

Hawkmoths' evolutionary history structures plant-pollinator interactions frequency, beyond its effect through ecological traits

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ABSTRACT

Multiple non-exclusive neutral or niche-based processes influence species interactions and shape the structure of ecological networks. Although evolutionary processes may influence interactions via shared inherited traits, their relative importance in comparison to ecological processes remain poorly understood. Here we investigate the effects of evolutionary history compared to ecological processes on both interaction frequencies and the emergence of modularity in a plant-hawkmoth interaction network. Finally, we tested the phylogenetic signals on hawkmoth ecological traits relevant to pollination interactions. We analyzed a plant-hawkmoth network on Pampa grasslands for which we collected morphological, phenological, abundance and phylogenetic data for every species in the network to evaluate neutral-based processes (i.e., random chance of encounter dictated by species abundances) or niche-based processes (i.e., morphological matching). The best model predicting interactions indicated that, beyond increasing interaction frequencies with increased morphological matching and phenological overlap, distantly related hawkmoth species also tended to interact with plants more frequently. Further, by evaluating whether hawkmoth composition differed among interaction modules, we found that modules encompassed phylogenetically distant and functionally distinct hawkmoth species. Thus, module composition suggests that, despite evolutionary history and ecological traits influencing interactions' frequency, modules emergence may be also potentially modulated by processes such as environmental filtering or stochastic factors acting on other traits than the ones evaluated here. Such processes may have influenced hawkmoths' species composition in the community and ultimately also shape the observed interaction patterns.

Keywords: Interaction networks, modularity, niche partitioning, phylogenetic signal, phylogeny, pollination

The interest on plant-hawkmoth interactions as a model system to understand coevolution is notorious since the 1800s (Darwin, 1862). However, only recently advances in concepts and analytical tools derived from complex networks approaches (Vázquez et al., 2009a) have allowed to understand more deeply what Charles Darwin defined as a “tangled bank of organisms that interact with each other according to laws that determine their associations” (Darwin, 1859). In fact, interactions between organisms that coexist in communities connect species forming interaction networks whose structures are shaped by multiple non-exclusive ecological, evolutionary and stochastic processes (Lewinsohn et al., 2006; Vázquez et al., 2009b).

Plant-hawkmoth interactions form a subnetwork that is part of the larger pollination network of a community, by including mostly nocturnal animals and flowers with nocturnal anthesis (Amorim et al., 2020; Lautenschleger et al., 2020). In plant-hawkmoth interaction networks, recent studies indicate that ecological processes, such as morphological matching between proboscis length and floral tube length, as well as phenological overlap, are often primary mechanisms structuring interaction frequencies (Sazatornil et al., 2016; Johnson et al., 2017; Lautenschleger et al., 2020). It means that hawkmoths tend to visit more frequently flowers with corolla tubes of similar length to their proboscis. It also means that morphological constraints shape interactions because long tubular flowers are not visited by short proboscis hawkmoths, and that potential partners that do not have overlapping phenologies also cannot interact (*i.e.*, forming forbidden links).

These mechanisms are examples of what has been called niche-based processes, in contrast with the neutral-based processes. Neutral-based processes (also called ‘neutrality’) postulates that partners interact in proportion to their relative chance of encounter, resulting in abundant species interacting more frequently than rarer species, and that the chance of encounter is not related to other traits different from abundance (*i.e.* other biological features do not determine abundance nor the chance of encounter) (Krishna et al., 2008). Recently it has been recognized that not only abundance should define neutral-based models as, for instance, partners that have longer overlapping flowering and foraging times are likely to interact more frequently as the extent of phenological overlap increases chances of encounter (Vizentin-Bugoni et al., 2022). On the other hand, a niche-based model of phenology would be expected when behavioral differences in the foraging time of some hawkmoth species and the time of some sphingophilous flowers anthesis indicate that time constraints may be mediated by pollinators.

Interestingly, in biological communities, niche partitioning is expected to be less pronounced in low-affinity mutualistic systems such as pollination networks (Guimarães et al., 2007). Furthermore, when considering pollination subnetworks composed by a phylogenetically related set of pollinators (*e.g.*, hawkmoths), than the entire community of plants and pollinators, trophic niche partitioning may be associated with reciprocal selection on groups of pollinators and plants species (*i.e.*, diffuse coevolution, resulting in the emergence of a modular network structure, Olesen et al., (2007)). The detection of modularity in the community supports the pollination niche partition (sensu Phillips et al. 2020) and this pattern is known to emerge in plant-hawkmoth networks possibly owing to morphological and phenological constraints (Amorim, 2020; Lautenschleger et al., 2020). Considering the wide variation in hawkmoths’ proboscis length, it is plausible that differentiation of feeding niches among species is importantly driven by this trait (Johnson et al. 2017), resulting in different trait compositions across modules. So, modules in such subnetworks could be expected to reflect variations in hawkmoth ecological traits such as proboscis length or phenology and their feeding efficiency. In this way, functionally similar species group into modules, but the investigation of module composition and their predictors on this system is still incipient.

Furthermore, functional traits may be phylogenetically conserved, as they do not change rapidly throughout the evolutionary process, or they may undergo rapid adaptation via niche divergence between sister species (Dormann et al., 2017). By testing whether phylogenetically close species show phenotypic similarity (phylogenetic signal) and interact with similar partners, it may be possible to unravel evolutionary and/or ecological processes underlying species interactions (Bascompte & Jordano, 2007). Concerning interaction frequencies, three scenarios considering the degree of evolutionary isolation (Pavoine et al., 2005) for plants and/or hawkmoths are possible: (i) the higher isolation hypothesis (H+) predicts that species with greater evolutionary divergence would have a greater chance of interaction, possibly at a scenario where the interactions of closer species are limited by competition and a greater evolutionary divergence allows development of novel traits that increase the chance of interaction; (ii) the inferior isolation

hypothesis (H-) predicts that species that diverged more recently would have a greater chance of interaction, possibly by the effect of co-occurring close and functionally similar species increasing the frequency of interactions and the number of partners or in a scenario where a poorly correlated trait reduces the chances of interactions ; and (iii) the hypothesis of intermediate isolation (H_{μ}) consider that the two previous ones would act together, leading the species that diverged in an intermediate time to have a greater probability of interaction (Vitória et al., 2018).

Concerning modularity, interactions between mutualistic plants and animals may present different phylogenetic patterns depending on the dominant process structuring the community: (i) in case of competition among plants mediated by pollinators when plants in the community have conserved functional traits, should be phylogenetically distant and interact with functionally and phylogenetically similar subsets of pollinators; and (ii) if plant traits are not conserved, modules should encompass functionally similar plants, regardless of phylogenetic relationships, interacting with functionally and phylogenetically similar partners. In case of plant-to-plant facilitation or pollinator-mediated filtering, (iii) when plants functional traits are conserved, modules are expected to encompass phylogenetically related plants that share functionally similar and phylogenetically related pollinators; and (iv) non-conserved plant traits favor modules that encompass phylogenetically distant plant species that share pollinators.

However, the role of phylogenetic relationships in pollination networks remains debated, with limited knowledge on the importance of evolutionary history in shaping the modularity observed in these networks (Peralta, 2016). To the best of our knowledge, no study so far has investigated phylogenetic signals in hawkmoth traits and the influence of plant and hawkmoth evolutionary history on observed patterns of pollination interactions' identity and frequency, as well as on a modular network structure. In this context, the aim of this study is to evaluate the influence of phylogenetic relationships on the structure of interactions and module composition in a plant-hawkmoth network from the Pampa grasslands. Studying these interactions provides valuable insights into the dynamics of Pampa ecosystems, contributing to understanding the ecological processes that sustain biodiversity in this important and neglected biome (Ellwanger et al., 2022). Specifically, we (i) tested the relative importance of species phylogenetic relationships in comparison to ecological processes in defining plant and hawkmoth interactions' identity and frequency; (ii) evaluated the phylogenetic signal of hawkmoth ecological traits; and (iii) tested whether module composition of the quantitative plant-hawkmoth network was associated with hawkmoths phylogenetic relationships and functional traits.

Given the importance of niche-based processes (morphological matching and temporal overlap) in structuring this interaction network (Lautenschleger et al., 2020) and that low evolutionary divergence can limit niche differentiation (Pavoine et al. 2005), we hypothesized that distantly related hawkmoths will show greater interaction frequencies and that traits related to morphology and temporal distribution of this group present phylogenetic signal. Still about the relative importance of the processes predicting interactions, but with regard to plant species, due to the ability to predict hawkmoth visitors based on converging floral traits (Oliveira et al., 2014), we expected that plants' phylogenetic relationship may have little or no association with their interactions with hawkmoths, but their morphologies and phenologies will be important in defining interactions identity and frequency. Furthermore, owing to the high functional specialization of this studied system (Oliveira et al., 2014), we expected that network modules will include phylogenetically close hawkmoths with similar functional traits. Thus, due to the trend of phylogenetically closer species to interact with similar partners (Thompson, 2005), modules composition should aggregate close relative hawkmoths with longer proboscis and shorter temporal distribution and, on the other hand, close relative hawkmoths with shorter proboscis and longer flowering duration.

Methods

INTERACTION NETWORK AND TRAITS DATA

We used interactions data from a plant-hawkmoth community in the Pampa grasslands (Lautenschleger et al., 2020). Interactions are described through a matrix of visitation frequencies between each pair of plant-pollinator species, composed of 17 hawkmoth species and 13 plants species. The quantitative network was tested for modularity using the metric Q and the optimization algorithm DIRTLPawb+ (Beckett, 2016), and presents three modules in which subgroups of species interact more strongly with each other than with species from other modules (Lautenschleger et al., 2020). We repeated the modularity analysis ten times and

accepted the highest value obtained, which was the result used for module composition interpretation. Statistical significance of modularity was accessed by comparing the observed Q value with the 95% confidence interval of the 1000 random matrices generated with the null model *r2dtable* which keeps the number of species in each trophic level and marginal totals as in the observed interaction matrix (Lautenschleger et al., 2020).

Sampling was carried out using a phytocentric approach, with focal observations of plants potentially pollinated by hawkmoths and capture of floral visitors, fortnightly, between November 2015 and April 2017. This method allows recording diurnal, crepuscular and nocturnal species interacting within an explicitly defined geographical area, while zoocentric sampling may result in the detection of more interactions without allowing knowledge on the spatial scales sampled (Freitas et al., 2014). Since a phytocentric approach may favor detection of diurnal and crepuscular species, the sampling covered also night periods (6:00 pm to 6:00 am) to optimize the potential to record species with exclusively nocturnal behavior. In addition, plant and hawkmoth species morphology, abundance and temporal distribution data were recorded.

For morphology, we calculated the average of proboscis and floral tube length measurements among individuals of each species of hawkmoth and plants. The relative abundance was measured as the total number of open flowers per plant species and the total number of individuals of each hawkmoth species, in relation to the total number of flowers and hawkmoths recorded during the study. The temporal distribution was measured as the presence or absence of flowers and hawkmoths in each month during the sampling period (18 months). As the sampling was phytocentric centered and we registered the hawkmoths by the interactions, every data of hawkmoth interaction is also considered for hawkmoth abundance and temporal distribution; on the other hand, to quantify the plants abundance and phenology, the sampled was conducted independent of interactions, through pre-established transects. For further sampling details, see Lautenschleger et al. (2020). Dataset is available at Cavalheiro, L (2023) and at supplementary material from Lautenschleger et al. (2020).

PHYLOGENETIC TREES AND SPECIES ORIGINALITY INDEX

Based on the phylogenetic hypothesis currently accepted for hawkmoths (Kawahara & Barber, 2015), phylogenetic trees of the species occurring in the community were constructed. The internal relationships of *Eumorpha* and *Manduca* genera were solved using phylogenies proposed by Ponce et al. (2014) and Kawahara et al. (2013), respectively. The phylogenetic tree was assembled by inserting species of the community as polytomies on the base phylogeny and subsequently clipping the surplus species. Nomenclature was according to the catalogue of the Sphingidae family provided by Kitching (2024). We used the packages *phytools* (Revell, 2012), *ape* (Paradis & Schliep, 2019) and *geiger* (Pennell et al., 2014) on the R software (R Core Team, 2021).

Plants phylogenetic tree was based on Megatree R20160415, which follows the classification system APG IV (2016). The length of the branches was estimated based on the *TimeTree* database (Hedges et al., 2006), using the *BLADJ* function of *Phylocom* software (v4.2, Webb et al., 2008), with an equal distribution of the undated nodes among the dated ones. All subsequent analyzes were performed in R (R Core Team, 2021).

We estimated the degree of evolutionary isolation of each species belonging to the community using the QE-based originality index (Pavoine et al., 2005), following Vitória et al. (2018). This index measures how much each species contributes to the quadratic entropy of Rao from the length of the branches between the focal species and its closest ancestor, and the patristic distance between this and all other species in the phylogeny (Pavoine et al., 2005).

RELATIVE IMPORTANCE OF NICHE-BASED AND NEUTRAL PROCESSES ON INTERACTIONS FREQUENCY

To assess the relative importance of each process in determining the frequencies of interaction, probabilistic matrices were produced according to the conceptual and analytical framework proposed by Vázquez et al. (2009b). This analysis aims to evaluate which matrix (or combination of matrices) is most suitable for predicting the frequency of interactions observed. A matrix was created for each structuring process (morphological matching **M**, phenological overlap **P**, abundance **A** and evolutionary history **H**; see below details on each matrix). Also, a null matrix **N** considers that the chances of interaction of all species are equiprobable. Since more than one mechanism may influence interaction concomitantly, models considering the possible combinations between these mechanisms were elaborated using Hadamard's product, resulting

in the following probabilistic models: **MP**, **MA**, **MH**, **PA**, **PH**, **AH**, **MPA**, **MPH**, **MAH**, **PAH**, and **MPAH** in addition to **M**, **P**, **A**, **H** and **N**. Each model was contrasted with the observed matrix using the *Maximum Likelihood Analysis* and evaluated using the *Akaike Information Criterion* (AIC) with the *mlik* function (Vázquez et al., 2009b) in the *bipartite* R package (Dormann et al., 2008). Parameters to weight for model complexity were equivalent to twice the number of mechanisms used to produce each probabilistic model, because each model included variables measured for both plants and animals. Parameter for **N**, the simplest model, was set to zero (Vizentin-Bugoni et al., 2014).

While in the context of network ecology ‘neutral models’ have been typically estimated based on species’ relative abundances (e.g., Krishna et al., 2008), we acknowledge that temporal overlap may also affect chances of encounter as species that occur in a community over longer periods of time may encounter (and thus interact) more partners (Vizentin-Bugoni et al., 2022). However, as stated in the introduction, in this case, phenological overlap appears to be related to pollinator-mediated selection in a diffuse coevolution scenario, and is therefore considered here as a niche-based process. Thus, while the models **M**, **P**, **H** and their combinations are associated with niche-based processes, the neutral model is the one comprehending only the matrix **A**, without any combination, as neutrality means that interactions are defined only by abundances – and not related to other biological traits.

PROBABILISTIC MATRICES

The **morphological matching** matrix (**M**) assumes that the probability of interaction is proportional to the correspondence between proboscis length and floral tube depth (Sazatornil et al., 2016), calculated as the Gower Similarity (Vizentin-Bugoni et al., 2019). In this case, a plant with a flower much longer than a proboscis results in a virtually impossible interaction, being a forbidden link. The **phenological overlap** matrix (**P**) assumes that the number of months a pair of species co-occurs defines probabilities of interaction and that species that never coexist over time cannot interact constituting, thus, a forbidden link. The **abundance** matrix (**A**) assumes that probabilities of interaction are defined by the product of the relative abundance of each pair of species (Lautenschlager et al., 2020).

The matrix **H** derives probabilities of interaction based on partners’ **evolutionary history**, following Vitória et al. (2018). Matrices were elaborated according to the three hypotheses on how the degree of evolutionary isolation (Pavoine et al., 2005) can potentially influence interaction frequencies: (i) to the **higher isolation** hypothesis (**H+**), cells on matrix were given by the value of the QE-based originality index; (ii) to the **inferior isolation** hypothesis (**H-**), the chance of interaction was considered as the complement value of the originality index (1-originality index); and (iii) to the hypothesis of **intermediate isolation** (**Hμ**), we calculated the deviation of each species QE-based original index value from the mean value of all species of the group and normalized the values, so the chance of interaction was considered as the multiplication of the deviation for both interaction species. As the evolutionary histories of plants and pollinators can influence interactions together or separately, both scenarios were considered in which only the evolutionary history of one group has an effect on interactions, as well as combined hypotheses between both groups. If only one group has an effect on interactions from phylogenetic relationships, the other group has **no influence** hypothesis (**H0**), and the probability value of interaction in this case is considered 1 (Vitória et al., 2018).

From this, 15 possible evolutionary scenarios were generated (**H+/H+**, **H+/Hμ**, **H+/H-**, **H+/H0**, **Hμ/H+**, **Hμ/Hμ**, **Hμ/H-**, **Hμ/H0**, **H-/H+**, **H-/Hμ**, **H-/H-**, **H-/H0**, **H0/H+**, **H0/Hμ**, **H0/H-**, where the first argument refers to pollinators and the second refers to plants). Each scenario leads to a matrix that assumes the chance of interaction as defined by the combination of QE-based originality index values of the respective species pair. Each scenario was then contrasted with the observed matrix using a *Maximum Likelihood Analysis* and the quality of its fit was evaluated by the *Akaike Information Criterion* (AIC). The scenario with the lowest AIC value is considered the most adjusted to the observed matrix, and it was selected as the **H** matrix (and then used in analyses described on “RELATIVE IMPORTANCE OF EACH PROCESS ON INTERACTIONS FREQUENCY” section, above).

PHYLOGENETIC SIGNAL OF HAWKMOTH ECOLOGICAL TRAITS

The phylogenetic signal for each hawkmoth ecological trait (morphology, temporal distribution and abundance) was evaluated using the K statistic (Blomberg et al., 2003), with the function *phylosignal* (Keck et al., 2016) of the R-package *picante* (Kembel et al., 2010). This approach allows testing whether there is a tendency for phylogenetically related species to have greater phenotypic similarity than would be expected

by neutral models of evolution for each ecological trait. The K values are obtained from the ratio between the observed phylogenetic signal and the value expected by the evolution of the attribute in Brownian evolutionary motion for the phylogeny. Thus, a trait with $K=1$ indicates that a given trait evolved following the Brownian evolutionary movement. $K<1$ indicates that related species have traits more different from each other than expected by the Brownian motion, and $K>1$ indicate more similar traits (Blomberg et al., 2003). Values were permuted 999 times and significance was assessed by $p\text{-value}<0.05$.

HAWKMOTH'S PHYLOGENETIC AND TRAIT SIMILARITY WITHIN AND BETWEEN INTERACTION MODULES

We then evaluated whether modules are composed of hawkmoth species more – or less – phylogenetically related than expected by chance. First, we calculated the cophenetic distance between species and then run subsequent analysis of the *Principal Coordinates of Phylogenetic Structure* (PCPS; Duarte et al., 2016) via ADONIS using the *matrix.p.sig* function (Duarte et al., 2016) of the R-package PCPS (Debastiani, 2020). PCPS is a phylobetadiversity ordination analysis, which allows assessing which axes – taxonomic levels – respond to the variation in modules composition, to access which hawkmoth clades are more strongly associated with plants on each module. The association between interaction matrix and floral composition on the modules generates an F value, whose significance is accessed with two null models: one model shuffles plant species that compose each module (*site.shuffle*) while the other model shuffles the terminal tips of hawkmoth phylogeny (*taxa.shuffle*). Statistically significant F values indicate that hawkmoth interactions differ between modules (*site.shuffle*) and/or that species are phylogenetically more similar within modules (*taxa.shuffle*).

Further, to assess whether differences in hawkmoths composition within and between modules result from similarity of ecological traits, the mean values of hawkmoths ecological traits were individually weighted using the CWM metric (community-weighted means, Lavorel et al., 2008). CWM is typically used to express which species or lineages are distributed in the different communities within a metacommunity. In the present study, this analysis was adapted to assess how species are distributed across distinct modules within the interaction network. Therefore, (i) the community matrix is here replaced by an interaction matrix; (ii) the functional traits in the traits matrix are replaced by data on hawkmoth morphology, temporal distribution and abundances; and (iii) the environmental matrix is replaced by the interaction modules, obtained from Lautenschleger et al. (2020). This allows accessing if the hawkmoths in a module are related to its plants composition (*CWM.site.shuffle*) and/or if modules composition depends on hawkmoth traits similarity (*CWM.trait.shuffle*) (Spaniol et al., 2019). In cases where there is a phylogenetic signal in the ecological trait and a phylogenetic influence on species distributed between modules, it is necessary to account for the effect of phylogeny in the analysis, which is implemented using the *CWM.sig* function, in order to avoid type I error (Duarte et al., 2018).

Results

PHYLOGENETIC TREES AND THE ORIGINALITY INDEX

Among the hawkmoths, the Dilophonotini tribe was represented in the community by the *Aellopos*, *Pachylia*, *Perigonia* and *Erinnyis* genera; while the Philampelini, Sphingini and Macroglossini were represented by *Eumorpha*, *Manduca* and *Xylophanes*, respectively; Acherontiini was represented by *Agrius cingulata* (Fabricius, 1775); and Ambulycini was represented by *Protambulyx stringilis* (Linnaeus, 1771). Fabaceae was the family with more hawkmoth-pollinated plant species, represented by *Calliandra brevipes* Benth., *Inga marginata* Willd. and *Inga vera* Willd.. Malvaceae and Verbenaceae had two hawkmoth-pollinated plant species, while Lamiaceae, Cactaceae, Convolvulaceae, Solanaceae, Zingiberaceae and Onagraceae had one hawkmoth-pollinated species.

Among hawkmoths, *Protambulyx stringilis* and *Agrius cingulata* presented greater evolutionary isolation (QEbased=0.16 and 0.11, respectively), while the closest relatives were *Manduca contracta* (Bütler, 1875) and *Manduca brasiliensis* (Jordan, 1911) (QEbased=0.02, for both) (Table S1). Among plants, species with greatest evolutionary isolation in the community were *Hedychium coronarium* J.Koenig (QEbased=0.28) and *Cereus hildmannianus* K.Schum. (QEbased=0.11), and those with the lowest evolutionary isolation were *Inga marginata*, *Inga vera*, *Lantana camara* L. and *Duranta erecta* L. (QEbased=0.03) (Table S1).

COMMUNITY STRUCTURING PROCESSES

The best evolutionary model for predicting observed interaction frequencies – selected **H** matrix – was the one with the higher evolutionary isolation in hawkmoths and neutral in plants (**H+/H0**; AIC=5882) (Table 1). The probabilistic model that presented the best performance combined the hawkmoths evolutionary history with the morphological matching and phenological overlap of species (**HMP** model; AIC=4052) (Table 2, Fig. 1).

Table 1 - Selection of evolutionary history models best predicting mutualistic plant-hawkmoth interaction frequencies at a community in the Pampa grasslands. For models names, the first argument (before /) refers to pollinators and the second refers to plants. Symbols: Higher isolation hypothesis (**H+**), inferior isolation hypothesis (**H-**), hypothesis of intermediate isolation (**Hμ**) and no influence hypothesis (**H0**). Model complexity was weighted using two parameters (*k*) for all models. For details, see Methods.

Evolutionary history model	AIC	ΔAIC
H+/H0	5882	0
H+/H-	5905	23
H+/Hμ	6095	213
H0/H-	6369	487
H-/H0	6406	524
H-/H-	6429	547
H0/Hμ	6559	677
H-/Hμ	6619	737
Hμ/H0	6851	969
Hμ/H-	6874	992
Hμ/Hμ	7065	1183
H+/Hμ	7105	1223
H0/Hμ	7569	1687
H-/Hμ	7629	1747
Hμ/Hμ	8075	2193

Table 2 - Model selection of best predicting hypotheses on mutualistic plant-hawkmoth interaction frequencies, at a community in the Pampa grasslands. Symbols: morphological matching **M**, phenological overlap **P**, abundance **A** and evolutionary history **H** (see below details on each matrix). **Null** is benchmark model for comparison which assumes that chances of interaction for all species are equiprobable; *k* indicates the number of parameters used to weight for model complexity.

Model	AIC	ΔAIC	<i>k</i>
HMP	4052	0	6
MP	4330	278	4
HP	4741	689	4
P	5046	994	2
HM	5153	1101	4
M	5581	1529	2
H	5881	1829	2
Null	6342	2290	0
MPA	7280	3228	6
PA	7710	3658	4
HMPA	7803	3751	8
HPA	8111	4059	6
MA	8328	4276	4
HMA	8599	4547	6
A	8910	4858	2
AH	8997	4945	4

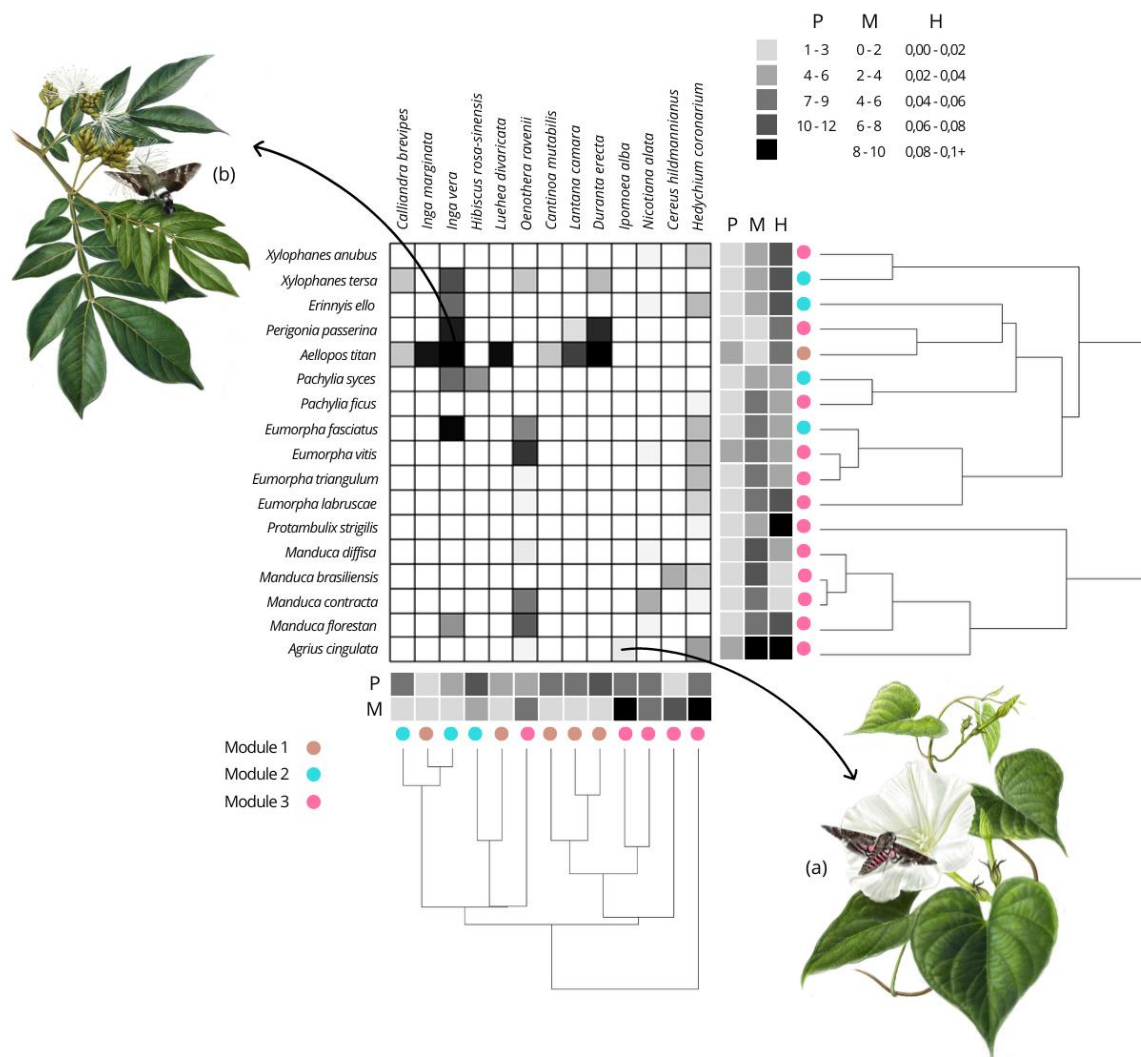


Figure 1 - Mutualistic plant-hawkmoth interaction networks in a community in the Pampas. Greyscale: indicate interaction frequencies inside the matrix and differences in relative phenology (P), proboscis or corolla lengths (M) and evolutionary originality (H) for either hawkmoths (right) or plants (bottom). Phylogenetic trees for hawkmoths (right) and plants (bottom) are indicated. Brown, blue and pink indicates to which interaction module each species belong. Emphasis in *Agrius cingulata* visiting *Ipomoea alba* (a) and *Aellopos titan* visiting *Inga vera* (b). Drawings: Natanael N. Santos.

HAWKMOTH'S PHYLOGENETIC SIGNAL

Hawkmoths' proboscis length showed phylogenetic signal as expected by the Brownian motion ($K=0.96$, $P<0.001$). There was no phylogenetic signal detected in both temporal distribution and abundance for hawkmoths ($K=0.25$, $P=0.38$ and $K=0.37$, $P=0.44$, respectively) in the community.

HAWKMOTH'S PHYLOGENETIC AND TRAIT SIMILARITY WITHIN AND BETWEEN INTERACTION NETWORK MODULES

According to the PCPS analysis, phylogeny contributed to the definition of hawkmoth composition within modules ($p.site.shuffle=0.012$). However, phylogenetic similarity of hawkmoth species within the modules was not higher than between modules ($p.taxa.shuffle=0.589$) (Fig. 2). Species taxonomic identity (PCPS 1) explained 58% of variation in hawkmoth composition in interaction modules, while 12% of this variation was explained by the genus identity (PCPS 2).

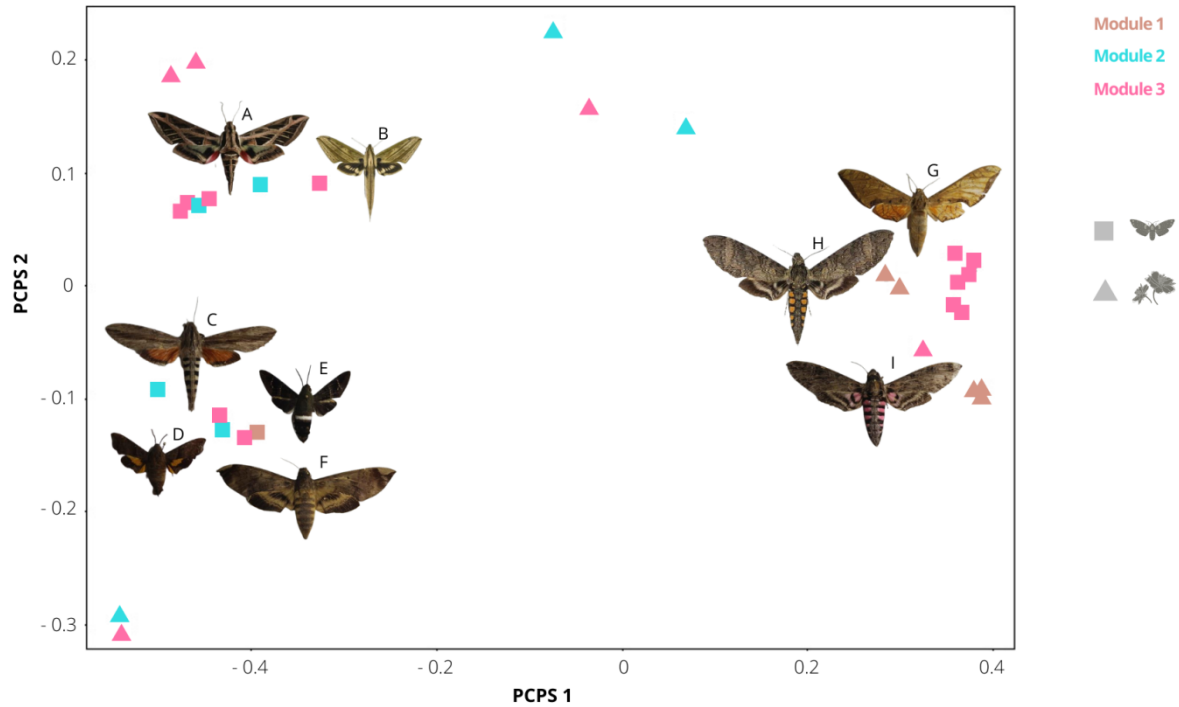


Figure 2 - Two main axis of the *Principal Coordinates of Phylogenetic Structure (PCPS)* for hawkmoths (squares) and plants (triangles) and the three modules they belong (colors) in the mutualistic plant-hawkmoth interaction network in a community in the Pampas (Lautenschleger et al., 2020). A=*Eumorpha vitis*, B=*Xylophanes anubus*, C=*Erinnyis ello*, D=*Perigonia passerina*, E=*Aellopos titan*, F=*Pachylia ficus*, G=*Protambulyx strigilis*, H=*Manduca diffisa*, I=*Agrius cingulata*.

When accounting for phylogenetic signal on species relationship and functional composition, phylogeny was also driving composition in interaction modules (Table 3: *p.site.shuffle*), while hawkmoths' morphology, abundance and temporal distribution were distributed independently among modules (Table 3: *p.trait.shuffle*), indicating that species are not functionally more similar within modules when the phylogenetical signal in traits is discounted.

Table 3 - Effects of hawkmoths' morphology (proboscis length), phenology (temporal distribution) and relative abundances on module composition in a mutualistic plant-hawkmoth network in a community in the Pampa grasslands.

Ecological trait	<i>F.model</i>	<i>p.site.shuffle</i>	<i>p.trait.shuffle</i>
Morphology	7,80	0,008	0,303
Relative abundance	23,10	0,001	0,129
Phenology	14,68	0,006	0,182

Discussion

To the best of our knowledge, we present here the first investigation on the effects of evolutionary history on interaction frequencies and modules emergence on mutualistic plant-hawkmoth networks. Through a case study, we contribute to advance the understanding of whether and how evolutionary history influences ecological traits that structure plant-pollinator interaction frequencies and modules composition. First, we show that hawkmoth species with greater evolutionary isolation tend to interact more frequently with plant species from the community than less phylogenetically isolated hawkmoth species, and that this results is independent from the plants evolutionary history (Evolutionary history H+/H0 model selection). Second, even we observed that hawkmoths tend to interact more frequently with plants matching their morphologies and with high phenological overlap (MPH model selection); interaction modules were not

determined by such hawkmoth traits when phylogenetic relatedness was taken into account. Third, in general, modules were neither composed by closely related hawkmoth species, nor by species sharing functional traits. These results, taken together with the detected phylogenetic signal of hawkmoth morphological traits and their niche partitioning that resulted in a modular network structure, suggest that competition for resources between these pollinators may be an important process structuring interactions within this functional group (Sargent & Ackerly, 2008).

EVOLUTIONARY ISOLATED HAWKMOTHS TEND TO INTERACT MORE FREQUENTLY

In addition to reiterating that morphological matching and temporal overlap are important processes shaping interaction frequencies in this system (Lautenschleger et al., 2020), our results indicate the importance of evolutionary history in shaping this microstructure (interaction frequencies) of plant-hawkmoth networks. Corroborating our hypothesis, we found that hawkmoths with greater evolutionary isolation tend to interact more frequently with plants, while interaction frequencies are not related to plants evolutionary isolation, indicating that they are selected by hawkmoths independently of their phylogenetic history.

This finding supports the notion of a hawkmoth-pollination syndrome (sphingophily), as phylogenetic distinct plant lineages have evolved a set of similar traits associated with such pollinators (Faegri & Van der Pijl, 1979). It is also consistent with previous studies showing an asymmetry of phylogenetic conservatism of interactions between plants and animals in complete pollination networks (reviewed in Peralta, 2016), and now demonstrated for a subnetwork, composed by a particular functional group (hawkmoths). In these cases, as we found here, evolutionary history of animals tends to be a more important determinant of interactions than the evolutionary history of plants (Fontaine & Thébault, 2015). However, in qualitative interaction networks (not considering interaction frequencies) the phylogeny of plants has been shown to be important for the occurrence of mutualistic interactions (Cirtwill et al., 2020). This suggests that while the evolutionary history of plants may influence which partners interact, it is less important in determining interaction frequencies. We caution here, anyhow, for the presence of two exotic plants in the community (*H. coronarius* and *Hibiscus rosa-sinensis*) which can at some extent downplay the detected role of plants' evolutionary history on interactions.

A few studies have evaluated phylogenetic effects on pollination interactions (reviewed in Peralta, 2016) but the role of evolutionary history in plant-hawkmoth interactions is still poorly understood. Here, the *hawkmoths higher isolation hypothesis* (**H+**) and the *plants no influence hypothesis* (**H0**) suggests a stronger effect of competition for resources than facilitation among hawkmoths as the main structuring process, since greater evolutionary distance tends to be related to greater functional divergence (Vitória et al., 2018). However, in communities where long-proboscis hawkmoths are less abundant, as the one studied here, competition for nectar is presumably weak and they preferentially interact with longer tubular flowers (Sazatornil et al., 2016). Thus, these results suggest that competition may be playing a role in structuring interaction in the studied community, but its role may not be directed related to the morphological traits.

The combination of traits and phylogeny is known to influence pollination interactions between bees and flowers (Stock et al., 2021), insects and orchids (Joffard et al., 2018), hummingbirds and flowers (Vitória et al., 2018) and, with our study, hawkmoths and flowers. For this study system, such interplay between phylogenetic history and traits in defining interaction frequencies may be illustrated by some examples. For instance, *Agrius cingulata* is a long-proboscis and temporally restricted hawkmoth specie that presented high evolutionary isolation in the community. It visited exclusively plants whose flowering period is relatively long and flowers are morphologically restrictive (long tubes): *Oenothera ravenii*, *Ipomoea alba* (Fig. 1a) and the exotic and introduced *Hedychium coronarium*. On the other hand, *Aellopos titan* interacted with a subset of abundant, morphologically generalist and long-flowering plants (Fig. 1b). It was the most abundant hawkmoth species, have a short proboscis, wide temporal distribution and low evolutionary isolation.

PHYLOGENETIC SIGNAL

As expected, hawkmoths' proboscis length showed phylogenetic signal, indicating that closely related hawkmoth species have similar proboscis lengths. This result suggests that, even with the influence of phylogeny on proboscis length, it adds information beyond those related to morphological matching, since both processes, **H** and **M**, were present on the best model selected (**HMP**). This occurs because simpler models are prioritized in likelihood analyzes and models containing variables with redundant information are

disfavored (Vázquez et al., 2009b; Vitória et al., 2018). On the other hand, the absence of a phylogenetic signal for temporal distribution and relative abundances of hawkmoths reinforces the importance of temporal overlap in structuring interaction frequencies as it integrates the selected model even without the influence of phylogeny. This indicates that, for an interaction to be strong, hawkmoths and plants need to co-occur considerably over time (which may also represent a component of neutrality; Vizentin-Bugoni et al., 2022) while the lack of phenological overlap constrains interaction between species by generating forbidden links. Thus, our results suggest that there is likely a hierarchy in the importance of the processes influencing interaction frequencies. While phenological overlap exerts a stronger effect on interactions and it is not phylogenetically determined, morphological matching – that is also of primary importance – can be, at least in part, attributed to the phylogenetic signal on proboscis length. Relative abundances, in turn, are not phylogenetically determined and are relatively less important than the other processes evaluated to define these interactions.

Furthermore, our evolutionary history quantification may indirectly encompass a series of phylogenetically determined (and *a priori* unknown) traits that may be important in defining interactions (Chamberlain et al., 2014). In this case, the relationship between hawkmoth phylogenetic history and interactions observed in the community would indirectly reflect the potential existence of phylogenetic signal on other, unmeasured, traits. Behavior may be one of these traits, for instance. Among all hawkmoths occurring in the community, *Aellopos titan* is the only one with exclusively diurnal foraging habits, which formed the smallest module of community, associated with flowers that exhibit diurnal anthesis. As pointed out by Amorim (2020) and Lautenschleger et al. (2020), the period of the day in which the hawkmoths forage may be associated with networks modularity. Thus, we recommend that future studies explicitly assess behavioral traits as structuring processes of communities.

EMERGENCE OF MODULARITY

When evaluating the drivers of network macrostructure (modularity), the PCPS analysis indicated that hawkmoth composition on each module is, at least, in part influenced by phylogeny. Although, contrary to our expectation, there was similar phylogenetic composition among different modules, which means that distantly related hawkmoth species were interacting with the same subsets of plants species. The hawkmoth phylogenetic influence on module composition may be guided by the clustering of *Manduca* spp. within the same module. While distinct species of the same genus (*Pachylia*, *Eumorpha* and *Xylophanes*) occupy different modules in the network, all species of *Manduca* genus were grouped together. *Manduca* species were rare, have long proboscis and interacted with four of the five flowers of the module 3 (on pink), all with restrictive morphology (long tubes): *Oenothera ravenii*, *Nicotiana alata*, *Cereus hildmannianus* and *Hedychium coronarium* (see Fig. 1). Thus, typically sphingophilous flowers ensure a ‘private niche’ for these individuals, reducing their competition with generalist hawkmoths for nectar on short-tubed flowers (Amorim, 2012; Lautenschleger et al., 2020). Since congeneric species of *Pachylia*, *Eumorpha* and *Xylophanes* interact with more generalist plants, niche partitioning (i.e., congeneric species belonging to different modules) may be a strategy to avoid competition with similar proboscis species. This indicates that at a coarser scale (macrostructure) of the network, species distribution of hawkmoths among modules is not related to phylogenetic proximity (cophenetic distance).

However, plant-pollinator interaction networks including different groups of pollinators often have phylogenetically determined modules, indicating that on broader phylogenetical scales (e.g., multiple groups of pollinators and plants considered) phylogeny explains the emergence of modularity (Dupont & Olesen, 2009). This may indicate that, in phylogenetically delimited interaction networks (i.e., a single group of pollinators considered, as in our study), the emergence of interaction modules is not only directly influenced by evolutionary history and phylogenetically conserved traits, but potentially also by a strong environmental filtering or stochastic factors (not measured here), that define assemblage composition and, possibly, may influence interactions. Environmental components may operate at distinct scales when considering small and local or big and broader communities (Kraft et al., 2015). In this sense, despite evolutionary history may influence interactions frequencies (Vitória et al., 2018), its contribution to the emergence of modules may be linked to other processes related to community assembling (Vázquez et al., 2009b), which requires attention of future studies on the drivers of interaction networks structure.

In conclusion, we demonstrate that plant-hawkmoth interaction frequencies (network microstructure) increase not only when partner species co-occur over longer periods of time and have morphologic traits

matching, but also for more phylogenetically unique hawkmoths. However, network modularity is only weakly related to phylogeny and evolutionary history, since a module often encompasses phylogenetically distant hawkmoth species. In turn, the tendency of closely related hawkmoths that present similar morphologies to occupy distinct modules (which results in interactions with distinct subsets of plants) may reinforce the importance of competition as a process underlying niche partitioning in this system. Furthermore, the traits considered here (morphologies, phenologies and abundances) may also be the result of other selective pressures than consumer-resource interactions, such as environmental filtering or stochasticity that defines community composition and, thus, influence interactions. The importance of such factors in shaping module composition may be tested in future studies that include multiple communities.

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Data, scripts, code, and supplementary information availability

Data and scripts are available online: <https://doi.org/10.5281/zenodo.7908194>

Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article.

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References

- Amorim FW (2012) A flora esfingófila de uma floresta ombrófila densa montana no sudeste brasileiro e relações mutualísticas com a fauna de Sphingidae. PhD thesis. Universidade Estadual de Campinas. 175 p. <https://doi.org/10.47749/T/UNICAMP.2012.882109>
- Amorim FW (2020) Are the New World hummingbird-hawkmoths functional equivalents of hummingbirds? *Ecology*, **101**, 12 <https://doi.org/10.1002/ecy.3161>
- Angiosperm Phylogeny Group (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, **181**(1), 1–20. <https://doi.org/10.1111/boj.12385>
- Bascompte J, Jordano P (2007) Plant-animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 567–593. <https://doi.org/10.1146/annurev.ecolsys.38.091206.095818>
- Beckett SJ (2016) Improved community detection in weighted bipartite networks. *Royal Society Open Science*, **3**, 140536. <https://doi.org/10.1098/rsos.140536>
- Bergamini LL, Lewinsohn TM, Jorge LR, Almeida-Neto M (2017) Manifold influences of phylogenetic structure on a plant-herbivore network. *Oikos*, **126**, 5, 703–712. <https://doi.org/10.1111/oik.03567>
- Blomberg SP, Garland JRT, Ives AR (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, **57**, 4, 717–745. <https://doi.org/10.1111/j.0014-3820.2003.tb00285.x>
- Cavalheiro L (2023) cavalheiro-l/sph-plants-grasslands: v2 (Version v2). Zenodo. <https://doi.org/10.5281/zenodo.7886325>

- Cirtwill AR, Riva GVD, Baker NJ, Ohlsson M, Norström I, Wohlfarth I, Thia JA, Stouffer DB (2020) Related plants tend to share pollinators and herbivores, but strength of phylogenetic signal varies among plant families. *New Phytologist*, **226**, 3, 909–920. <https://doi.org/10.1111/nph.16420>
- Chamberlain S, Vázquez DP, Carneiro L, Elle E, Vamasi JC (2014) Phylogenetic tree shape and the structure of mutualistic networks. *Journal of Ecology*, **102**, 5, 1234–1243. <https://doi.org/10.1111/1365-2745.12293>
- Darwin CR (1859) On the origin of species by means of natural selection or the preservation of favoured races in the struggle for life. John Murray, London.
- Darwin CR (1862) On the Various Contrivances by Which British and Foreign Orchids Are Fertilized by Insects. John Murray, London.
- Debastiani VJ (2020) PCPS: Principal Coordinates of Phylogenetic Structure. R package version 1.0.7. <https://CRAN.R-project.org/package=PCPS>
- Dormann CF, Gruber B, Fruend J (2008) Introducing the bipartite Package: Analysing Ecological Networks. *R News*, **8**, 2, 8–11.
- Dormann CF, Frund J, Schaefer HM (2017) Identifying Causes of Patterns in Ecological Networks: Opportunities and Limitations. *The Annual Review of Ecology, Evolution, and Systematics*, **48**, 559–84. <https://doi.org/10.1146/annurev-ecolsys-110316-022928>
- Duarte LDS, Debastiani VJ, Carlucci MB, Diniz-Filho JAF (2018) Analyzing community-weighted trait means across environmental gradients: should phylogeny stay or should it go? *Ecology*, **99**, 2, 385–398. <https://doi.org/10.1002/ecy.2081>
- Duarte LDS, Debastiani VJ, Freitas AVL, Pillar VD (2016) Dissecting phylogenetic fuzzy weighting: theory and application in metacommunity phylogenetics. *Methods in Ecology and Evolution*, **7**, 8, 937–946. <https://doi.org/10.1111/2041-210X.12547>
- Dupont YL, Olesen JM (2009) Ecological modules and roles of species in heathland plant–insect flower visitor networks. *Journal of Animal Ecology*, **78**, 2, 346–353. <https://doi.org/10.1111/j.1365-2656.2008.01501.x>
- Ellwanger JH, Ziliotto M, Chies JAB (2022) Protect Brazil's overlooked Pampa biome. *Science*, **377**, 720–720. <https://doi.org/10.1126/science.ade1838>
- Faegri K, Van Der Pijl L (1979) Principles of Pollination Ecology (3rd ed.). Elsevier.
- Fontaine C, Thébaud E (2015) Comparing the conservatism of ecological interactions in plant–pollinator and plant–herbivore networks. *Population Ecology*, **57**, 1, 29–36. <https://doi.org/10.1007/s10144-014-0473-y>
- Freitas L, Vizin-Bugoni J, Wolowski M, Souza JMT, Varassin IG (2014) As interações planta-polinizador e a estruturação das comunidades. In: AR Rech, K Agostini, PE Oliveira, IC Machado (Org.), *Biologia da Polinização*. Rio de Janeiro: Projeto Cultural.
- Guimarães PR, Rico-Gray V, Oliveira PS, Izzo TJ, Reis SF, Thompson JN (2007) Interaction Intimacy Affects Structure and Coevolutionary Dynamics in Mutualistic Networks, *Current Biology*, **17**, 20, 1797–1803. <https://doi.org/10.1016/j.cub.2007.09.059>
- Hedges SB, Dudley J, Kumar S (2006) TimeTree: A public knowledge-base of divergence times among organisms. *Bioinformatics*, **22**, 23, 2971–2972. <https://doi.org/10.1093/bioinformatics/btl505>
- Joffard N, Massol F, Grenié M, Montgelard C, Schatz B (2018) Effect of pollination strategy, phylogeny and distribution on pollination niches of Euro-Mediterranean orchids. *Journal of Ecology*, **107**, 1, 478–490. <https://doi.org/10.1111/1365-2745.13013>
- Johnson SD, More M, Amorim FW, Haber WA, Frankie GW, Stanley DA, Cocucci AA, Raguso RA (2017) The long and the short of it: a global analysis of hawkmoth pollination niches and interaction networks. *Functional Ecology*, **31**, 1, 101–115. <https://doi.org/10.1111/1365-2435.12753>
- Kawahara AY, Barber JR (2015) Tempo and mode of antibat ultrasound production and sonar jamming in the diverse hawkmoth radiation. *Proceedings of the National Academy of Sciences*, **112**, 20, 6407–6412. <https://doi.org/10.1073/pnas.1416679112>
- Kawahara AY, Breinholt JW, Ponce FV, Haxaire J, Xiao L, Lamarre GPA, Rubinoff D, Kitching IJ (2013) Evolution of *Manduca sexta* hornworms and relatives: Biogeographical analysis reveals an ancestral center of diversification in Central America. *Molecular Phylogenetics and Evolution*, **68**, 3, 381–386. <http://dx.doi.org/10.1016/j.ympev.2013.04.017>
- Keck F, Rimet F, Bouchez A, Franc A (2016) Phylosignal: an R package to measure, test, and explore the phylogenetic signal. *Ecology and Evolution*, **6**, 9, 2774–2780. <https://doi.org/10.1002/ece3.2051>

- Kembel S, Cowan P, Helmus M, Cornwell W, Morlon H, Ackerly D, Blomberg S, Webb C (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 11, 1463–1464. <https://doi.org/10.1093/bioinformatics/btq166>
- Kitching IJ (2024) Sphingidae Taxonomic Inventory. Available at <http://sphingidae.myspecies.info/>
- Kraft NJB, Adler PB, Godoy O, James EC, Fuller S, Levine JM (2015) Community assembly, coexistence and the environmental filtering metaphor. *Functional Ecology*, **29**, 5, 592–599. <https://doi.org/10.1111/1365-2435.12345>
- Krishna A, Guimarães Jr PR, Jordano P, Bascompte J. (2008). A neutral-niche theory of nestedness in mutualistic networks. *Oikos*, **117**, 11, 1609–1618. <https://doi.org/10.1111/j.1600-0706.2008.16540.x>
- Lautenschlager A, Vizentin-Bugoni J, Cavaleiro L, Iserhard CA (2020) Morphological matching and phenological overlap promote niche partitioning and shape a mutualistic plant-hawkmoth network. *Ecological Entomology*, **46**, 2, 292–300. <https://doi.org/10.1111/een.12961>
- Lavorel S, Grigulis K, McIntyre S, Williams NSG, Garden D, Dorrough J, Berman S, Quétier F, Thébaud A, Bonis A (2008) Assessing functional diversity in the field – Methodology matters! *Functional Ecology*, **22**, 1, 134–147. <https://doi.org/10.1111/j.1365-2435.2007.01339.x>
- Lewinsohn TM, Prado PI, Jordano P, Bascompte J, Olesen JM (2006) Structure in Plant-Animal Interaction Assemblages. *Oikos*, **113**, 1, 174–184. <https://doi.org/10.1111/j.0030-1299.2006.14583.x>
- Olesen JM, Bascompte J, Dupont YL, Jordano P (2007) The modularity of pollination networks. *Proceedings of the National Academy of Sciences*, **104**, 50, 19891–19896. <https://doi.org/10.1073/pnas.0706375104>
- Oliveira R, Duarte Jr. JA, Rech AR, De Avila Jr. RS (2014) Polinização por Lepidopteros. In: AR Rech, K Agostini, PE Oliveira, IC Machado (Org.), *Biologia da Polinização*. Rio de Janeiro: Projeto Cultural.
- Paradis E, Schliep K (2019) Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, **35**, 3, 526–528. <https://doi.org/10.1093/bioinformatics/bty633>
- Pavoine S, Ollier S, Dufour A (2005) Is the originality of a species measurable? *Ecology Letters*, **8**, 6, 579–586. <https://doi.org/10.1111/j.1461-0248.2005.00752.x>
- Pennell MW, Eastman JM, Slater GJ, Brown JW, Uyeda JC, Fitzjohn RG, Alfaro ME, Harmon LJ (2014) Geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics*, **30**, 15, 2216–2218. <https://doi.org/10.1093/bioinformatics/btu181>
- Peralta G (2016) Merging evolutionary history into species interaction networks. *Functional Ecology*, **30**, 2, 1917–1925. <https://doi.org/10.1111/1365-2435.12669>
- Phillips RD, Peakall R, van der Niet T, Johnson SD (2020) Niche perspectives on plant–pollinator interactions. *Trends in Plant Science*, **25**, 8, 779–793. <https://doi.org/10.1016/j.tplants.2020.03.009>
- Ponce FV, Breinholt JW, Hossie T, Barber JR, Janzen DH, Hallwachs W, Kawahara AY (2014) A molecular phylogeny of Eumorphia (Lepidoptera: Sphingidae) and the evolution of anti-predator larval eyespots. *Systematic Entomology*, **40**, 2, 401–408. <https://doi.org/10.1111/syen.12111>
- Queiroz JA, Diniz UM, Vázquez DP, Quirino ZM, Santos FAR, Mello MAR, Machado IC (2021) Bats and hawkmoths form mixed modules with flowering plants in a nocturnal interaction network. *Biotropica*, **53**, 2, 596–607. <https://doi.org/10.1111/btp.12902>
- R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at <https://www.R-project.org/>
- Rech AR, Brito VLG (2012) Mutualismos Extremos de Polinização: História Natural e Tendências Evolutivas. *Oecologia Australis*, **16**, 2, 297–310. <http://dx.doi.org/10.4257/oeco.2012.1602.08>
- Revell LJ (2012) Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**, 2, 217–223. <https://doi.org/10.1111/j.2041-210X.2011.00169.x>
- Sargent RD, Ackerly DD (2008) Plant–pollinator interactions and the assembly of plant communities. *Trends in Ecology & Evolution*, **23**, 3, 123–130. <https://doi.org/10.1016/j.tree.2007.11.003>
- Sazatornil FD, Moré M, Benítez-Vieyra S, Cocucci AA, Kitching IJ, Schlumpberger BO, Oliveira PE, Sazima M, Amorim FW (2016) Beyond neutral and forbidden links: morphological matches and the assembly of mutualistic hawkmoth–plant networks. *Journal of Animal Ecology*, **85**, 6, 1586–1594. <https://doi.org/10.1111/1365-2656.12509>
- Spaniol RL, Duarte LS, Mendonça Jr. MS, Iserhard CA (2019) Combining functional traits and phylogeny to disentangling Amazonian butterfly assemblages on anthropogenic gradients. *Ecosphere*, **10**, 8. <https://doi.org/10.1002/ecs2.2837>

- Stock M, Piot N, Vanbesien S, Meys J, Smagghe G, De Baets B (2021) Pairwise learning for predicting pollination interactions based on traits and phylogeny. *Ecological Modelling*, **451**.
<https://doi.org/10.1016/j.ecolmodel.2021.109508>
- Thompson JN (2005) The Geographic Mosaic of Coevolution. University of Chicago Press.
<https://doi.org/10.7208/9780226118697>
- Vázquez DP, Bluthgen N, Cagnolo L, Chacoff NP (2009a) Uniting pattern and process in plant-animal mutualistic networks: a review. *Annals of Botany*, **103**, 9, 1445–1457.
<https://doi.org/10.1093/aob/mcp057>
- Vázquez DP, Chacoff NP, Cagnolo L (2009b) Evaluating multiple determinants of the structure of plant–animal mutualistic networks. *Ecology*, **90**, 8, 2039–2046. <https://doi.org/10.1890/08-1837.1>
- Vitória RS, Vizentin-Bugoni J, Duarte LS (2018) Evolutionary history as a driver of ecological networks: a case study of plant–hummingbird interactions. *Oikos*, **127**, 4, 561–569. <https://doi.org/10.1111/oik.04344>
- Vizentin-Bugoni J, Debastiani VJ, Bastazini VAG, Maruyama PK, Sperry JH (2019) Including rewiring in the estimation of the robustness of mutualistic networks. *Methods in Ecology and Evolution*, **11**, 1, 106–116.
<https://doi.org/10.1111/2041-210X.13306>
- Vizentin-Bugoni J, Maruyama PK, Sazima M (2014) Processes entangling interactions in communities: forbidden links are more important than abundance in a hummingbird–plant network. *Proceeding of the Royal Society*, **281**, 1780. <http://dx.doi.org/10.1098/rspb.2013.2397>
- Vizentin-Bugoni J, Sperry JH, Kelley JP, Foster JT, Drake DR, Case SB, ..., Tarwater CE (2022) Mechanisms underlying interaction frequencies and robustness in a novel seed dispersal network: lessons for restoration. *Proceedings of the Royal Society B*, **289**, 1982, 20221490.
<https://doi.org/10.1098/rspb.2022.1490>
- Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics*, **24**, 18, 2098–2100.
<https://doi.org/10.1093/bioinformatics/btn358>