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Unravelling Darwin's entangled bank: architecture and robustness of mutualistic networks with multiple interaction types

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Trying to unravel Darwin's entangled bank further, we describe the architecture of a network involving multiple forms of mutualism (pollination by animals, seed dispersal by birds and plant protection by ants) and evaluate whether this multi-network shows evidence of a structure that promotes robustness. We found that species differed strongly in their contributions to the organization of the multi-interaction network, and that only a few species contributed to the structuring of these patterns. Moreover, we observed that the multi-interaction networks did not enhance community robustness compared with each of the three independent mutualistic networks when analysed across a range of simulated scenarios of species extinction. By simulating the removal of highly interacting species, we observed that, overall, these species enhance network nestedness and robustness, but decrease modularity. We discuss how the organization of interlinked mutualistic networks may be essential for the maintenance of ecological communities, and therefore the long-term ecological and evolutionary dynamics of interactive, species-rich communities. We suggest that conserving these keystone mutualists and their interactions is crucial to the persistence of species-rich mutualistic assemblages, mainly because they support other species and shape the network organization.

1. Introduction

Within the natural environment, there is a high diversity of interaction types between plant and animal species, including herbivory, pollination, ant protection and seed dispersal [1]. These ecological interactions regulate populations and biological communities, and play a key role in structuring biodiversity [2]. Fascinated by the variety of life forms and interactions between them, Darwin [3] called this complexity the 'entangled bank' in his seminal book *On the origin of species*.

In recent decades, studies have attempted to unravel the interaction structure of Darwin's entangled bank [4–6]. Tools derived from network science have been used to investigate how the complex organization of these species interactions varies over space and time, and the degree to which they are susceptible to perturbations [7–10]. In ecological networks, species are depicted as nodes and their interactions as links [11]. Such studies have focused on the structural properties of these networks in different ecosystems, and have advanced our understanding of the ecological and evolutionary dynamics of plant–animal interactions [4,12].

Traditionally, studies of ecological networks have considered only one type of ecological interaction (e.g. plant–pollinator or plant–disperser) within bipartite subnetworks (i.e. networks consisting of two interacting guilds or trophic levels; reviewed in [6]). There is no doubt that these studies have contributed to our current and comprehensive view of species interactions. However, in ecological communities, species are involved in multiple kinds of interactions [2,13,14]. For example, a plant species can be visited by pollinators, herbivores and seed dispersers, and generate complex networks of merged interactions [14]. The challenge is to understand how these coupled ecological networks are linked and the dynamical consequences for the resulting multi-interaction networks [14]. Despite the need to merge different types of interactions, only a few studies have evaluated these merged ecological multi-interaction networks [13].

Theoretical approaches to the study of coupled antagonism–mutualism networks have suggested that ecological networks involving different types of interactions would promote community robustness to perturbations [2]. We extended this view by evaluating whether an empirical, species-rich network involving multiple interaction types, but all mutualisms, would also show evidence of a structure that promotes robustness. We hypothesize that the multiple interaction types in the same ecological network beget robustness in the system, more so than the effects of a single interaction type, owing to the increase in connectance or species richness [2,15]. Owing to the high diversity of species and interactions in the tropics, tropical ecosystems could give us a system to study structure–robustness relationships by merging different types of mutualistic subnetworks. In tropical environments, approximately 90% of the woody plant species depend on the interaction with pollinators and seed dispersers to complete their life cycles [16]. Moreover, in such environments, many plant species bear extrafloral nectaries (EFNs) to attract ants that protect their host plants against herbivory [17].

Specifically, we tackled the following issues. What is the structure of an ecological network combining different types of mutualistic interactions? Which mutualism types contribute most to the patterns of organization of a mutualistic network with multiple, coupled types of interactions? Are multi-interaction mutualistic networks more robust to loss of species than bipartite plant–animal mutualistic subnetworks? What are the effects of each of the three types of mutualism on the robustness of this multi-interaction network? We tested the hypothesis that merging different types of mutualistic subnetworks would increase robustness in the system by studying a species-rich multi-interaction network involving different types of mutualism (animal pollination, seed dispersal by frugivorous birds and antiherbivore defence by protective ants) sampled by us in a coastal tropical environment in Mexico [18–20].

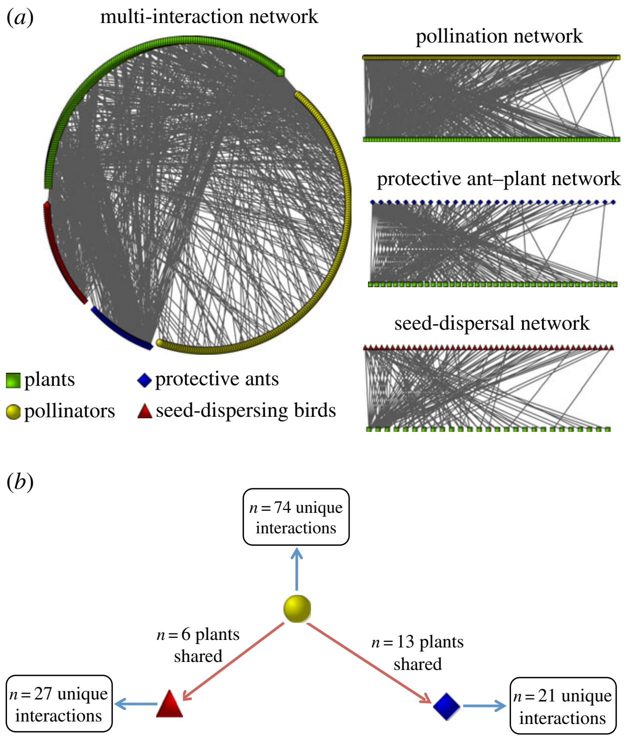

Figure 1. (*a*) A multi-interaction network of coupled plant–animal mutualisms recorded at Centro de Investigaciones Costeras La Mancha (CICOLMA), located on the central coast of Gulf of Mexico, state of Veracruz, Mexico. Each node represents one plant or animal species, and lines represent the presence of pairwise plant–animal interactions. (*b*) Number of unique and shared plant species between each network. (Online version in colour.)

2. Material and methods

(a) Datasets

Our study compiled a series of surveys carried out at Centro de Investigaciones Costeras La Mancha (CICOLMA), located on the central coast of the Gulf of Mexico, Veracruz, Mexico (19°36′ N, 96°22′ W; elevation less than 100 m) [18]. The dataset compiled by our research group involved three general types of plant–animal mutualistic interaction: pollination (by both insects and hummingbirds) [20], seed dispersal by frugivorous birds [19], and the protective mutualisms between ants and plants with EFNs [18]. Observations of all these plant–animal interactions were conducted by walking along six representative pre-established trails that covered the different vegetation associations present in the field station and surrounding area. Each of these broad categories includes a variety of ways in which species interact, but our goal here was to evaluate how the three main, fundamentally different ways in which plants interact mutualistically with animals fit together within a community. The resulting database is one of the largest compiled so far with respect to species richness, number of interactions and sampling effort. It comprises 141 plant species, 173 pollinator species, 46 frugivorous bird species and 30 ant species (figure 1). The dataset comprises 753 interactions in our plant–animal mutualistic multi-interaction network, 417 representing plant–pollinator interactions (55% of all recorded interactions), 128 plant–disperser interactions (17% of all recorded interactions) and 208 ant–plant interactions (28% of all recorded interactions). No plant species was involved in all three types of mutualistic interaction, and 122 plant species had only one type of mutualism (86.5% of the total plant species). Among the plant species with only one type of mutualistic interaction, plant–pollinator was the most common interaction ($n = 74$ species), followed by disperser–plant (27 species) and ant–plant (21 species). Nineteen plant species had two types of mutualistic interactions (13.5% of the total plant species): 13 interacted with ants and pollinators and six interacted with seed-dispersing birds and pollinators (figure 1*b*). No plant species interacted with both ants and seed-dispersing birds. No animal was involved in more than one type of mutualism. This study is therefore a step in evaluating the structure and dynamics of multiple forms of interaction networks in species-rich communities, combining a unique set of studies and years to assess the patterns that emerge at a single locality. In that sense, the results and conclusions can serve as a working hypothesis for future studies that may be in a better position to undertake multi-year, multi-interaction data collection and analyses that hold more variables constant. Detailed information on sampling of mutualistic interactions and study area is presented in electronic supplementary material, appendix S1.

(b) Data analysis

We used only qualitative networks (binary data), because this approach allowed us to compare the effect of each type of mutualism without bias based on different types of sampling. Moreover, this is a conservative approach, because characterization of interaction strengths is always difficult, especially when addressing distinct types of interaction modes over multiple years of sampling. Considering all plant–animal interactions compiled, we built an interaction matrix A, in which elements $a_{ij} = 1$ represent the presence of an interaction between plant species i and animal species j, and zero for no observed interaction [11]. Initially, we built a matrix for each type of mutualism (pollination, seed-dispersing birds and protective ants) and one matrix including all types of mutualisms together (mutualistic multi-interaction network). We then characterized the structure of each of the four mutualistic networks using the following network descriptors (calculated using the bipartite package in R): nestedness, modularity and robustness.

Nestedness (NODF-metric) describes a pattern of interaction in which species with fewer interactions often interact with a proper subset of the partners of more connected species [21]. Moreover, we tested whether within each network there were groups of species interacting more strongly with each other than with the species in the other groups in the network (i.e. modular pattern). For this, we calculated the modularity index (M) proposed by Barber [22] (range from 0, no subgroups, to 1, totally separated subgroups). Then, we generated random matrices ($n = 1000$ randomizations for each network) to test the significance of nestedness and modularity according to a null model in which the probability of an interaction occurring is proportional to the number of species with which a focal species is observed to interact [11]. We calculated the nestedness and modularity values, standardizing the difference in richness, connectance and heterogeneity of interactions among the networks, using z-scores to allow cross-network comparisons [21].

Because our mutualistic multi-interaction network was significantly nested and modular (see Results), we explored whether the three types of mutualisms contribute equally to these non-random patterns. For this analysis, we estimated the degree to which the interactions of plant or animal species increase or decrease the network's overall nestedness (cn_i) and compared it with our random expectations [23]. Additionally, we recorded the network roles of species in the modular structure by computing (i) the standardized within-module degree (z_i), which is a measure of the extent to which each species is connected to the other species in its module, and (ii) the among-module connectivity (c_i), which describes how evenly distributed are the interactions of a given species across modules [24]. We then used a one-way ANOVA with Bonferroni's correction for multiple comparisons to assess differences in the mean values of cn_{i}, c_i and z_i among the three types of mutualism. See electronic supplementary material, appendix S2 for details on methods of calculation for all metrics, descriptors and null model. Additionally, we used a principal component analysis (PCA) on the correlation matrix among k_i (number of interactions), c_i (among-module connectivity), z_i (standardized within-module degree) and cn_i (contribution to nestedness) values to synthesize the species' contributions to connectivity, nestedness and modularity according to Vidal *et al.* [25]. The first principal component (PC1) was used as a new descriptor summarizing species' contribution to network structure, and the higher scores assigned to each species indicate greater contributions to all analysed structural aspects described above. Biologically, species with a higher contribution to the network structure are those with many interactions in an environment and tend to have the highest niche overlap.

Robustness (R) of each of the two trophic levels (plants and animals) to the loss of species of the other trophic level was calculated based on the area below the extinction curve after simulations of cumulative removals of species from the network. Robustness values range from 0 (less robust network) to 1 (more robust network) [26]. We removed either plants or animals from networks based on three different extinction scenarios: (i) systematic removal from least to most connected species (e.g. expected by differences in abundance among species, where less abundant species have a higher extinction risk); (ii) systematic removal from most to least connected species (e.g. expected in a catastrophic scenario, where most connected species have a higher extinction risk); and (iii) random species deletion, which represents a benchmark (null model) to compare with the two types of systematic removals. Afterwards, we compared the values of robustness between the multi-interaction network and the three independent mutualistic subnetworks: pollination, seed-dispersing birds and protective ants. Therefore, if the multi-interaction network has higher values of robustness than each mutualistic subnetwork, then these mutualistic subnetworks together could contribute to the robustness of the multi-interaction network over and above their individual contribution. The measure of robustness as performed here assumes that if all the mutualistic partners of one species for a given type of interaction were removed, but not the mutualist partners for another type of interaction, then this species would still persist. In reality, it is possible that a species might require different types of mutualisms simultaneously in order to persist. However, many of these mutualistic interactions are 'facultative' (i.e. characterized by low specificity), so that the loss of an interaction involves a fitness reduction but not necessarily extinction in ecological time. This approach does not necessarily represent real extinctions in nature, but is a first approximation for understanding the robustness of networks to loss of species in different extinction scenarios.

We further analysed how each of the three types of mutualism contributes to the architecture and robustness of our mutualistic multi-interaction network by removing the central core of highly interacting plant and animal species from the multi-interaction network and from each subnetwork independently. We removed only the central core of highly interacting species because these are species that are relatively more important than others for maintaining community structure (high number of interactions), and have the potential to drive the ecological and coevolutionary dynamics within species-rich networks [12]. For this analysis, we first defined the core species of a certain network as the species with a standardized degree higher than 1, following Dáttilo *et al.* [27] (electronic supplementary material, appendix S3 and S4). In other words, a core species has more interactions compared with the mean (weighted by the standard deviation) number of the interactions of species in the network. We then performed four independent analyses in which we removed the core species of the multi-interaction network or of each of the three subnetworks, and quantified the change in the multi-interaction network descriptors. We expected

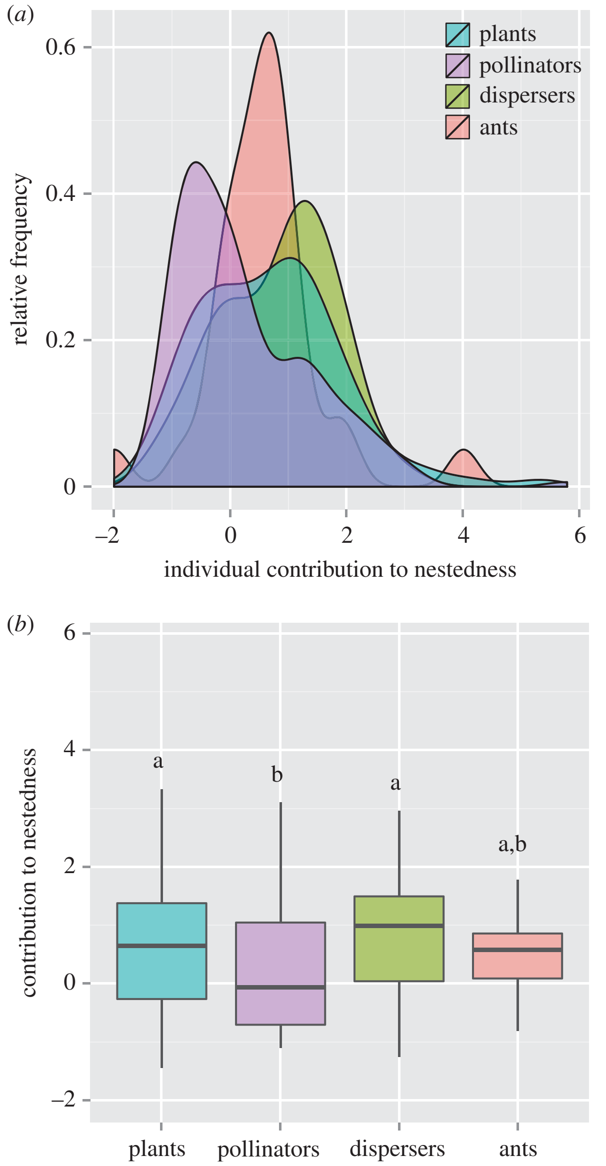

Figure 2. (*a*) Relative frequency and (*b*) the absolute values of individual nestedness contribution for all species within each of the four groups of partners (plants, pollinators, seed-dispersal birds and protective ants) in the mutualistic multi-interaction network. Boxplots sharing the same case letters are not significantly different according to *post hoc* tests. (Online version in colour.)

that removing the core species would cause a decrease in nestedness and robustness, and an increase in modularity, mainly because the exclusion of this central core of highly interacting species will disconnect modules within the network. For each core species removal analysis, we performed 100 simulations in which we randomly removed the same number of species and calculated the network descriptors for the 100 randomly species-rarefied networks. With those simulations, we tried to answer the following question: do the core species of a given network contribute more to the multi-interaction network structure and robustness than randomly chosen species from that network? We quantified the contribution of the core species to each network descriptor by calculating standardized (i.e. z-score) network descriptors and p-values, using the randomly rarefied networks (details in electronic supplementary material, appendix S3). We accounted for the changes in network connectance by performing four additional analyses in which we removed only the links between the core species and quantified the change in the multi-interaction network descriptors (electronic supplementary material, appendix S3).

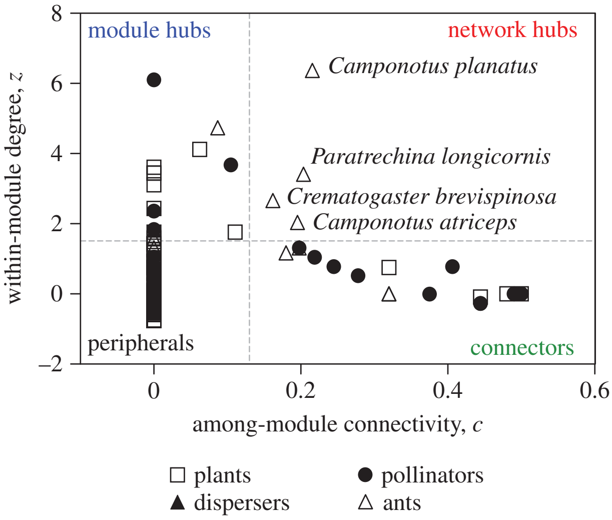

Figure 3. Network roles of different species of plants, pollinators, seed-dispersal birds and protective ants in the multi-interaction network. Within-module degree (z) describes the standardized number of interactions of a species compared with other species in its module. Among-module connectivity (c) describes the distribution of interactions of a given species across partner species in different modules. (Online version in colour.)

3. Results

Our multi-interaction network exhibited a significantly nested ($\text{NODF}_{\text{s.d.}} = 14.77$) and modular ($M_{\text{s.d.}} = 12.01$) pattern of interactions ($p < 0.05$). All three mutualistic subnetworks were also significantly nested (pollination: $\text{NODF}_{\text{s.d.}} = 10.57$, seed-dispersing birds: $\text{NODF}_{\text{s.d.}} = 6.07$ and protective ants: $\text{NODF}_{\text{s.d.}} = 9.60$). However, only the pollination subnetwork was significantly modular (pollination: $M_{\text{s.d.}} = 3.01$, seed-dispersing birds: $M_{\text{s.d.}} = -0.33$ and protective ants: $M_{\text{s.d.}} = -2.00$). Only a few animal species contributed strongly to the nested pattern within each individual network (figure 2*a*; electronic supplementary material, appendix S3). Animal species also differed greatly in the degree to which they contributed to nestedness in the mutualistic multi-interaction network (ANOVA: $F_{3,387} = 5.556$; $p < 0.001$). In general, seed-dispersing birds (mean ± s.e.: 0.83 ± 0.14) and plants (0.71 ± 0.08) contributed more to nestedness than pollinators (0.23 ± 0.08) (figure 2*b*). Protective ants (0.58 ± 0.18), seed-dispersing birds, plants and pollinators contributed equally to nestedness.

For the multi-interaction network, contributions to modularity also differed greatly among plant and animal species. Most plant and animal species were peripherals ($n = 129$ plant species, 159 pollinator species, 45 seed-dispersing birds and 21 ant species), followed by connectors ($n = 10$ pollinator species, four plant species and three ant species) and module hubs ($n = 8$ plant species, four pollinator species, two ant species and one seed-dispersing bird). Only four species, all ants, were network hubs (figure 3). We found no significant differences in the among-module connectivity (c) values between the four groups (plants, pollinators, seed dispersers and ants, $p = 0.14$). However, within-module degree (z) values differed among the four groups ($p < 0.001$). When compared separately, z-values did not differ between plants and pollinators ($p > 0.05$), plants and seed-dispersing birds ($p > 0.05$), and pollinators and seed-dispersing birds ($p > 0.05$). Ants had higher z-values (mean ± s.e.: 0.881 ±

Table 1. Robustness (*R*) of plants and animals to the loss of species based on three different extinction scenarios: (i) systematic removal from least to most connected species; (ii) systematic removal from most to least connected species and (iii) random species deletion, calculated for the original mutualistic networks with the central core (CC) of highly interacting species and for the resultant interaction networks after removal of the central core of highly interacting species (without CC).

mutualistic network	trophic level	least to most connected species		most to least connected species		random species deletion	
		with CC	without CC	with CC	without CC	with CC	without CC
multi-interaction network	plants	0.886	0.775	0.319	0.357	0.655	0.575
	animals	0.934	0.822	0.411	0.494	0.731	0.678
seed-dispersal network	plants	0.904	0.871	0.323	0.326	0.678	0.643
	animals	0.909	0.930	0.427	0.419	0.710	0.724
protective ant–plant network	plants	0.927	0.881	0.481	0.304	0.791	0.651
	animals	0.957	0.929	0.459	0.438	0.790	0.707
pollination network	plants	0.869	0.781	0.292	0.378	0.619	0.617
	animals	0.923	0.830	0.418	0.473	0.709	0.719

0.293) than plants (-0.012 ± -0.084, $p < 0.05$), pollinators (-0.152 ± 0.054, $p < 0.05$) and seed-dispersing birds (0.063 ± 0.072, $p < 0.05$).

In addition, we found that species differed strongly in their contribution to the organization of the multi-interaction network, and that only a few species contributed to the structuring of these patterns in the multi-interaction network (electronic supplementary material, appendix S5). The first principal component (PC1) resulting from the PCA was positively associated with k_i, cn_i, z_i and c_i, retaining much of the information provided by network measurements (96.1%). We found that k_i, z_i and cn_i were almost always positively correlated with each other (Pearson's $r > 0.42$, $p < 0.0001$). In other words, plant and animal species with many links tend to establish interactions within modules and to exhibit a greater contribution to nestedness. Moreover, these species tended to be classified as module hubs (electronic supplementary material, appendix S6). A list of all species recorded in this study with their values of contribution to network structure is presented in electronic supplementary material, appendix S6.

We found that the robustness to loss of species varied disproportionately among the three different mutualistic networks (pollination, seed-dispersal or protective ant–plant networks) and over different extinction scenarios (table 1). In general, the multi-interaction network was not greater in robustness compared with each independent mutualistic network. In fact, the protective ant–plant network was the most robust to loss of species across all models of extinction.

As expected, the removal of the central core of the multi-interaction network decreased nestedness (electronic supplementary material, appendix S7). This change in nestedness was significant relative to random species removal (electronic supplementary material, appendix S7). Removing either the core of the pollination subnetwork or the protective ants subnetwork significantly decreased nestedness (electronic supplementary material, appendix S7). Removal of the seed-dispersal core decreased modularity (electronic supplementary material, appendix S7). Overall, removal of the multi-interaction network, pollination or seed-dispersal core species significantly decreased network robustness, except for the most to least connected species extinction scenario (table 2). Surprisingly, for the most to least connected species extinction scenario, robustness greatly increased after removal of the core species (table 2). Finally, removal of the protective ants core had an overall weak effect on network robustness except for two extinction scenarios (table 2: random animal extinction and most to least connected animal extinction). Results for the core links removal simulations are presented in electronic supplementary material, appendix S8 and S9.

4. Discussion

Our study shows that the overall organization of mutualistic interactions involving plants and animals depends, in part, on the types of mutualism in which the plants participate (pollination by animals, seed dispersal by birds and plant protection by ants), and how these species integrate in the multi-interaction network. In general, we found that our mutualistic network with multiple interaction types exhibited a nested and modular pattern of species interactions. Seed-dispersing birds and plants contributed more to nestedness than did pollinators, whereas ants tended to decrease modularity. Additionally, we found that the multi-interaction network did not promote community robustness over different simulated scenarios of species extinction compared with each of the three independent mutualistic networks, possibly owing to low overlap of mutualism types among plant species. However, when the central core of the multi-interaction network is removed the network robustness collapses (except for the most to least extinction scenario). Moreover, few species contribute to the multi-interaction network central core, and loss of these species results in network structures that are likely more vulnerable than networks with these generalists. These results indicate that merging different types of mutualism can change our estimates of the relative importance of the species to the organization of mutualistic networks when compared with isolated networks. Our results suggest that the organization of coupled mutualistic networks within larger

Table 2. Robustness values, standardized robustness values (*z*-score) with the associated *p*-values (see electronic supplementary material, appendix S3 for explanation) and the number of species removed for each simulation analysis of core species removal. The network robustness values were quantified for the intact multi-interaction network (no core removal) or for the multi-interaction network after the removal of its own core (general core removal) or the core of one of the subnetworks (pollination core removal, ants core removal and dispersal core removal).

extinction scenario	trophic level for extinction	core removal scenario	robustness value	robustness *z*-score	*p*-value	number of species removed
random	plants	no core removal	0.651	—	—	0
		general core removal	0.574	−9.096	0	39
		pollination core removal	0.624	−3.746	0	27
		ants core removal	0.640	−0.982	0.18	10
		dispersal core removal	0.638	−2.510	0	10
random	animals	no core removal	0.728	—	—	0
		general core removal	0.679	−5.211	0	39
		pollination core removal	0.718	−1.330	0.1	27
		ants core removal	0.707	−2.408	0.03	10
		dispersal core removal	0.733	1.712	0.97	10
least to most connected	plants	no core removal	0.884	—	—	0
		general core removal	0.774	−11.237	0	39
		pollination core removal	0.769	−13.602	0	27
		ants core removal	0.884	−0.068	0.38	10
		dispersal core removal	0.858	−8.051	0	10
least to most connected	animals	no core removal	0.934	—	—	0
		general core removal	0.835	−8.591	0	39
		pollination core removal	0.818	−11.023	0	27
		ants core removal	0.932	0.238	0.57	10
		dispersal core removal	0.907	−5.434	0	10
most to least connected	plants	no core removal	0.320	—	—	0
		general core removal	0.354	3.813	1	39
		pollination core removal	0.382	7.212	1	27
		ants core removal	0.303	−1.783	0.04	10
		dispersal core removal	0.328	2.401	0.99	10
most to least connected	animals	no core removal	0.409	—	—	0
		general core removal	0.499	5.567	1	39
		pollination core removal	0.485	5.770	1	27
		ants core removal	0.432	2.593	1	10
		dispersal core removal	0.420	2.134	0.99	10

and more diversified multi-interaction networks could be essential to the maintenance of ecological communities as shown in previous studies on multi-interaction networks [13,28,29], once the multi-interaction network exhibited non-random patterns that promote persistence of biological communities.

When we evaluated each type of mutualism as independent networks, we observed that all networks were significantly nested. By contrast, only the pollination network exhibited a modular pattern of interaction. The structure of these independent networks was consistent with previously described patterns in the literature (see [11,29–31]). In addition, despite our large sampling effort, no species of plant was involved in all three types of mutualism, and most plants are involved in only one type of mutualism. This is probably because plants with EFNs are only a minor subset of the plant community in a given environment, and the presence of ants foraging on these plants could repel pollinators and frugivorous birds by aggressive attacks, affecting pollination efficiency [17]. Moreover, there is evidence that some plants with biotic pollination tend to have abiotic seed dispersal and vice versa in the study area (W.D. and V.R-G. 2014, personal data), which also decreases the shared record of mutualistic interactions, suggesting a trade-off between animal pollination and animal dispersal.

Probably owing to high specialization of species interactions within each mutualism and the low overlap between types of mutualisms, the multi-interaction network did not promote community robustness over different extinction scenarios. Thus, our original hypothesis was not supported. However, our mutualistic multi-interaction network was both nested and modular. This combined structure plays an important role in the persistence of biological communities, beyond the ability to support high levels of biodiversity [7,32,33] for two main reasons. First, because there is a core of highly connected species in nested networks, when one of the central core species goes extinct other species can 'dampen' the system [34,35]. Second, in modular networks, any perturbation that occurs within a module decreases the likelihood of cascading effects propagating to other modules [13,36,37]. Therefore, both non-random patterns may enhance the stability of plant–animal mutualisms in complementary ways [38].

Nestedness describes the organization of niche breadth, in which more nested networks tend to have the highest niche overlap [39]. Here, we observed that frugivorous birds and protective ants were the mutualistic agents that contribute most to nestedness. This is possibly related to interactions between plants and seed-dispersing birds, and plant and ants tending to be more functionally redundant and generalized compared with interactions with pollinators [40]. Moreover, ants also had an important role in network connectivity and in the robustness of the modular pattern, and were classified as network hubs. In this case, EFN is a seasonal resource and ants can use other resources available on foliage, which makes ant–plant interactions less specialized and more facultative [41], and therefore, more robust to loss of species over different extinction scenarios as shown in this study. Therefore, ant–plant interactions could have a remarkable impact on the architecture and robustness of mutualistic multi-interaction networks. However, the high plant specificity among pollinators could explain the high frequency of functional peripheral roles of pollinating species within the modular structure. Despite the importance of nestedness and modularity to the robustness and species coexistence in mutualisms, only a few species contributed to the structuring of these patterns in the multi-interaction network. Therefore, if the goal is to conserve mutualistic interactions within an environment, then a key task is to identify the 'keystone mutualists'. These species and their interactions play disproportionately important roles in the community either through many direct or indirect links to other species that help guarantee the persistence of a mutualistic community rich in species [42,43]. The loss of keystone mutualists has important consequences for the ecological and evolutionary dynamics of the system, because the extinction of these highly connected species can lead to co-extinctions of other species and reduce the long-term overall species persistence [23,25].

Using different approaches, some recent studies have highlighted the importance of identifying key positions within ecological networks (i.e. highly connected species) in order to conserve the ecological and evolutionary processes in an environment [44,45]. This is mainly because different types of ecological networks vary disproportionately in their robustness over different extinction scenarios and types of interactions [42]. We further explored the role of these highly connected species in structuring and promoting robustness in the multi-interaction network by removing the central core of highly interacting species. We observed that the core species of our mutualistic multi-interaction network, as well as the core species of our pollination and protective ants subnetworks contribute to a nested pattern of interactions. These patterns, in turn, promoted robustness to most extinction scenarios studied here. However, the removal of the core species of our multi-interaction network or of each of the three types of mutualisms led to an increase in network robustness in a scenario where the most connected species have a higher extinction risk. Therefore, environmental impacts that selectively affect these highly connected core species [25] may disrupt network organization and make the network very susceptible to future impacts of the same kind that target the remaining species in the community. We did not observe important changes in network robustness when removing only the interactions between the core species from any of our networks. Thus, we suggest that interactions between core and periphery species, rather than just the interactions among the core species, are important to maintain the multi-interaction network robust to extinctions.

Finally, our results suggest that studying only one type of mutualistic network does not necessarily lead us to erroneous conclusions about system stability. Many of the network statistics have similar values in the multi- versus single-mutualism type networks. Moreover, the multi-mutualism network is no more robust than the mutualistic subnetworks considered individually. However, multi-interaction networks can more clearly show the relative importance of the species to maintenance of ecological communities, mainly because species can differ in their contributions to network structure. In summary, our study provides one of the few empirical examples available in the literature highlighting the importance of combining different interaction types within ecological multi-interaction networks to better characterize their architectural patterns of plant–animal mutualisms of free-living species. The next step is to assess the determinants of these mutualistic multi-interaction network structures, such as phylogenetic constraints and trait matching, and how they vary over space and time, and whether these multi-interaction networks respond collectively to perturbations.

Data accessibility. The datasets and codes supporting this article are deposited in Figshare (https://dx.doi.org/10.6084/m9.figshare.3443210.v2) and GitHub (https://github.com/lucaspdmedeiros/multi-network_core_removal).

Authors' contributions. All the authors conceived and designed the experiments, and wrote the paper. W.D., N.L.-R., R.O.-P. and L.P.M. performed the experiments and/or data analysis.

Competing interests. The authors declare no conflict of interest.

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References

1. Thompson JN. 2009 The coevolving web of life (American Society of Naturalists Presidential Address). *Am. Nat.* **173**, 125–140. (doi:10.1086/595752)
2. Mougi A, Kondoh M. 2012 Diversity of interaction types and ecological community stability. *Science* **337**, 349–351. (doi:10.1126/science.1220529)
3. Darwin C. 1859 *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. London, UK: John Murray.
4. Bascompte J, Jordano P. 2007 The structure of plant-animal mutualistic networks: the architecture of biodiversity. *Annu. Rev. Ecol. Evol. Syst.* **38**, 567–593. (doi:10.1146/annurev.ecolsys.38.091206.095818)
5. Montoya JM, Yvon-Durocher G. 2007 Ecological networks: information theory meets Darwin's entangled bank. *Curr. Biol.* **17**, R128–R130. (doi:10.1016/j.cub.2007.01.028)
6. Ings TC *et al.* 2009 Ecological networks—beyond food webs. *J. Anim. Ecol.* **78**, 253–269. (doi:10.1111/j.1365-2656.2008.01460.x)
7. Memmott J, Waser NM, Price MV. 2004 Tolerance of pollination networks to species extinctions. *Proc. R. Soc. Lond. B* **271**, 2605–2611. (doi:10.1098/rspb.2004.2909)
8. Tylianakis JM, Tscharntke T, Lewis OT. 2007 Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature* **445**, 202–205. (doi:10.1038/nature05429)
9. Dupont YL, Padrón B, Olesen JM, Petanidou T. 2009 Spatio-temporal variation in the structure of pollination networks. *Oikos* **118**, 1261–1269. (doi:10.1111/j.1600-0706.2009.17594.x)
10. Benítez-Malvido J, Martínez-Falcón AP, Dáttilo W, del Val E. 2014 Diversity and network structure of invertebrate communities associated to *Heliconia* species in natural and human disturbed tropical rain forests. *Glob. Ecol. Conserv.* **2**, 107–117. (doi:10.1016/j.gecco.2014.08.007)
11. Bascompte J, Jordano P, Melián CJ, Olesen JM. 2003 The nested assembly of plant–animal mutualistic networks. *Proc. Natl Acad. Sci. USA* **100**, 9383–9387. (doi:10.1073/pnas.1633576100)
12. Guimarães Jr PR, Jordano P, Thompson JN. 2011 Evolution and coevolution in mutualistic networks. *Ecol. Lett.* **14**, 877–885. (doi:10.1111/j.1461-0248.2011.01649.x)
13. Melián CJ, Bascompte J, Jordano P, Krivan V. 2009 Diversity in a complex ecological network with two interaction types. *Oikos* **118**, 122–130. (doi:10.1111/j.1600-0706.2008.16751.x)
14. Fontaine C, Guimarães PR, Kéfi S, Loeuille N, Memmott J, van der Putten W, van Veen F, Thébault E. 2011 The ecological and evolutionary implications of merging different types of networks. *Ecol. Lett.* **14**, 773–781. (doi:10.1111/j.1461-0248.2011.01688.x)
15. Sauve AMC, Fontaine C, Thébault E. 2014 Structure–stability relationships in networks combining mutualistic and antagonistic interactions. *Oikos* **123**, 378–384. (doi:10.1111/j.1600-0706.2013.00743.x)
16. Jordano P. 2000 Fruits and frugivory. In *Seeds: the ecology of regeneration in natural plant communities* (ed. M Fenner), pp. 125–166. Wallingford, UK: Commonwealth Agricultural Bureau International.
17. Rico-Gray V, Oliveira PS. 2007 *The ecology and evolution of ant-plant interactions*. Chicago, IL: University of Chicago Press.
18. Rico-Gray V. 1993 Use of plant-derived food resources by ants in the dry tropical lowlands of coastal Veracruz, Mexico. *Biotropica* **25**, 301–315. (doi:10.2307/2388788)
19. Ortiz-Pulido R, Laborde J, Guevara S. 2000 Frugivoría por aves en un paisaje fragmentado: consecuencias en la dispersión de semillas. *Biotropica* **32**, 473–488. (doi:10.1111/j.1744-7429.2000.tb00494.x)
20. Hernández-Yáñez H, Lara-Rodríguez N, Díaz-Castelazo C, Dáttilo W, Rico-Gray V. 2013 Understanding the complex structure of a plant-floral visitor network from different perspectives in coastal Veracruz, Mexico. *Sociobiology* **60**, 329–336. (doi:10.13102/sociobiology.v60i3.329-336)
21. Almeida-Neto M, Guimaraes P, Guimaraes PR, Loyola RD, Urlich W. 2008 A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. *Oikos* **117**, 1227–1239. (doi:10.1111/j.2008.0030-1299.16644.x)
22. Barber MJ. 2007 Modularity and community detection in bipartite networks. *Phys. Rev. E.* **76**, 006102. (doi:10.1103/PhysRevE.76.066102)
23. Saavedra S, Stouffer DB, Uzzi B, Bascompte J. 2011 Strong contributors to network persistence are the most vulnerable to extinction. *Nature* **478**, 233–235. (doi:10.1038/nature10433)
24. Olesen JM, Bascompte J, Dupont YL, Jordano P. 2007 The modularity of pollination networks. *Proc. Natl Acad. Sci. USA* **104**, 19 891–19 896. (doi:10.1073/pnas.0706375104)
25. Vidal MM, Hasui E, Pizo MA, Tamashiro JY, Silva WR, Guimarães PRJr. 2014 Frugivores at higher risk of extinction are the key elements of a mutualistic network. *Ecology* **95**, 3440–3447. (doi:10.1890/13-1583)
26. Burgos E, Ceva H, Perazzo RP, Devoto M, Medan D, Zimmermann M, Delbue AM. 2007 Why nestedness in mutualistic networks? *J. Theor. Biol.* **249**, 307–313. (doi:10.1016/j.jtbi.2007.07.030)
27. Dáttilo W, Guimarães PR, Izzo TJ. 2013 Spatial structure of ant–plant mutualistic networks. *Oikos* **122**, 1643–1648. (doi:10.1111/j.1600-0706.2013.00562.x)
28. Albrecht J, Berens DG, Jaroszewicz B, Selva N, Brandl R, Farwig N. 2014 Correlated loss of ecosystem services in coupled mutualistic networks. *Nat. commun.* **5**, 3810. (doi:10.1038/ncomms4810)
29. Kéfi S, Berlow EL, Wieters EA, Joppa LN, Wood SA, Brose U, Navarrete SA. 2015 Network structure beyond food webs: mapping non-trophic and trophic interactions on Chilean rocky shores. *Ecology* **96**, 291–303. (doi:10.1890/13-1424.1)
30. Mello MAR, Marquitti FMD, Guimarães PR, Kalko EKV, Jordano P, de Aguiar MAM. 2011 The modularity of seed dispersal: differences in structure and robustness between bat– and bird–fruit networks. *Oecologia* **167**, 131–140. (doi:10.1007/s00442-011-1984-2)
31. Dáttilo W, Díaz-Castelazo C, Rico-Gray V. 2014 Ant dominance hierarchy determines the nested pattern in ant–plant networks. *Biol. J. Linn. Soc.* **113**, 405–414. (doi:10.1111/bij.12350)
32. Okuyama T, Holland JN. 2008 Network structural properties mediate the stability of mutualistic communities. *Ecol. Lett.* **11**, 208–216. (doi:10.1111/j.1461-0248.2007.01137.x)
33. Bastolla U, Fortuna MA, Pascual-García A, Ferrera A, Luque B, Bascompte J. 2009 The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature* **458**, 1018–1020. (doi:10.1038/nature07950)
34. Thébault E, Fontaine C. 2010 Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* **329**, 853–856. (doi:10.1126/science.1188321)
35. Dáttilo W. 2012 Different tolerances of symbiotic and nonsymbiotic ant–plant networks to species extinctions. *Net. Biol.* **2**, 127–138.
36. Krause AE, Frank KJ, Mason DM, Ulanowicz RE, Taylor WW. 2003 Compartments revealed in food-web structure. *Nature* **426**, 282–285. (doi:10.1038/nature02115)
37. Teng J, McCann KS. 2004 Dynamics of compartmented and reticulate food webs in relation to energetic flow. *Am. Nat.* **164**, 85–100. (doi:10.1086/421723)
38. Fortuna MA, Stouffer DB, Olesen JM, Jordano P, Mouillot D, Krasnov BR, Poulin R, Bascompte J. 2010 Nestedness versus modularity in ecological networks: two sides of the same coin? *J. Anim. Ecol.* **79**, 811–817. (doi:10.1111/j.1365-2656.2010.01688.x)
39. Blüthgen N. 2010 Why network analysis is often disconnected from community ecology: a critique and an ecologist's guide. *Basic Appl. Ecol.* **11**, 185–195. (doi:10.1016/j.baae.2010.01.001)
40. Blüthgen N, Menzel F, Hovestadt T, Fiala B, Blüthgen N. 2007 Specialization, constraints, and conflicting interests in mutualistic networks. *Curr. Biol.* **17**, 341–346. (doi:10.1016/j.cub.2006.12.039)
41. Schoereder JH, Sobrinho TG, Madureira MS, Ribas CR, Oliveira PS. 2010 The arboreal ant community visiting extrafloral nectaries in the Neotropical cerrado savanna. *Terr. Arthropod Rev.* **3**, 3–27. (doi:10.1163/187498310X487785)
42. Gilbert LE. 1980 Food web organization and the conservation of neotropical diversity.

In *Conservation biology* (eds ME Soule, BA Wilcox), pp. 11–33. Sunderland, MA: Sinauer Associates.

43. Terborgh JW. 1986 Keystone plant resources in the tropical forest. In *Conservation biology: an evolutionary-ecological perspective* (eds ME Soule, BA Wilcox), pp. 330–344. Sunderland, MA: Sinauer Associates.
44. Pocock MJO, Evans DM, Memmott J. 2012 The robustness and restoration of a network of ecological networks. *Science* **335**, 973–977. (doi:10.1126/science.1214915)
45. Mello MAR, Costa LF, Rodrigues FA, Marquitti FMD, Kissling WD, Sekercioglu CH, Kalko EKV. 2015 Keystone species in seed dispersal networks are mainly determined by dietary specialization. *Oikos* **124**, 1031–1039. (doi:10.1111/oik.01613)