press.
- 443 Baker, N. J., Kaartinen, R., Roslin, T., & Stouffer, D. B. (2015). Species' roles in food webs
444 show fidelity across a highly variable oak forest. *Ecography*, *38*(2), 130–139. doi:
445 10.1111/ecog.00913
- 446 Bastolla, U., Fortuna, M. A., Pascual-García, A., Ferrera, A., Luque, B., & Bascompte, J.
447 (2009). The architecture of mutualistic networks minimizes competition and increases
448 biodiversity. *Nature*, *458*(7241), 1018–1020. doi: 10.1038/nature07950
- 449 Canard, E. F., Mouquet, N., Mouillot, D., Stanko, M., Miklisova, D., & Gravel, D. (2014).
450 Empirical evaluation of neutral interactions in host-parasite networks. *American*
451 *Naturalist*, *183*(4), 468–479. doi: 10.1086/675363
- 452 Carvalheiro, L. G., Biesmeijer, J. C., Benadi, G., Fründ, J., Stang, M., Bartomeus, I., ...
453 Kunin, W. E. (2014). The potential for indirect effects between co-flowering plants via
454 shared pollinators depends on resource abundance, accessibility and relatedness.
455 *Ecology Letters*, *17*(11), 1389–1399. doi: 10.1111/ele.12342
- 456 Charlebois, J. A., & Sargent, R. D. (2017). No consistent pollinator-mediated impacts of alien
457 plants on natives. *Ecology Letters*, *20*(11), 1479–1490. doi: 10.1111/ele.12831
- 458 Chittka, L., & Schürkens, S. (2001). Successful invasion of a floral market: An exotic Asian
459 plant has moved in on Europe's river-banks by bribing pollinators. *Nature*, *411*(6838),
460 653. doi: 10.1038/35079676
- 461 Dalsgaard, B., Trøjelsgaard, K., Martín González, A. M., Nogués-Bravo, D., Ollerton, J.,
462 Petanidou, T., ... Olesen, J. M. (2013). Historical climate-change influences modularity
463 and nestedness of pollination networks. *Ecography*, *36*(12), 1331–1340. doi:
464 10.1111/j.1600-0587.2013.00201.x
- 465 Dormann, C. F., Frund, J., Bluthgen, N., & Gruber, B. (2009). Indices, Graphs and Null
466 Models: Analyzing Bipartite Ecological Networks. *The Open Ecology Journal*, *2*(1), 7–
467 24. doi: 10.2174/1874213000902010007
- 468 Flanagan, R. J., Mitchell, R. J., & Karron, J. D. (2010). Increased relative abundance of an
469 invasive competitor for pollination, *Lythrum salicaria*, reduces seed number in *Mimulus*
470 *ringens*. *Oecologia*, *164*(2), 445–454. doi: 10.1007/s00442-010-1693-2
- 471 Guimarães, P. R., Pires, M. M., Jordano, P., Bascompte, J., & Thompson, J. N. (2017).
472 Indirect effects drive coevolution in mutualistic networks. *Nature*, *550*(7677), 511–514.
473 doi: 10.1038/nature24273
- 474 Hochkirch, A., Mertes, T., & Rautenberg, J. (2012). Conspecific flowers of *sinapis arvensis*

- 475 are stronger competitors for pollinators than those of the invasive weed *bunias orientalis*.
476 *Naturwissenschaften*, 99(3), 217–224. doi: 10.1007/s00114-012-0888-2
- 477 Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric
478 models. *Biometrical Journal*, Vol. 50, pp. 346–363. doi: 10.1002/bimj.200810425
- 479 Jordano, P. (2016a). Chasing Ecological Interactions. *PLoS Biology*, 14(9). doi:
480 10.1371/journal.pbio.1002559
- 481 Jordano, P. (2016b). Sampling networks of ecological interactions. *Functional Ecology*, Vol.
482 30, pp. 1883–1893. doi: 10.1111/1365-2435.12763
- 483 Kaiser-Bunbury, C. N., Mougai, J., Whittington, A. E., Valentin, T., Gabriel, R., Olesen, J.
484 M., & Blüthgen, N. (2017). Ecosystem restoration strengthens pollination network
485 resilience and function. *Nature*, 542(7640), 223–227. doi: 10.1038/nature21071
- 486 Martínez, D., García, D., & Herrera, J. M. (2014). Consistency and reciprocity of indirect
487 interactions between tree species mediated by frugivorous birds. *Oikos*, 123(4), 414–
488 422. doi: 10.1111/j.1600-0706.2013.00558.x
- 489 Maruyama, P. K., Vizentin-Bugoni, J., Oliveira, G. M., Oliveira, P. E., & Dalsgaard, B.
490 (2014). Morphological and spatio-temporal mismatches shape a neotropical savanna
491 plant-hummingbird network. *Biotropica*, 46(6), 740–747. doi: 10.1111/btp.12170
- 492 Mitchell, R. J., Flanagan, R. J., Brown, B. J., Waser, N. M., & Karron, J. D. (2009). New
493 frontiers in competition for pollination. *Annals of Botany*, 103(9), 1403–1413.
- 494 Moeller, D. A. (2004). Facilitative interactions among plants via shared pollinators. *Ecology*,
495 85(12), 3289–3301. doi: 10.1890/03-0810
- 496 Morales, C. L., & Traveset, A. (2009). A meta-analysis of impacts of alien vs. native plants
497 on pollinator visitation and reproductive success of co-flowering native plants. *Ecology*
498 *Letters*, 12(7), 716–728. doi: 10.1111/j.1461-0248.2009.01319.x
- 499 Olesen, J. M., & Jordano, P. (2002). Geographic patterns in plant-pollinator mutualistic
500 networks: Comment. *Ecology*, 83(9), 2416–2424. doi: 10.1890/03-3016
- 501 Olito, C., & Fox, J. W. (2015). Species traits and abundances predict metrics of plant-
502 pollinator network structure, but not pairwise interactions. *Oikos*, 124(4), 428–436. doi:
503 10.1111/oik.01439
- 504 Simmons, B. I., Cirtwill, A. R., Baker, N. J., Wauchope, H. S., Dicks, L. V., Stouffer, D. B.,
505 & Sutherland, W. J. (2019). Motifs in bipartite ecological networks: uncovering indirect
506 interactions. *Oikos*, 128(2), 154–170. doi: 10.1111/oik.05670
- 507 Simmons, B. I., Sweering, M. J. M., Schillinger, M., Dicks, L. V., Sutherland, W. J., & Di
508 Clemente, R. (2019). bmotif: A package for motif analyses of bipartite networks.
509 *Methods in Ecology and Evolution*, 10(5), 695–701. doi: 10.1111/2041-210X.13149
- 510 Simmons, B. I., Vizentin-Bugoni, J., Maruyama, P. K., Cotton, P. A., Marin-Gomez, O. H.,
511 Lara, C., ... Sutherland, W. J. (2018). Abundance drives broad patterns of generalisation
512 in plant-hummingbird pollination networks. *BioRxiv*. doi: 10.1101/339762
- 513 Simmons, B. I., Vizentin-Bugoni, J., Maruyama, P. K., Cotton, P. A., Marín-Gómez, O. H.,
514 Lara, C., ... Sutherland, W. J. (2019). Abundance drives broad patterns of generalisation
515 in plant-hummingbird pollination networks. *Oikos*, 128(9), 1287–1295. doi:

- 516 10.1111/oik.06104
- 517 Sonne, J., Vizentin-Bugoni, J., Maruyama, P. K., Araujo, A. C., Chávez-González, E.,
518 Coelho, A. G., ... Dalsgaard, B. (2020a). *Ecological mechanisms explaining*
519 *interactions within plant-hummingbird networks: morphological matching increases*
520 *towards lower latitudes*, v5, *Dryad, Dataset*. doi:
521 <https://doi.org/10.5061/dryad.dncjsxkw2>
- 522 Sonne, J., Vizentin-Bugoni, J., Maruyama, P. K., Araujo, A. C., Chávez-González, E.,
523 Coelho, A. G., ... Dalsgaard, B. (2020b). Ecological mechanisms explaining interactions
524 within plant-hummingbird networks: morphological matching increases towards lower
525 latitudes. *Proceedings of the Royal Society B: Biological Sciences*, 287(1922),
526 20192873. doi: 10.1098/rspb.2019.2873
- 527 Sotomayor, D. A., & Lortie, C. J. (2015). Indirect interactions in terrestrial plant
528 communities: Emerging patterns and research gaps. *Ecosphere*, 6(6), 103. doi:
529 10.1890/ES14-00117.1
- 530 Strauss, S. Y. (1991). Indirect Effects in Community Ecology - Their Definition, Study and
531 Importance. *Trends in Ecology & Evolution*, 6(7), 206–210. doi: Doi 10.1016/0169-
532 5347(91)90023-Q
- 533 Thébault, E., & Fontaine, C. (2010). Stability of ecological communities and the architecture
534 of mutualistic and trophic networks. *Science*, 329(5993), 853–856. doi:
535 10.1126/science.1188321
- 536 Vandermeer, J., Hazlett, B., & Rathcke, B. (1985). Indirect facilitation and mutualism. In D.
537 H. Boucher (Ed.), *The Biology of Mutualism: Ecology and Evolution* (pp. 326–343).
538 New York: Oxford University Press.
- 539 Vázquez, D. P., Chacoff, N. P., & Cagnolo, L. (2009). Evaluating multiple determinants of
540 the structure of plant-animal mutualistic networks. *Ecology*, 90(8), 2039–2046. doi:
541 10.1890/08-1837.1
- 542 Vázquez, D. P., Ramos-Jiliberto, R., Urbani, P., & Valdovinos, F. S. (2015). A conceptual
543 framework for studying the strength of plant-animal mutualistic interactions. *Ecology*
544 *Letters*, Vol. 18, pp. 385–400. doi: 10.1111/ele.12411
- 545 Vieira, M. C., & Almeida-Neto, M. (2015). A simple stochastic model for complex
546 coextinctions in mutualistic networks: Robustness decreases with connectance. *Ecology*
547 *Letters*, 18(2), 144–152. doi: 10.1111/ele.12394
- 548 Vizentin-Bugoni, J., Maruyama, P. K., & Sazima, M. (2014). Processes entangling
549 interactions in communities: forbidden links are more important than abundance in a
550 hummingbird-plant network. *Proceedings of the Royal Society B: Biological Sciences*,
551 281(1780), 20132397. doi: 10.1098/rspb.2013.2397
- 552 Vizentin-Bugoni, Jeferson, Tarwater, C. E., Foster, J. T., Drake, D. R., Gleditsch, J. M.,
553 Hruska, A. M., ... Sperry, J. H. (2019). Structure, spatial dynamics, and stability of
554 novel seed dispersal mutualistic networks in Hawai'i. *Science*, 364(6435), 78–82. doi:
555 10.1126/science.aau8751
- 556 Weinstein, B. G., & Graham, C. H. (2017). Persistent bill and corolla matching despite
557 shifting temporal resources in tropical hummingbird-plant interactions. *Ecology Letters*,
558 Vol. 20, pp. 326–335. doi: 10.1111/ele.12730

- 559 Wootton, J. T. (1994). The Nature and Consequences of Indirect Effects in Ecological
560 Communities. *Annual Review of Ecology and Systematics*, 25(1), 443–466. doi:
561 10.1146/annurev.es.25.110194.002303
- 562 Wootton, J. T. (2002). Indirect effects in complex ecosystems: recent progress and future
563 challenges. *Journal of Sea Research*, 48(2), 157–172. doi: 10.1016/S1385-
564 1101(02)00149-1
- 565 Ye, Z. M., Dai, W. K., Jin, X. F., Gituru, R. W., Wang, Q. F., & Yang, C. F. (2014).
566 Competition and facilitation among plants for pollination: Can pollinator abundance
567 shift the plant-plant interactions? *Plant Ecology*, 215(1), 3–13. doi: 10.1007/s11258-
568 013-0274-y
- 569
- 570