

Research article

Cheating interactions favor modularity in mutualistic networks

Lucas A. Camacho¹ , Cecilia Siliansky de Andreazzi² , Lucas P. Medeiros³ , Irina Birskis-Barros⁴ , Carine Emer⁵ , Carolina Reigada⁶  and Paulo R. Guimarães Jr.⁷ 

¹Programa de Pós-graduação em Ecologia, Depto de Ecologia – Inst. de Biociências, USP, São Paulo, SP, Brasil

²Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, Inst. Oswaldo Cruz, Fundação Oswaldo Cruz, Manguinhos, Rio de Janeiro, RJ, Brasil

³Dept of Civil and Environmental Engineering, MIT, Cambridge, MA, USA

⁴School of Natural Sciences, Univ. of California, Merced, EUA

⁵Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rua Pacheco Leão, 915. Jardim Botânico, Rio de Janeiro - RJ. Brasil. CEP 22460-000

⁶Centro de Ciências Biológicas e da Saúde, Depto de Ecologia e Biologia Evolutiva, Univ. Federal de São Carlos, UFSCAR, São Carlos, SP, Brasil

⁷Dept de Ecologia – Inst. de Biociências, USP, Rua do Matão, São Paulo, SP, Brasil

Correspondence: Lucas A. Camacho (lucas.camacho@alumni.usp.br)

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A fundamental fact about mutualisms is that these mutually beneficial interactions often harbor cheaters that benefit from the use of resources and services without providing any positive feedback to other species. The role of cheaters in the evolutionary dynamics of mutualisms has long been recognized, yet their broader impacts at the community level, beyond species they directly interact with, is still poorly understood. Because mutualisms form networks often involving dozens of species, indirect effects generated by cheaters may cascade through the whole community, reshaping trait evolution. Here, we study how cheating interactions can influence coevolution in mutualistic networks. We combined a coevolutionary model, empirical data on animal–plant mutualistic networks and numerical simulations to show that high trait disparity emerges as a consequence of the negative effect of cheaters on victim fitness, which in turn fuels selection favoring victim traits that are increasingly different from the cheaters' traits. Intermediate levels of cheating interactions in a network can lead to the formation of groups of species phenotypically similar to each other and distinct from species in other groups, generating clustered trait patterns. The resulting clustered trait pattern, in turn, changes the pattern of interaction in simulated networks, fostering the formation of modules of interacting species and reducing nestedness. Our results indicate that directional selection imposed by cheaters on their victims counteracts selection for trait convergence imposed by mutualists, leading to the emergence of modules of phenotypically similar interacting species but phenotypically distinct from other modules. Based on these results, we suggest that cheaters might be a fundamental missing element for our understanding of how multispecies selection shapes the trait distribution and structure of mutualistic networks.

Keywords: coevolution, ecological network, modularity, mutualism, network structure



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Introduction

Selection imposed by ecological interactions is one of the main forces impacting phenotypic patterns observed across levels of biological organization (Thompson 2005). Selection imposed by ecological interactions partially shapes trait matching among interacting individuals (Zhang et al. 2013), trait distributions within interacting populations (Bronstein et al. 2003), trait variation across populations of the same species (Thompson 2005), and trait patterns at the community level (Strauss and Irwin 2004). At the community level, ecological interactions can be a driving force of selection leading to trait convergence (Wilson et al. 2012) and trait disparity (Siepielski and Benkman 2010). In this sense, theoretical and empirical results indicate that distinct ecological interactions (e.g. mutualistic, antagonistic) may favor different trait outcomes due to coevolution, i.e. the reciprocal evolutionary change between interacting species (Thompson 1994, Yoder and Nuismer 2010, Wechsler and Bascompte 2022).

Mutualistic interactions, for example, can favor trait matching (Thompson 2005), which is the trait similarity between interacting species, e.g. the size of the proboscis of a pollinator matching the depth of the flower it pollinates. Mutualisms may also generate other trait patterns, such as traits of extraordinary proportions when there is a coevolutionary arms race (Anderson and Johnson 2008). However, trait matching is observed in a variety of mutualistic systems, such as pollination by flies and bees (Santamaría and Rodríguez-Girónés 2007, Zhang et al. 2013), seed dispersal by bats and birds (Mello et al. 2011, Galetti et al. 2013) and Müllerian rings in velvet ants (Wilson et al. 2012). Hence, trait matching is an expected outcome of reciprocal selection that may favor higher interaction efficiency in mutualisms (Thompson 1994, Zhang et al. 2013). In contrast, under antagonistic interactions, such as among parasites and their hosts, higher similarity between parasite and host traits leads to a higher fitness to the parasite and lower fitness to the host, while trait mismatching leads to a higher fitness to the host but a lower fitness to the parasite. In fact, antagonisms may lead to coevolutionary dynamics such as alternation and escalation (Nuismer et al. 1999), which can result in the maintenance or increase in trait variation within and across species (Thompson 2005, Andreazzi et al. 2017).

Antagonisms and mutualisms are not isolated in nature, in fact, they can coexist in many ways. Studies have explored the effects of combining mutualisms and antagonisms on population dynamics (Melián et al. 2009), community stability (Wilson et al. 2003) and network structure (Genini et al. 2010). In some cases, individuals of the same species can be antagonistic or mutualistic partners of the same interacting species, e.g. seed-caching rodents can be either seed predators, or seed dispersers of the same plant species (Loayza et al. 2014). In other cases, individuals may act as antagonists of some species and as mutualists of others (Gómez et al. 2014, 2018, Montesinos-Navarro et al. 2017). Finally, there are species that are specialized cheaters, exploiting mutualistic interactions without providing any benefit in return, like

some fig wasps (Bronstein 2001). Thus, selection may favor the evolution of life histories that exploit the resources and services provided by mutualistic partners without giving back any benefits, creating cheating interactions (Bronstein 2001, Vieira et al. 2003).

The theory on how cheating interactions may affect evolution in mutualisms is growing (Wechsler and Bascompte 2022), especially when considering how multiple interactions could fuel different coevolutionary outcomes with distinct local adaptations. For example, the interaction between the plant *Lithophragma parviflorum* and the floral-parasitic moth *Gryea politella* changes from parasitism to mutualism if the other pollinators of *L. parviflorum* are absent in the community, affecting the coevolutionary dynamics of both species (Thompson and Cunningham 2002). By exploring the role of cheaters in three-species systems, we progressed in our understanding on how cheaters may fuel novel evolutionary dynamics (Anderson 2005). A next step in our understanding of the importance of cheaters in mutualisms is to explore what are the evolutionary roles that cheaters play in species-rich communities. In species-rich communities, networks generate pathways connecting species that do not interact directly with each other, allowing evolutionary cascades to affect how species traits evolve (Guimarães et al. 2017). Yet, the impact of cheating interactions at the community level, beyond their impact on the species they directly interact with, is still poorly understood.

Here, we combine a single trait coevolutionary model, empirical networks of species interactions and numerical simulations to investigate how cheating interactions may affect coevolution in mutualistic networks (Fig. 1a). Specifically, we explore two main questions: 1) how does the frequency of cheating interactions affect coevolutionary dynamics? Because arms race dynamics are favored by directional antagonistic selection, we expect a higher trait mismatch in scenarios with elevated frequencies of cheating interactions. 2) what is the effect of cheating interactions on the structure of mutualistic networks? By assuming that cheating interactions may favor arms race dynamics, we expect higher trait disparity fueled by the presence of cheaters. Assuming that trait matching is essential for interactions to occur, higher trait disparity will result in a sparse network in which we observe less interactions compared to a community without cheaters. By disrupting interactions, trait disparity may generate clusters of species with similar traits, leading to a disruption of nestedness and the emergence of modularity (Fig. 1b).

Methods

Evolutionary model

Our discrete-time, evolutionary model describes how the average trait of a species i , Z_i , evolves due to reciprocal selection imposed by ecological interactions and other environmental factors (e.g. abiotic conditions). In our model, the selection differential, S , and the additive genetic variance

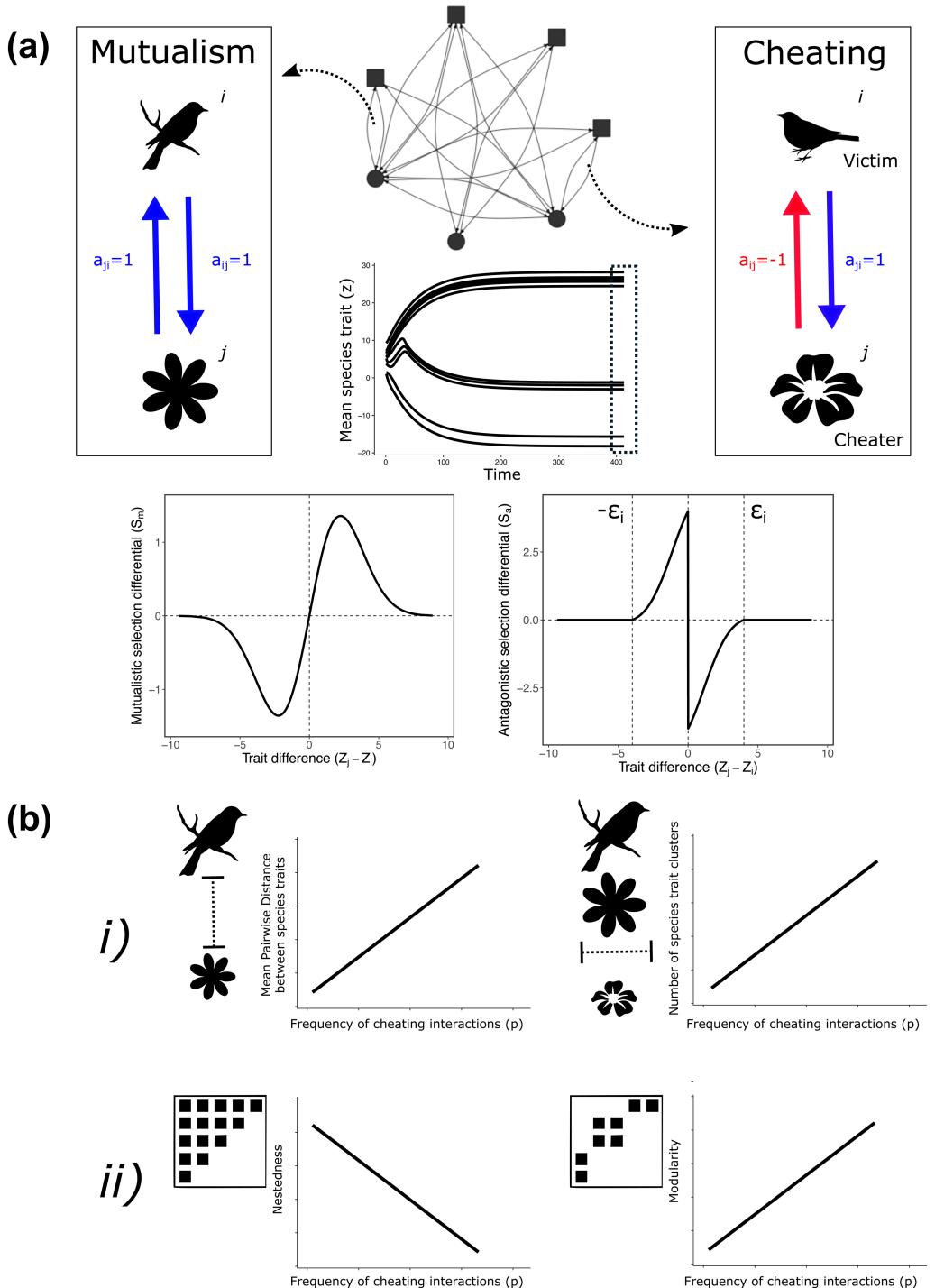


Figure 1. Interplay between mutualism and cheating interactions may drive the coevolutionary process in mutualistic networks. (a) Using a network of interactions (squares are animals and circles are plants), we define two types of interactions between species i and j : mutualism with positive effects and cheating as positive and negative effects together. Then, we simulate how the species mean trait value Z changes in time due to coevolution, as shown in the central plot. The simulations run based on the selection differentials shown in the graphs where mutualisms will favor trait matching for both i and j species (left plot) and cheating interactions will favor trait matching for the cheater j (left plot) and trait mismatch for the victim i (right plot). (b) We show our expectations for 1) trait matching and 2) network structure considering the frequency of cheating interactions. We test these predictions with numerical simulations using a trait-based coevolution model and empirical mutualistic matrices of interactions.

of the trait govern trait change across generations (Lande 1976). We assume that species abundances are at equilibrium and there is no ecological feedback in trait evolution. Having said that, we show that variation in abundances across species in a network does not qualitatively change the results we report here (Supporting information). We assume that S has three components potentially affecting the evolution of the trait Z_i : the selection imposed by 1) mutualisms, 2) cheating interactions and 3) an environmental factor (Supporting information). As a first approximation, we assume that, for a given interaction, species are either mutualistic partners or cheaters, but the same species i may behave as a cheater for species j but as a mutualistic partner to species k . A given species i may have multiple interactions and each interaction may contribute differently to selection, where the contribution of species j to selection on species i is described by $q_{ij}^{(t)}$. Thus, $q_{ij}^{(t)}$ represents the evolutionary effect between i and j and depends on the trait matching between these species as explained below.

The mutualism component, S_{m_i} is defined as the sum of selection effects caused by all mutualistic partners. Because exploiting victims also lead to fitness benefits to a given species, we added the selection effects imposed by the victims of species i on S_{m_i} . We assume that selection imposed by mutualism and from victims favors trait matching and we also assume that perfect trait matching between species i and j occurs if $|Z_j - Z_i| = 0$ (Guimarães et al. 2011). Thus, the total contribution of mutualistic interactions and exploitation of victims to selection on Z_i at time t is defined as:

$$S_{m_i} = \sum_{j=1, a_{ij}=1}^N q_{ij}^{(t)} \left(Z_j^{(t)} - Z_i^{(t)} \right) \quad (1)$$

where N is the total number of species in the community, a_{ij} is an element of the adjacency matrix \mathbf{A} , $q_{ij}^{(t)}$ is the contribution of partner j to selection on species i at time t and $Z_i^{(t)}$ is the mean trait of species i at time t . Elements in \mathbf{A} , a_{ij} , can be either 0 if there is no interaction between species i and j , or 1 if species j is a mutualistic partner or a victim of species i , or -1 if species j is a cheater. The summation condition ($j=1, a_{ij}=1$) ensures that the sum runs only over species that have mutualistic interactions with i or are exploited by i .

In the cheating component S_{a_i} , selection favors trait mismatch for the victim species i , defined as the species exploited by the cheater. The contribution of a cheater j for the selection on species i is given by $q_{ij}^{(t)}$. Thus, selection for trait mismatching of cheater j on victim i is given by:

$$S_{a_i} = \sum_{j=1, a_{ij}=-1}^N q_{ij}^{(t)} g_{ij}^{(t)} \quad (2)$$

where the summation condition ($j=1, a_{ij}=-1$) represents a sum only over species that are cheaters of i and $g_{ij}^{(t)}$ is a function of $Z_i^{(t)}$ and $Z_j^{(t)}$ given by Eq. 3:

$$g_{ij}^{(t)} = \begin{cases} 0 & \text{if } Z_j - Z_i < -\varepsilon_{ij} \\ Z_j - Z_i + \varepsilon_{ij} & \text{if } -\varepsilon_{ij} < Z_j - Z_i < \varepsilon_{ij} \\ Z_j - Z_i - \varepsilon_{ij} & \text{if } 0 < Z_j - Z_i < \varepsilon_{ij} \\ 0 & \text{if } Z_j - Z_i > \varepsilon_{ij} \end{cases} \quad (3)$$

where ε_{ij} represents a trait barrier (Santamaría and Rodríguez-Girónés 2007), preventing the antagonistic effects of the interaction to impose selective pressures on the victim. That is, we assume that if the trait difference between i and j is higher than or equal to ε_{ij} , then the cheater species imposes no selection on the victim, but the victim still imposes a positive effect on the cheater. This outcome resembles a commensalistic interaction with no effect for one of the individuals and a positive effect for the other individual. However, if trait difference between i and j is smaller than ε_{ij} , selection on victim i will favor the increase or decrease of trait values depending on $Z_j^{(t)} - Z_i^{(t)}$. Finally, we assume that the environmental component S_{e_i} is the combined effects of all other selective pressures, which favor an optimum environmental trait value for each species, θ_i :

$$S_{e_i} = \theta_i - Z_i^{(t)} \quad (4)$$

Combining the three selection components described above (Eq. 1, 2 and 4), the evolutionary change of Z_i from time t to $t+1$ is given by:

$$Z_i^{(t+1)} = Z_i^{(t)} + \varphi_i \left[(1 - \gamma_i) (S_{m_i} + S_{a_i}) + \gamma_i (S_{e_i}) \right] \quad (5)$$

in which φ_i ($\varphi_i > 0$) is a compound parameter formed by additive genetic variance ($\sigma_{G_z}^2$) and a scaling constant affecting the adaptive landscape ρ_i , which will result in a scaling factor $\varphi_i = \rho_i \sigma_{G_z}^2$ (Guimarães et al. 2017), and the S terms are the selection differentials. The parameter γ_i ($0 \leq \gamma_i \leq 1$) dictates the importance of ecological interactions versus environmental factors as selective pressures. Both φ_i and γ_i assume a single value in our simulations (Table 1) and sensitivity analysis show that these parameters do not qualitatively affect the overall patterns reported here (Supporting information).

The evolutionary effect $q_{ij}^{(t)}$ that affects the magnitude of trait change due to the mutualistic and cheating interactions is defined as the relative effect of species j on i :

$$q_{ij}^{(t)} = \frac{e^{-\alpha(Z_j^{(t)} - Z_i^{(t)})^2}}{\sum_{k, i \neq k} |a_{ik}| e^{-\alpha(Z_k^{(t)} - Z_i^{(t)})^2}} \quad (6)$$

where the parameter α controls the sensitivity of the evolutionary effect due to trait matching between species i and j where $|a_{ik}| = 1$ if there is a mutualistic or a cheating

Table 1. Variables and parameters of the model and their baseline values.

Parameter	Description	Baseline values
Z_i	Initial mean trait value of species i	$(0 \leq Z_i \leq 10)$
φ_i	Composed parameter formed by additive genetic variance and a scaling constant affecting the adaptive landscape, which will result in a single scaling factor	0.2 [0, 1]
ε_{ij}	Trait barrier for the cheating interaction between species i and j	5
γ_i	Strength of abiotic selection for trait change of species i	0.1 [0, 1]
θ_i	Z_i optimum value for the environmental selection	$(0 \leq \theta_i \leq 10)$
α	Sensitivity of evolutionary effect due to the trait matching between interacting species	0.2
p	Probability of a positive effect to become negative in a mutualistic network	$0.01 \leq p \leq 1$
b	Trait barrier for any interaction to happen between species in the network	7

interaction between i and k , and $|a_{ik}|=0$, otherwise. The numerator of Eq. 6 is the evolutionary effect between species i and j considering the trait difference between them, whereas the denominator is the sum of evolutionary effects of all species that interact with species i . Thus, q_{ij} is the normalized evolutionary effect between i and j due to the trait difference between these species. The values and the description of the model parameters are depicted in Table 1 and the sensitivity analysis of the model parameters can be found in the Supporting information.

Mutualistic networks

We use 24 empirical mutualistic networks available at the databases Web of Life (www.web-of-life.es/) and Interaction Web Database (<http://ecologia.ib.usp.br/iwdb/index.html>). These 24 networks include eight plant–pollinator networks, eight plant–frugivore networks and eight ant–myrmecophyte networks. Each network is represented by an adjacency matrix (**A**) in which each species is represented by a single row and a single column of the matrix; each element of this matrix represents the presence ($a_{ij}=1$, $a_{ij}=-1$) or absence ($a_{ij}=0$) of the corresponding animal–plant interaction (Supporting information). Ant–myrmecophyte networks are commonly less connected, more modular and less nested in comparison to seed dispersal and pollination networks, while seed dispersal networks are usually more nested and have a higher connectance than pollination and ant–myrmecophyte networks (Supporting information). We used these 24 networks to parametrize the network structure in our simulations. We emphasize that we did not have information if the recorded interactions are cheating interactions, therefore we only used the empirical information to parameterize the structure of the mutualistic networks.

Simulations

Our simulations described how the mean trait Z_i evolves in time (Fig. 1a). Each simulation started with the sampling of a single Z_i value for each species and ended after at most 1000 timesteps, which was enough time to generate asymptotic trait values. In most simulations, however, the equilibrium was reached before 1000 timesteps. The simulation stopped when the condition of equilibrium, $|Z_i^{(t+1)} - Z_i^{(t)}| < 10^{-4}$, was achieved for every species i in the network. We

ran 48 000 simulations, 2000 per empirical network, where each simulation tracked how species traits changed in time due to coevolution and the selective pressures from the environment (Fig. 1a). Initial trait values and parameter values used in our simulations are described in Table 1. All the simulations were performed in R ver. 3.5.3 (www.r-project.org). In what follows, we explain how we used this modeling approach to explore our two questions. All the simulation results are available at Zenodo under open access license (Camacho et al. 2022).

How does the frequency of cheating interactions affect coevolutionary dynamics?

We ran simulations with different frequencies of cheating interactions for each mutualistic network to evaluate the impact of cheaters on the coevolutionary process. In each simulation, we defined a probability p for an interaction within a ‘mutualistic network’ to be a cheating interaction (i.e., $a_{ij}=-1$) and $(1-p)$ the probability of an interaction to be a mutualistic interaction. We explored values of p ranging from 0.01 to 1 to test how different frequencies of cheating interactions affect trait evolution and network structure. We assumed that cheating interactions are randomly distributed across all interactions in the mutualistic network and that the frequency of cheating interactions in the network is fixed over a given simulation. We started with each pair of elements describing an interaction and each interaction coded as a mutualism, e.g., $a_{ij}=a_{ji}=1$. Then, for each pairwise interaction, we shift it to a cheating interaction with probability p . Because a cheating interaction is an interaction with a negative effect on one partner and a positive effect on the other one, we prohibited the occurrence of double-negative effects between two species (i.e., $a_{ij}=a_{ji}=-1$). Double-negative effects describe competitive-like interactions that are out of the scope of this paper.

To assign cheating interactions, we used the square adjacency matrix **A**, which has all the species in the rows and all the species in the columns (Supporting information). We sampled nonzero elements to transform from 1 to -1 and randomized whether species i or j will have its effect transformed from positive to negative, allowing every species in the network the chance to have at least one of its interactions changed from 1 to -1 . Thus, both animals and plants could be cheaters and the identity of the cheaters could change between simulations because the process of sampling

elements were made before every simulation. The process of defining the outcome of interactions based on p generates a network with both positive and negative elements (i.e., $a_{ij} = 1$ or $a_{ij} = -1$), merging the effect of mutualists and cheaters in a single network (Melián et al. 2009). We also assumed that the outcome of the interaction does not change over time. Although such an approach did not allow us to explore the effects of conditional outcomes of many interactions, it was a starting point to unravel how cheating interactions change the outcome of coevolution in mutualisms. We performed a set of sensitivity analyses where we relaxed this assumption by allowing interactions to shift from positive and negative outcomes during simulations. These analyses suggest that temporal variability on the interaction outcome does not influence our main results (Supporting information).

We characterized the outcome of coevolutionary dynamics by describing patterns in trait distributions across species. We first measured the average trait distance between pairs of species as a proxy for trait disparity in the network. We computed the mean pairwise distance (D) as the sum of the Euclidean distances of species traits of all possible pairwise combinations between all species divided by the total number of pairwise combinations (Ciampaglio et al. 2001):

$$D = \frac{\sum_i^N \sum_{j \neq i}^N \sqrt{(Z_i^{(t)} - Z_j^{(t)})^2}}{N(N-1)} \quad (7)$$

Because antagonisms are expected to generate trait mismatching in networks (Andreazzi et al. 2017), whereas some mutualisms are expected to generate trait matching (Guimarães et al. 2017), we performed an analysis to detect the number of trait clusters (i.e., groups of species with similar traits) following coevolutionary dynamics. By doing so, we measured how species traits can become different due to cheating or become similar due to mutualism. In this way, we could measure trait disparity (D) and the number of trait clusters in a single network. To compute the number of trait clusters among interacting species, we used Ward's hierarchical clustering analysis (Ward 1963) along with the GAP validation index (following Tibshirani et al. 2001). Both algorithms organize trait values and create clusters which minimize intra-cluster variation, generating clusters of species with high trait similarity. We then explored how the frequency of cheating interactions in the network (p) affects the coevolutionary outcome (i.e., trait disparity or number of trait clusters).

We performed a sensitivity analysis to compare the results of our baseline simulations, where cheating interactions are randomly distributed across species with simulations in which highly connected, central species harbor most of the cheating interactions (Supporting information). The outcomes of these analyses led to similar results, suggesting the lack of a strong effect of the distribution of cheating interactions across species in shaping the network-level patterns of trait distributions (Supporting information).

What is the effect of cheating interactions on the structure of mutualistic networks?

In our baseline coevolutionary model, the trait barrier \mathcal{E}_{ij} indicates whether the evolutionary effects of a cheater species on a victim becomes negligible. To explore the effect of cheating interactions on the network structure, we changed our baseline coevolutionary model to add an additional trait barrier, b_{ij} , defining the maximum absolute trait mismatch between two species traits so the species can interact. If $|Z_i^{(t)} - Z_j^{(t)}| > b_{ij}$, then a_{ij} is set to 0. While $|Z_i^{(t)} - Z_j^{(t)}| \leq b_{ij}$, a_{ij} remains at its original value of -1 or 1 .

Note that \mathcal{E}_{ij} from Eq. 3 represents a trait barrier that dictates if there is a selection on a victim by a cheater species. In contrast, b_{ij} describes that a potential mutualism or cheating interaction cannot occur because of a large trait dissimilarity between partners. By incorporating b_{ij} in our model we explored how network structure changes through time as an outcome of the coevolutionary process. With this approach, at each time t in our simulations, we verified if there were interacting species with differences in trait values higher than b_{ij} and we disconnected those interactions generating an interaction loss. Because we focused on how the network changes due to interaction loss, once the interaction is lost, it remains lost and cannot reconnect during the simulations. Thus, at the end of a simulation, we may have an interaction network with a different structure generated through the removal of links. We then compared the initial and final network structure in each simulation. We performed a sensitivity analysis allowing the reconnection of interactions that led to qualitatively similar results (Supporting information).

We characterized the structure of each network by calculating two common measures of network structure: nestedness and modularity (Almeida-Neto et al. 2008, Blondel et al. 2008). For each simulation, we computed the number of interactions lost, here called F . These interactions are lost due to the simulated coevolutionary dynamics as described in the previous paragraph. We computed measures based on nestedness and modularity describing structural changes between the final and initial network for each simulation. We used two measures of network structural changes: $\Delta NODF$ and ΔQ . For each network, these measures were calculated as the difference between the network nestedness (NODF, Almeida-Neto et al. 2008) and modularity (Q , Blondel et al. 2008) at the end and at the beginning of the simulations. Thus, $\Delta NODF$ is defined as $NODF_{final} - NODF_{initial}$ and ΔQ is defined as $Q_{final} - Q_{initial}$.

Thus, coevolutionary dynamics in our model may reduce the total number of interactions, favoring specialization. We then explored if the removal of interactions by coevolutionary dynamics deviates from random removal of interactions from the initial network. To do so, in addition to each initial and final network, we created a third network generated by randomly removing F interactions from the initial network. This network, therefore, has the same number of interactions as the coevolved network but the set of interactions removed was randomly defined. Finally, we compared how

the network structure changes with the random loss of interactions and the loss of interactions due to trait dissimilarity from the coevolutionary dynamics.

Results

How does the frequency of cheating interactions affect coevolutionary dynamics?

We found that the higher the proportion of cheating interactions, the higher the trait disparity observed across animal and plant species in the networks (Fig. 2a–c). This effect of cheating

interactions is similar for the three types of mutualisms studied here (Table 2). Thus, cheating interactions increase trait disparity across species (Fig. 2), and this effect did not change among networks of different types of mutualism.

We observed that the frequency of cheating interactions affects the number of species trait clusters. At low levels of cheating interactions ($p=0.01$, with p being the frequency of cheating interactions in the networks), we found a low number of clusters (2.37 ± 0.60 , Fig. 2d–f). At intermediate levels of cheating interactions, the number of trait clusters increased ($p=0.5$, 2.99 ± 0.79). Finally, if most of the network was formed by cheating interactions then, on average, we saw a slight decrease in the numbers of species trait

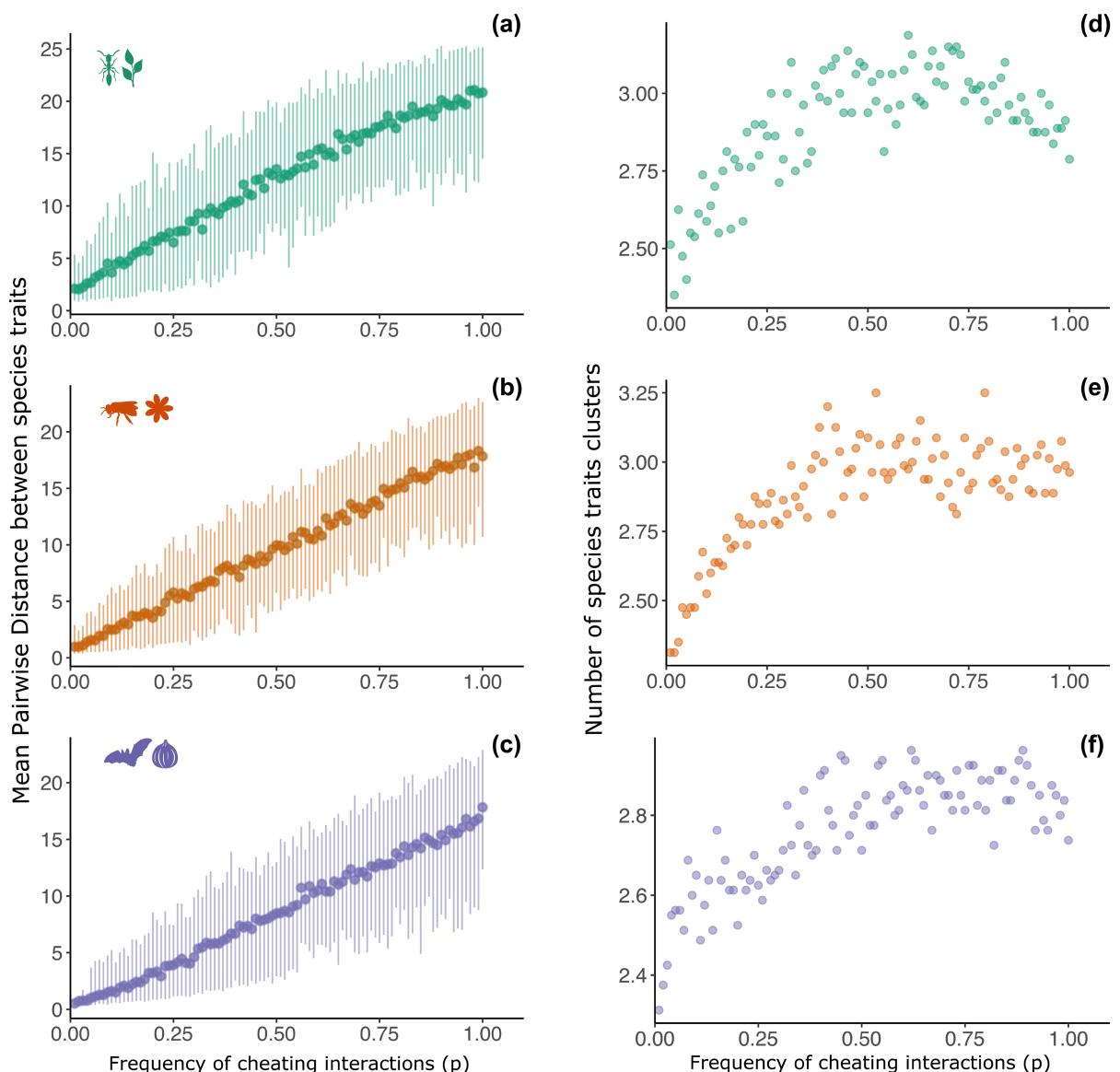


Figure 2. How does the frequency of cheating interactions affect coevolutionary dynamics? Each point in the plot is the average value depicting the mean pairwise distance between all species in the network (D) and the number of species trait clusters in different frequencies of cheating interactions (p). Each error bar is the 0.05 and 0.95 quantile from our simulation results. Ant–myrmecophyte interactions are represented by the green plots, pollination by orange plots and seed dispersal interactions are represented by purple plots.

Table 2. Average \pm SD values of mean pairwise distance (D) and statistical fit of a linear model between D and frequency of cheating interactions in the networks for three types of mutualisms. D is the mean pairwise distance calculated as the sum of the Euclidean distances of species traits of all possible pairwise combinations divided by the total number of pairwise combinations between species traits from our numerical simulations.

Mutualisms	Average $D \pm$ SD	Slope	R^2
Ant-myrmecophyte (n=8)	12.39 ± 6.72	19.56	0.70
Pollination (n=8)	9.58 ± 5.92	17.31	0.75
Seed dispersal (n=8)	8.55 ± 5.89	17.81	0.75

clusters with a more pronounced effect in ant-myrmecophyte networks (2.89 ± 0.50 , $p=0.9$, Fig. 2d–f). Thus, increasing the frequency of cheating interactions fuels trait disparity in mutualistic networks by promoting the emergence of trait clusters. However, under high frequencies of cheating interactions, several arms races happened at the same time and victim's trait evolution inevitably increased trait matching for one of their several cheater's partners. Thus, trait disparity and the number of trait clusters decreased under a very high frequency of cheating interactions, mainly in ant-myrmecophyte networks (Fig. 2d–f). Running simulations

incorporating species abundances does not qualitatively influence the patterns of high trait disparity and species trait clusters (Supporting information).

What is the effect of cheating interactions on the structure of mutualistic networks?

We found that when assuming $p=0$ (no cheating interactions), coevolutionary dynamics led to no qualitative change in the measured structural features of the network ($\Delta Q \approx 0$; $\Delta NODF \approx 0$). In contrast, as we increase the frequency of cheating interactions, mutualistic networks become more modular and less nested (Fig. 3, Table 3). Despite the pattern of increasing modularity and reduced nestedness being the same between simulation scenarios, the magnitude of increasing modularity and reduced nestedness observed was not reproduced by randomly removing interactions (colored versus black points in Fig. 3). In other words, mutualistic networks tend to become modular with interactions removal, but networks become more modular when considering interaction lost due to cheater coevolution than random interaction lost.

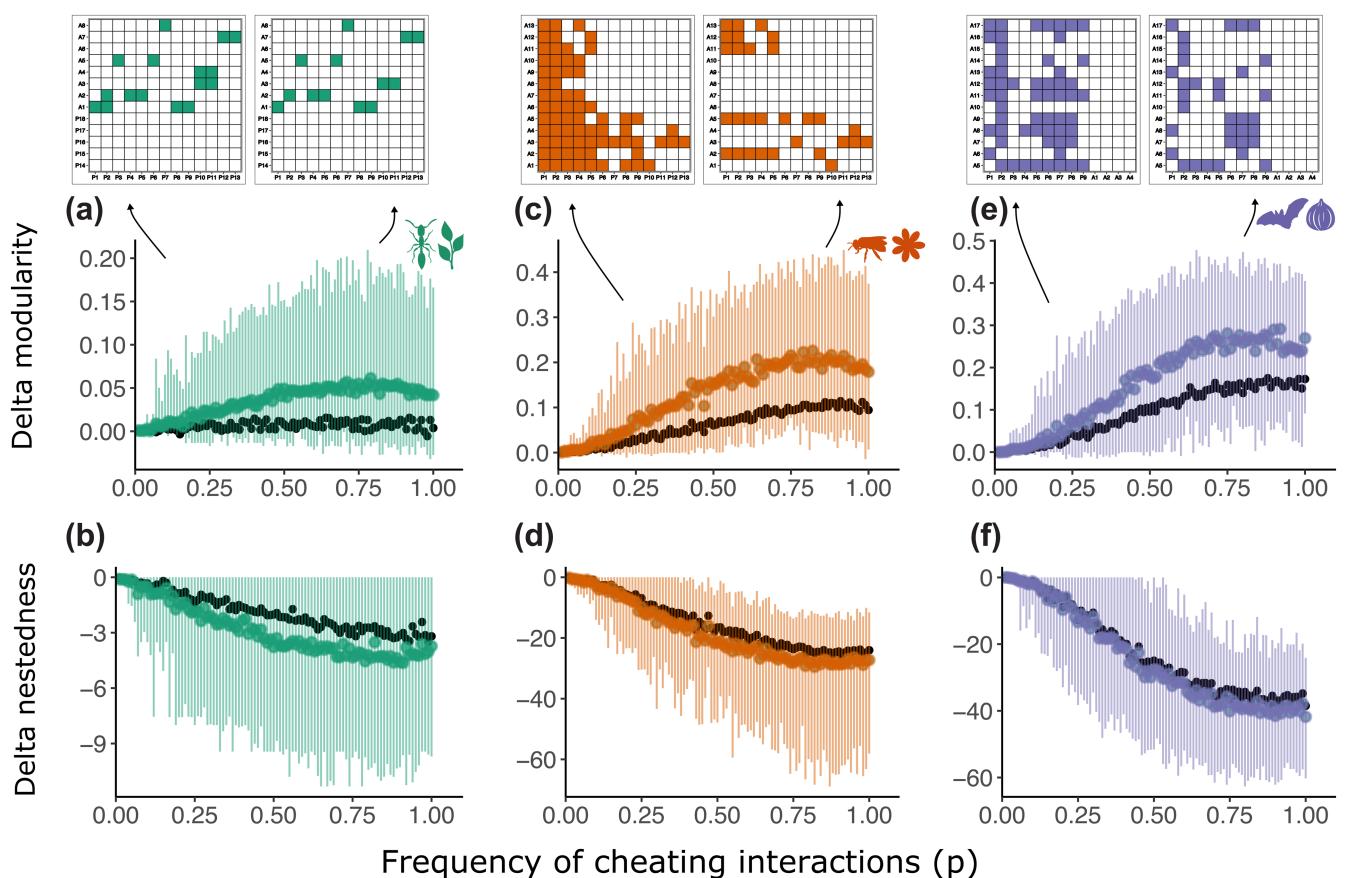


Figure 3. What is the effect of cheating interactions on the structure of mutualistic networks? Each point in the plot is the average value of ΔQ and $\Delta NODF$ in different frequencies of cheating interactions (p). Each error bar is 0.05 and 0.95 quantile from our simulation results. The black points are values of ΔQ and $\Delta NODF$ from networks where we removed interactions randomly. Ant-myrmecophyte interactions are represented by the green plots, pollination by orange plots and seed dispersal interactions are represented by purple plots. The matrices above the graphs are examples of binary adjacency matrices to compare the loss of interactions in simulations from different scenarios.

Table 3. Average \pm SD ΔQ and $\Delta NODF$ for random interaction removal and trait barrier interaction removal for three types of mutualisms. The average and SD value of ΔQ and $\Delta NODF$ were calculated using all the simulations (ranging from $p=0.01$ to $p=1$).

Mutualisms	$\Delta Q \pm SD$		$\Delta NODF \pm SD$	
	Random	Simulations	Random	Simulations
Ant-myrmecophyte ($n=8$)	0.0063 ± 0.04	0.035 ± 0.053	-1.89 ± 2.74	-2.8 ± 3.3
Pollination ($n=8$)	0.060 ± 0.075	0.12 ± 0.11	-15.26 ± 13.87	-18.22 ± 15.2
Seed dispersal ($n=8$)	0.093 ± 0.085	0.16 ± 0.14	-22.53 ± 17.48	-24.62 ± 18.92

The increase in modularity and reduction in nestedness was not equally distributed across mutualisms. In mutualisms where the empirical network was initially very modular and not nested (e.g. ant-myrmecophyte networks) changes in network structure were weaker than in mutualisms that initially showed higher nestedness (e.g. pollination and seed dispersal). Hence, in mutualistic networks containing a high frequency of cheating interactions, coevolution can enhance modularity and decrease nestedness (Table 3). Having said that, for networks composed of almost only cheaters, the levels of modularity showed a small decline (Fig. 3). Sensitivity analysis taking into account variation in species abundances does not change qualitatively our results of simulated networks showing a higher trait disparity and modularity (Supporting information). Finally, considering species rewiring during the simulations also did not change the main pattern of increased modularity and reduced nestedness depicted in our results. Thus, even with species gaining new interactions during simulations, the networks tend to become more modular and less nested (Supporting information).

Discussion

In this study, we explored the coevolutionary outcomes of cheating interactions in mutualistic networks. Our results showed that trait evolution and network structure can change due to cheating interactions that emerge in mutualistic systems. Previous studies have already explored the effect of mutualistic and cheating interactions on population dynamics (Law et al. 2001, Bronstein et al. 2003, Wilson et al. 2003, Lee 2015) and phenotypic evolution (Ferriere et al. 2002). In this context, our work contributes to further understanding of the coevolutionary dynamics of multispecies assemblages in three different ways.

First, we showed that cheating interactions promoted higher community-level trait disparity in mutualistic networks. By imposing selection favoring trait mismatching, the presence of cheating interactions led to an increase in species trait disparity in mutualistic assemblages. The arms race dynamics promoted by cheaters partially offset selection favoring convergence and trait matching in mutualisms (Guimarães et al. 2011, Zhang et al. 2013). Therefore, cheating interactions may provide one of the mechanisms preventing the emergence of perfect trait matching in empirical mutualistic communities (Law et al. 2001). Yet, trait disparity cannot increase indefinitely due to factors related to the environmental pressures and distinct selection regimes (Andreazzi et al. 2017). For instance, limited genetic

variation or limited anatomical and physiological mechanisms restricts fruit sugar content and flower size (Jordano 1995), which could limit the potential for trait change in time and, consequently, restrict trait matching. In addition, annual variation in soil nutrients limit the pulp composition of fleshy fruits and, consequently, represents a restriction for the development of sugar-rich fruits that are highly attractive to frugivores (Herrera 1998), limiting trait matching in plant-frugivore systems. Finally, the way species are organized in mutualistic networks can change the resulting trait matching or trait disparity of species. However, we explored the effects of cheater species centrality on trait coevolution and did not find a clear effect on either trait matching or disparity of species (Supporting information).

Second, we found that cheating interactions promoted, under intermediate frequencies, an increase in the number of trait clusters in mutualistic networks. Under intermediate frequencies of cheating interactions, the joint effect of mutualistic selection favoring trait matching and cheating interactions favoring trait mismatching created clusters of species traits. There is evidence that the proportion of positive and negative effects between species is similar in empirical communities (Dodds 1997). That is, communities have the equal proportions of mutualistic and cheating interactions. Applying these empirical proportions to our results, equal proportions of cheating and mutualism interactions may generate a higher trait disparity due to the formation of trait clusters. This prediction can be tested by measuring the level of trait disparity and the frequency of cheating interactions across different interacting assemblages. In high frequencies of cheating interactions there is a slight fall in the number of species trait cluster in ant-myrmecophyte networks. Ant-myrmecophyte networks are sparse, smaller and more modular than pollination and seed dispersal networks (Supporting information). In high frequency of cheaters, smaller and modular networks could diminish the potential for victims find mutualistic partners. Thus, the weaker formation of clusters observed in ant-myrmecophyte could happen due to the effect of cheater's arms race in networks with low number of species.

It is noteworthy that the presence of cheaters may be underestimated in empirical networks (Genini et al. 2010), partially because there is great behavioral plasticity among interacting individuals (Bronstein 2001). Thus, behavioral plasticity generating intraspecific variation may play an important role in individual fitness and may lead to occasional cheating. Also, it is important to point out that the patterns of higher trait disparity and cluster formation were observed at the network-level and not necessarily a single pairwise interaction will have higher trait disparity in this

context. It is possible that for a single pairwise interaction the cheater and victim may have an increase in trait matching but since other interacting victims could be favoring extreme trait values due to cheaters interactions, the result, at the network level, might be a higher trait disparity. Finally, we observed the growth of trait disparity in our simulations but we emphasize that the conflicting selection from cheater and mutualism and environment selection imposes a limit to this growth of trait disparity.

Third, we showed that the presence of cheating interactions in mutualistic networks led to the reorganization of network patterns due to the increased trait dissimilarity. In particular, we found that cheating led to increased modularity and reduced nestedness in mutualistic networks. The emergence of modularity was also observed as an outcome of coevolutionary dynamics in antagonistic networks depending on the selection intensity between victim and exploiter species (Andreazzi et al. 2017). Our results propose an alternative path to the emergence of modularity in mutualistic networks, which depends on how many cheating interactions the mutualistic network contains. The structure of interaction networks can be explained by several factors, such as species abundance distribution (Dátilo et al. 2014), differences in species richness between species sets (Guimarães et al. 2007), match and mismatch between traits of plants and animals (Stang et al. 2007), phylogenetic and phenology-based constraints (Jordano 1995, Jordano et al. 2003) and ecological niche adaptation (Cai et al. 2020). Here we have shown that cheating interactions can also change the structure of mutualistic networks through the coevolutionary process. Cai and collaborators (2020) showed that, in mutualistic networks showing simultaneously modularity and nestedness, both structures can change the robustness of the network when facing either invasion or extinction. Future studies could test the influence of cheating interactions on network robustness due to the re-organization of modularity and nestedness.

Overall, our results suggest that the incorporation of the selection imposed by cheating interactions that naturally emerge from mutualisms is pivotal to a deep understanding of the coevolutionary dynamics in species-rich mutualisms. Cheating interactions changed the outcomes of mutualistic coevolution, leading to increased interspecific trait variation and clustering. We provided insights on the effect of multiple interaction types in a network and how it may shape trait diversity by the contrasting selective forces favoring convergence and disparity across interacting species (Sauve et al. 2016). The structure of simulated networks significantly changed when we increased the frequency of cheating interactions, and this result opens new questions on what is the balance of positive and negative effects that maintains the structure of empirical networks. There is theoretical evidence showing that nestedness can, in part, increase network robustness to species extinction and invasions (Lever et al. 2014) but also modularity can moderately increase network stability (Stouffer and Bascompte 2011, Grilli et al. 2016). Also, theoretical and empirical evidence shows that the identity and type of interactions change over time, consequently

changing the robustness of the network (Montesinos-Navarro et al. 2017, Ponisio et al. 2017, Baruah 2022). If so, different interaction types or the initial loss of nestedness with higher frequencies of cheaters that we observed could impact the robustness of communities, impacting ecosystem functioning and services. Here, we contributed to understanding by which factors and how the structure of mutualistic networks may change. Our theoretical predictions may also help us to understand how networks might evolve under a rapidly changing world, creating modularity and nestedness depending on, for instance, historical factors (Dalsgaard et al. 2013). All in all, our work suggests that interaction changes may have important consequences for trait evolution and, consequently, for the reorganization of network structure in multispecies mutualistic systems.

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Author contributions

Lucas A. Camacho: Conceptualization (lead); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Software (equal); Visualization (equal); Writing – original draft (equal); Writing – review and editing (equal). **Cecilia S. Andreazzi:** Conceptualization (equal); Methodology (equal); Software (equal); Supervision (equal); Writing – review and editing (equal). **Lucas P. Medeiros:** Formal analysis (equal); Investigation (equal); Methodology (equal); Software (equal); Writing – review and editing (equal). **Irina Birskis-Barros:** Formal analysis (equal); Investigation (equal); Methodology (equal); Software (equal); Writing – review and editing (equal). **Carine Emer:** Validation (equal); Writing – review and editing (equal). **Carolina Reigada:** Validation (equal); Writing – review and editing (equal). **Paulo R. Guimarães Jr.:** Funding acquisition (equal); Methodology (equal); Project administration (equal); Resources (equal); Software (equal); Supervision (equal); Validation (equal); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Zenodo Digital Repository: <https://doi.org/10.5281/zenodo.6480140> (Camacho et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

References

- Almeida-Neto, M., Guimarães, P., Guimarães Jr., P. R., Loyola, R. D. and Ulrich, W. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. – *Oikos* 117: 1227–1239.
- Anderson, B. and Johnson, S. D. 2008. The geographical mosaic of coevolution in a plant–pollinator mutualism. – *Evolution* 62: 220–225.
- Anderson, B., Johnson, S. and Carbut, C. 2005. Exploitation of a specialized mutualism by a deceptive orchid. – *Am. J. Bot.* 92: 1342–1349.
- Andreazza, C. S., Thompson, J. N. and Guimarães, P. R. 2017. Network structure and selection asymmetry drive coevolution in species-rich antagonistic interactions. – *Am. Nat.* 190: 99–115.
- Baruah, G. 2022. The impact of individual variation on abrupt collapses in Andreazzi mutualistic networks. – *Ecol. Lett.* 25: 26–37.
- Blondel, V. D., Guillaume, J.-L., Lambiotte, R. and Lefebvre, E. 2008. Fast unfolding of communities in large networks. – *J. Stat. Mech. Theory Exp.* 2008: P10008.
- Bronstein, J. L. 2001. The exploitation of mutualisms. – *Ecol. Lett.* 4: 277–287.
- Bronstein, J. L., Wilson, W. G. and Morris, W. F. 2003. Ecological dynamics of mutualist/antagonist communities. – *Am. Nat.* 162: 24–39.
- Cai, W., Snyder, J., Hastings, A. and D’Souza, R. M. 2020. Mutualistic networks emerging from adaptive niche-based interactions. – *Nat. Commun.* 11: 5470.
- Camacho, L. A., de Andreazzi, C. S., Medeiros, L. P., Birskis-Barros, I., Emer, C., Reigada, C. and Guimarães Jr., P. R. 2022. Data from: Cheating interactions favor modularity in mutualistic networks. – Zenodo Digital Repository, <https://doi.org/10.5281/zenodo.6480140>.
- Ciampaglio, C. N., Kemp, M. and McShea, D. W. 2001. Detecting changes in morphospace occupation patterns in the fossil record: characterization and analysis of measures of disparity. – *Paleobiology* 27: 695–715.
- Dalsgaard, B., Trøjelsgaard, K., Martín González, A. M., Nogués-Bravo, D., Ollerton, J., Petanidou, T., Sandel, B., Schleuning, M., Wang, Z., Rahbek, C., Sutherland, W. J., Svenning, J.-C. and Olesen, J. M. 2013. Historical climate-change influences modularity and nestedness of pollination networks. – *Ecography* 36: 1331–1340.
- Dátillo, W., Marquitti, F. M. D., Guimarães, P. R. and Izzo, T. J. 2014. The structure of ant–plant ecological networks: is abundance enough? – *Ecology* 95: 475–485.
- Dodds, W. K. 1997. Interspecific interactions: constructing a general neutral model for interaction type. – *Oikos* 78: 377–383.
- Ferriere, R., Bronstein, J. L., Sergio, R., Richard, L. and Mathias, G. 2002. Cheating and the evolutionary stability of mutualisms. – *Proc. R. Soc. B* 269: 773–780.
- Galetti, M., Guevara, R., Côrtes, M. C., Fadini, R., Matter, S., Leite, A., Labecca, F., Ribeiro, T., Carvalho, C., Collevatti, R., Pires, M., Guimarães Jr., P. R., Brancalion, P., Ribeiro, M. and Jordano, P. 2013. Functional extinction of birds drives rapid evolutionary changes in seed size. – *Science* 340: 1086–1090.
- Genini, J., Morellato, L. P. C., Guimarães, P. R. and Olesen, J. M. 2010. Cheaters in mutualism networks. – *Biol. Lett.* 6: 494–497.
- Gómez, J. M., Perfectti, F. and Klingenberg, C. P. 2014. The role of pollinator diversity in the evolution of corolla-shape integration in a pollination-generalist plant clade. – *Phil. Trans. R. Soc. B* 369: 20130257.
- Gómez, J. M., Schupp, E. W. and Jordano, P. 2018. Synzoochory: the ecological and evolutionary relevance of a dual interaction. – *Biol. Rev. Camb. Phil. Soc.* 94: 874–902.
- Grilli, J., Rogers, T. and Allesina, S. 2016. Modularity and stability in ecological communities. – *Nat. Commun.* 7: 12031.
- Guimarães, P. R., Machado, G., de Aguiar, M. A. M., Jordano, P., Bascompte, J., Pinheiro, A. and dos Reis, S. F. 2007. Build-up mechanisms determining the topology of mutualistic networks. – *J. Theor. Biol.* 249: 181–189.
- Guimarães Jr., P. R., Jordano, P. and Thompson, J. N. 2011. Evolution and coevolution in mutualistic networks. – *Ecol. Lett.* 14: 877–885.
- Guimarães Jr., P. R., Pires, M. M., Jordano, P., Bascompte, J. and Thompson, J. N. 2017. Indirect effects drive coevolution in mutualistic networks. – *Nature* 550: 511–514.
- Herrera, C. M. 1998. Long-term dynamics of Mediterranean frugivorous birds and fleshy fruits: a 12-year study. – *Ecol. Monogr.* 68: 511–538.
- Jordano, P. 1995. Angiosperm fleshy fruits and seed dispersers: a comparative analysis of adaptation and constraints in plant–animal interactions. – *Am. Nat.* 145: 163–191.
- Jordano, P., Bascompte, J. and Olesen, J. M. 2003. Invariant properties in coevolutionary networks of plant–animal interactions. – *Ecol. Lett.* 6: 69–81.
- Lande, R. 1976. Natural selection and random genetic drift in phenotypic evolution. – *Evolution* 30: 314–334.
- Law, R., Bronstein, J. L. and Ferrière, R. 2001. On mutualists and exploiters: plant–insect coevolution in pollinating seed–parasite systems. – *J. Theor. Biol.* 212: 373–389.
- Lee, C. T. 2015. Inherent demographic stability in mutualist–resource–exploiter interactions. – *Am. Nat.* 185: 551–561.
- Lever, J. J., van Nes, E. H., Scheffer, M. and Bascompte, J. 2014. The sudden collapse of pollinator communities. – *Ecol. Lett.* 17: 350–359.
- Loayza, A. P., Carvajal, D. E., García-Guzmán, P., Gutierrez, J. R. and Squeo, F. A. 2014. Seed predation by rodents results in directed dispersal of viable seed fragments of an endangered desert shrub. – *Ecosphere* 5: 1–9.
- Melián, C. J., Bascompte, J., Jordano, P. and Krivan, V. 2009. Diversity in a complex ecological network with two interaction types. – *Oikos* 118: 122–130.
- Mello, M. A. R., Marquitti, F. M. D., Guimarães, P. R., Klara, E., Kalko, V., Jordano, P. and de Aguiar, M. A. M. 2011. The modularity of seed dispersal: differences in structure and robustness between bat– and bird–fruit networks. – *Oecologia* 167: 131–140.
- Montesinos-Navarro, A., Hiraldo, F., Tella, J. L. and Blanco, G. 2017. Network structure embracing mutualism–antagonism continuums increases community robustness. – *Nat. Ecol. Evol.* 1: 1661–1669.
- Nuismer, S. L., Thompson, J. N. and Gomulkiewicz, R. 1999. Gene flow and geographically structured coevolution. – *Proc. Biol. Sci.* 266: 605–609.
- Ponisio, L. C., Gaiarsa, M. P. and Kremen, C. 2017. Opportunistic attachment assembles plant–pollinator networks. – *Ecol. Lett.* 20: 1261–1272.

- Santamaría, L. and Rodríguez-Gironés, M. A. 2007. Linkage rules for plant–pollinator networks: trait complementarity or exploitation barriers? – *PLoS Biol.* 5: e31.
- Sauve, A. M. C., Fontaine, C. and Thébaud, E. 2016. Stability of a diamond-shaped module with multiple interaction types. – *Theor. Ecol.* 9: 27–37.
- Siepielski, A. M. and Benkman, C. W. 2010. Conflicting selection from an antagonist and a mutualist enhances phenotypic variation in a plant. – *Evolution* 64: 1120–1128.
- Stang, M., Klinkhamer, P. G. L. and van der Meijden, E. 2007. Asymmetric specialization and extinction risk in plant–flower visitor webs: a matter of morphology or abundance? – *Oecologia* 151: 442–453.
- Stouffer, D. B. and Bascompte, J. 2011. Compartmentalization increases food–web persistence. – *Proc. Natl Acad. Sci. USA* 108: 3648–3652.
- Strauss, S. Y. and Irwin, R. E. 2004. Ecological and evolutionary consequences of multispecies plant–animal interactions. – *Annu. Rev. Ecol. Evol. Syst.* 35: 435–466.
- Thompson, J. N. 1994. The coevolutionary process. – Univ. of Chicago Press, www.press.uchicago.edu/ucp/books/book/chicago/C/bo3637930.html.
- Thompson, J. N. 2005. The geographic mosaic of coevolution. – Univ. of Chicago Press, www.press.uchicago.edu/ucp/books/book/chicago/G/bo3533766.html.
- Thompson, J. N. and Cunningham, B. M. 2002. Geographic structure and dynamics of coevolutionary selection. – *Nature* 417: 735–738.
- Tibshirani, R., Walther, G. and Hastie, T. 2001. Estimating the number of clusters in a data set via the gap statistic. – *J. R. Stat. Soc. B* 63: 411–423.
- Vieira, E., Pizo, M. and Izar, P. 2003. Fruit and seed exploitation by small rodents of the brazilian atlantic forest. – *Mammalia* 67: 533–539.
- Ward, J. H. 1963. Hierarchical grouping to optimize an objective function. – *J. Am. Stat. Assoc.* 58: 236–244.
- Wechsler, D. and Bascompte, J. 2022. Cheating in mutualisms promotes diversity and complexity. – *Am. Nat.* 199: 393–405.
- Wilson, J. S., Williams, K. A., Forister, M. L., von Dohlen, C. D. and Pitts, J. P. 2012. Repeated evolution in overlapping mimicry rings among North American velvet ants. – *Nat. Commun.* 3: 1272.
- Wilson, W. G., Morris, W. F. and Bronstein, J. L. 2003. Coexistence of mutualists and exploiters on spatial landscapes. – *Ecol. Monogr.* 73: 397–413.
- Yoder, J. B. and Nuismer, S. L. 2010. When does coevolution promote diversification? – *Am. Nat.* 176: 802–817.
- Zhang, F., Hui, C. and Pauw, A. 2013. Adaptive divergence in Darwin’s race: how coevolution can generate trait diversity in a pollination system. – *Evol. Int. J. Org. Evol.* 67: 548–560.