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Inferring coevolution in a plant–pollinator network

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Mutualistic interactions are at the core of community dynamics, determining dispersal, colonization and differential survival and reproduction among individuals and species. Mutualistic interactions therefore affect the fitness of interaction partners, hence modifying their respective evolutionary trajectories reciprocally, potentially leading to coevolution. Although mathematical models predict coevolution in mutualistic interaction networks, no empirical data are available. By taking into account the patterns of interactions and reconstructing evolutionary change in plant and pollinator traits, we tested the hypothesis that coevolution occurs between plants and insects that interact more frequently, or more symmetrically. To test this hypothesis, we built an interaction network with data from five flowering seasons, measured plant and insect morphology, mapped morphology on the plant and insect phylogenies, and reconstructed ancestral character changes based on maximum parsimony. We calculated an index, called the coevolutionary ratio, which represents the amount of correlated change in traits that mediate the interaction between plants and pollinators (i.e. proboscis versus corolla length, and body width and corolla aperture). Our results suggest that high frequency of interaction, i.e. the number of times two species interact, does not lead to coevolution. Instead, symmetry of interaction strength, i.e. the reciprocal similarity in the mutual effect of interaction partners, may lead to coevolution, in spite of a pervasive lack of reciprocal specialization and high interaction frequency. Although the statistical signal is quite weak, our results hold for three statistical tests of very different nature. The most specialized species, expected to be under directional selection, do not show more evolutionary change than do generalist species, expected to be under different, perhaps opposing, selective pressures. By dissecting the complexity of an interaction network we show that coevolution may partially shape functional morphology of interaction partners, thus providing the closest evidence to date of mutualistic adaptation of organisms within a community.

Keywords: Argentina, ecological interactions, evolutionary ecology, Monte desert, mutualism, pollinator community

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'A fallacy continually perpetuated is the notion that mutualisms are coevolved'

(Schemske 1983)

Introduction

Mutualistic interactions are at the core of community dynamics, because they help determine dispersal and colonization of new habitats, defining ecological patterns such as species diversity in tropical forests (Howe and Miriti 2000, 2004). Mutualisms also affect survival and reproduction of individuals, driving ecological dynamics (Mougi and Kondoh 2012), evolutionary trajectories, and morphological and taxon diversity (Jordano 1995, Rønsted et al. 2008, Lomáscolo et al. 2010). All these effects are reciprocal between mutualists and, if they are somehow equivalent as mutual selective pressures, they may lead to coevolution. Understanding whether and how coevolution in mutualisms shapes biodiversity is one of the major challenges of evolutionary biology (Schemske 1983, Thompson 1999a). A paradigmatic example of the importance of coevolution in shaping biodiversity (Thompson 1999a) is the purported role of flower–pollinator interactions in angiosperm diversification (Kiester et al. 1984, Eriksson and Bremer 1992). However, to our knowledge, studies dealing with coevolution in plant–animal networks are mostly based on mathematical models (Guimarães et al. 2007, 2011, 2017, Nuismer et al. 2013, Ponisio and M'Gonigle 2017, Medeiros et al. 2018). They do not offer empirical evidence on whether coevolution has shaped the diversity of functional traits that mediate mutualistic interactions within a community, except between rare (Waser et al. 1996), hyper-specialized mutualists (Darwin 1862, Kiester et al. 1984, Smith et al. 2008).

The conceptual basis of the coevolutionary process is mostly based on the above extreme, reciprocally specialized interactions (Darwin 1862, Janzen 1979, Jousselin et al. 2003), which leads to the prediction that coevolution occurs only between reciprocally specialized interactions. Yet it is widely acknowledged that reciprocal specialization in mutualistic interactions, historically considered a prerequisite for coevolution (Schemske 1983), is extremely rare in natural communities (Waser et al. 1996, Vázquez and Aizen 2004, Bascompte et al. 2006). The geographic mosaic theory of coevolution (GMTC) (Thompson 1999b, Thompson and Cunningham 2002, Medeiros et al. 2018) incorporated the spatial dimension of the outcome and strength of interactions. The GMTC does not require reciprocal specialization at the species level but it does at the population level, because it predicts that interaction tightness will vary geographically, as the context in which the interaction takes place determines its outcome in the fitness of interaction partners (Thompson 1997). Hence, as species interact with varying intensity throughout their geographical range, coevolution between interaction partners will occur mainly in coevolutionary hotspots where the species are mutually specialized. Besides the tight specialization model and the GMTC, a third idea on how coevolution occurs, diffuse coevolution, often invoked in studies at the community level, might be more appropriate (Janzen 1980) to understand the coevolutionary process in complex mutualistic networks, as it incorporates the idea that multiple interacting species are exposed to the ecological effect of several other species. As a result, both groups of mutualists (insects and plants) evolve under diverse selective pressures. A problem with diffuse coevolution is that, with few notable exceptions (Iwao and Rausher 1997, Stinchcombe and Rausher 2001, Irwin and Strauss 2005), it is often used as a vague term with no specific predictions as to what would be the evolutionary outcome of multiple interactions. We test a fourth idea that has recently drawn attention to evolutionary biologists interested in how coevolution occurs, based on the structure of mutualistic interactions networks, for which we pose unexplored predictions on the coevolutionary outcomes of interacting species.

Network studies in plant–animal mutualisms have identified certain patterns in the structure of interactions. A first outstanding pattern is that frequency of interaction, the number of times a certain insect species is recorded on a plant species, is unevenly distributed among all interactions in the network (Bascompte and Jordano 2007), that is some species interact very frequently and most interact infrequently. A second pattern of mutualistic networks is that symmetry (Vázquez and Aizen 2004, Bascompte et al. 2006, Vázquez et al. 2007) and reciprocal specialization (Waser et al. 1996) are rare, seemingly leaving little opportunity for coevolutionary coupling of interacting populations (Vázquez and Aizen 2006). Symmetry relates to the similarity in the relative visitation frequency between two interacting species, while specialization is basically related to the number of species with which the focal species interacts. We propose that both frequency and symmetry of interactions define the reciprocal selective pressures between interaction partners. Because interaction frequency has been argued to represent well the population-level effect of the interacting species on each other's fitness (Vázquez et al. 2005), and has been shown to lead to morphological adaptation (Pauw et al. 2017), we expect that frequent interactions should show stronger evidence of coevolution than infrequent interactions. We also expect that the uncommon symmetric interactions, those with comparable reciprocal effects on the fitness of each interaction partner, will show greater evidence of coevolution than asymmetric interactions. We expect coevolution in both symmetrically strong and symmetrically weak interactions (Fig. 1), albeit perhaps at different rates. Symmetrically strong interactions are those in which each interaction partner represents a similarly high percentage of its partner's total interactions. Those, by definition, are reciprocally specialized interactions, in the sense that they represent a large proportion of the total interaction frequency of those species. In addition, it is reasonable to expect coevolution also between symmetrically weak interactions, even if at a much slower rate because, albeit weak, the

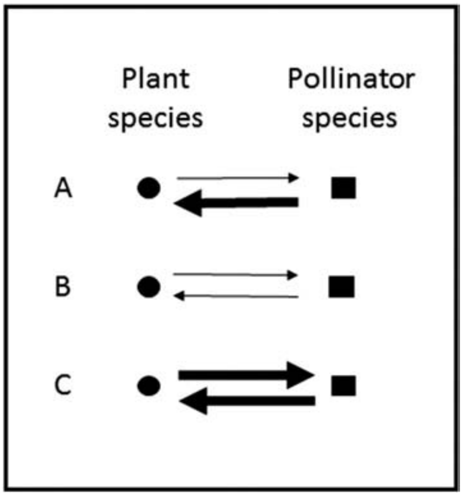

Figure 1. Illustration of symmetry of interaction strength. Circles represent plant species, while squares represent pollinator species that interact with them. This figure only represents the detail of pairwise interactions, but it should be understood that each of these hypothetical species interact with many other hypothetical species. All interactions with other species are not drawn for simplification. Arrows represent the effect of one interactor on the other, measured as the proportion of visits represented by that species to the other, also called interaction strength. The thickness of the arrow is proportional to the strength of the interaction. In the first pair, (A) the interaction is asymmetric, as the thickness of both arrows is different. In the second pair, (B) the interaction is symmetrically weak, as interaction strength is similar for both species, but its low. That means that these species mostly interact with other species, yet, the reciprocal effect is similar. In the third pair, (C) the interaction is symmetrically strong, which means that both interaction partners represent a similarly high proportion of all visits of the other partner.

reciprocal effects are similar in magnitude between partners and have thus the potential to represent comparable selection pressures for the interacting organisms. Thus, consideration of the above network structure of interactions leads to testable predictions on the expected coevolutionary outcomes of mutualistic interactions that occur in a community. Previous studies of reciprocal effects focus on measures of fitness (e.g. pollen transfer, number of offspring) of one or both interaction partners (Vázquez et al. 2012), but not on the resulting change in functional morphology. And studies in network evolution per se are theoretical (Guimarães et al. 2007, 2017, Ponisio and M'Gonigle 2017). The unique and novel aspect of what we propose here is that we focus on the resulting evolutionary morphological change in functional traits that mediate pollination interactions, and that we pose testable empirical predictions about the drivers of such morphological change, incorporating the complexity of interactions in a community.

Recent mathematical models predict that coevolution shapes species traits in mutualistic networks (Guimarães et al. 2007, 2011, Nuismer et al. 2013), leading to trait complementarity among interaction partners and trait convergence among species in the same trophic level (Guimarães et al. 2011). Coevolution may even alter the patterns of species interactions in ecological networks (Nuismer et al. 2013) or may leave a weak signal in network topology, specifically nestedness and modularity (Ponisio and M'Gonigle 2017). However, although coevolution has been thoroughly studied in pairs of reciprocally specialized interactions (Darwin 1862, Janzen 1979, Weiblen 2002), a recent study shows that, in order to understand the coevolutionary process, it is key to progress from pairs of interacting species, to a network of interactions within a community, as coevolution may occur even among species that do not interact, via indirect effects (Guimarães et al. 2017). To our knowledge, no empirical evidence exists for reciprocal evolutionary change, i.e. coevolution at the community level. Based on a phylogenetic reconstruction of ancestral state character evolution using empirical data on plant and insect traits, we analyze whether evolutionary changes in plant and insect traits that mediate pollination interactions evolved reciprocally. Our general hypothesis, that coevolution in a community is governed by the patterns of interaction, leads to the expectation that the strongest reciprocal selective pressures and the most clearly defined morphological response to those selective pressures will come from 1) the most frequent interaction partners, 2) partners with highly symmetric interaction strength and 3) the most specialized interactions. This is in line with predictions discussed in previous studies (Vázquez et al. 2007). We test two specific evolutionary hypotheses with field data from a plant pollinator community from the Monte desert of Argentina. The first one, H1, is that pollinating insects and the plants they visit more frequently, or with more symmetrical strength, will experience simultaneous changes in their morphology, i.e. they will coevolve. Coevolution was calculated as the similarity in evolutionary change of matching morphological traits (proboscis versus corolla length, body width versus corolla aperture) between interacting species. Thus, the perceived morphological adjustment between pollinators and flowers would be consistent with improved fitness on both sides. In the context of this hypothesis, we test two specific predictions, expected from the reciprocal selective pressure between plants and pollinators in the network: the evolutionary change in traits that mediate plant–pollinator interactions will coevolve: 1) between species that interact more frequently and 2) between species that interact with more symmetrical strength in an interaction network. Specifically we will focus on coevolution between length of a pollinator's proboscis and corolla length, and between width of a pollinator's body and corolla aperture. A second hypothesis, H2, is that, because generalists are exposed to variable, often conflicting, selective pressures, species that interact with a wide range of mutualists in a plant–pollinator interaction network will show less net evolutionary change (i.e. stasis) than species that interact with few mutualists (expected due to directional selection). The prediction tested under this hypothesis is that the amount of matching evolutionary change in proboscis, body width, corolla length and aperture is positively correlated with the degree of specialization of the species.

We found that, for some traits, high symmetry of interaction is associated with stronger coevolution between plant–pollinator interacting pairs. Although the statistical signal is quite weak, so we cautiously call this a trend, our results hold for three statistical tests of very different nature. Neither mutual specialization nor high interaction frequency are prerequisites for coevolution, as expected under all previous models of coevolution.

Methods

To test for coevolution in our plant–pollinator network, H1, we need to know whether historical changes in traits that mediate the interaction have occurred in a correlated manner in plant and pollinator species that interact with each other. To this end, we first recorded plant–pollinator interactions for five consecutive years in a Monte desert site, measured species traits that we considered important in mediating the interaction, and reconstructed the historical changes of those traits based on a phylogeny of the plants and pollinators involved. If changes in plant and pollinator traits, e.g. proboscis and corolla length, were correlated, we considered that as a sign of coevolution. In order to know whether frequency or symmetry of interaction strength were drivers of plant–pollinator coevolution, we built an interaction network to detect interaction patterns within the community. The network structure allowed us to obtain quantitative measures of frequency and symmetry needed for the first hypothesis, and of specialization, which allowed us to address our second hypothesis. To test H2, whether generalists, expected to be under stasis showed less evolutionary change than specialists, expected to be under directional selection, we correlated the amount of change in traits as reconstructed in the phylogeny to the degree of specialization.

Network structure and species traits

Interactions

Data were collected in the lowlands of Villavicencio Natural Reserve, which represent a well conserved Monte desert site in Mendoza, Argentina. Plant–pollinator interactions were sampled over five consecutive flowering seasons (from September to December in 2006 to 2010), twice a week. We performed observations of the interactions on focal plants species during 5 min (more details on how we gathered plant–pollinator visitation data can be found in Supplementary material Appendix 1 and in Chacoff et al. (2012), the resulting interaction matrix is shown in Supplementary material Appendix 1 Table A1). Of all the species published in the cited study, we included here only those for which we were able to quantify morphology. The interaction network was built with over 18 000 interaction records observed between the 38 plant species and 71 insect species (Fig. 2) (see details in Supplementary material Appendix 1) for which we measured morphological traits (Supplementary material Appendix 1 Fig. A1).

Network parameters

To quantify symmetry, we used Bascompte et al.'s (2006) index, which is actually an index of asymmetry and is calculated as $\mathrm{AS}(i, j) = |d_{ij} - d_{ji}|/\max(d_{ij}, d_{ji})$, where d_{ij} is the proportion of all observations of insect species i recorded on plant species j, hereafter called interaction strength of species i on species j (Vázquez et al. 2012), and d_{ji} is the proportion of all visits recorded to plant species j done by insect species i, that is, the interaction strength of species j on species i. These are called relative dependences by Bascompte et al. (2006). Thus, AS_{ij} is low (close to zero) when the interaction between a pair of species i and j represents a similar proportion of all interactions of both species (i.e. the interaction is symmetric), and high when the proportions are different (i.e. the interaction is asymmetric). Note that AS_{ij} will tend to zero whenever mutual interaction strengths are similarly high or similarly low. We discussed above why this is useful in our study, as we expect coevolution in symmetrically strong and symmetrically weak interactions, as both will show low AS_{ij}. Because we think it is conceptually simpler to focus on symmetry instead of asymmetry, as we did in the Introduction, we performed the analysis using $1 - \mathrm{AS}_{ij}$, which is the exact inverse of Bascompte et al.'s index, and report and discuss our results in terms of symmetry.

Frequency of visits of a specific insect to a plant was represented by the number of times that an insect species was seen on a plant species, summed up throughout the five years of the study (Fig. 2, Supplementary material Appendix 1 Table A1). An interaction was recorded only when the insect touched the flower's reproductive parts.

Morphological data

Plant traits

We collected data on corolla dimensions, specifically, corolla length and aperture (Supplementary material Appendix 1 Fig. A1, Table A2) as these have been shown to vary due to selection by pollinators (Nilsson 1988, Muchhala and Thomson 2009), and were easy to measure as were the pollinator traits that we considered to be the matching traits when considering reciprocal selective forces (proboscis length and body width, see Pollinator traits). Plant traits were measured using a caliper. We included 38 plant species in the network (Supplementary material Appendix 1 Table A1) and measured at least six flowers per species, with the exception of two species for which we only measured three flowers (Supplementary material Appendix 1 Table A2). For shrubs, trees, and herbaceous plants for which individuals can readily be identified, we randomly selected three individuals on which we measured traits of three flowers. We believe that this number represented a good compromise between incorporating intraspecific variation and being able to include more species. We considered that three individuals were enough to represent the morphology of the species, as intraspecific variation

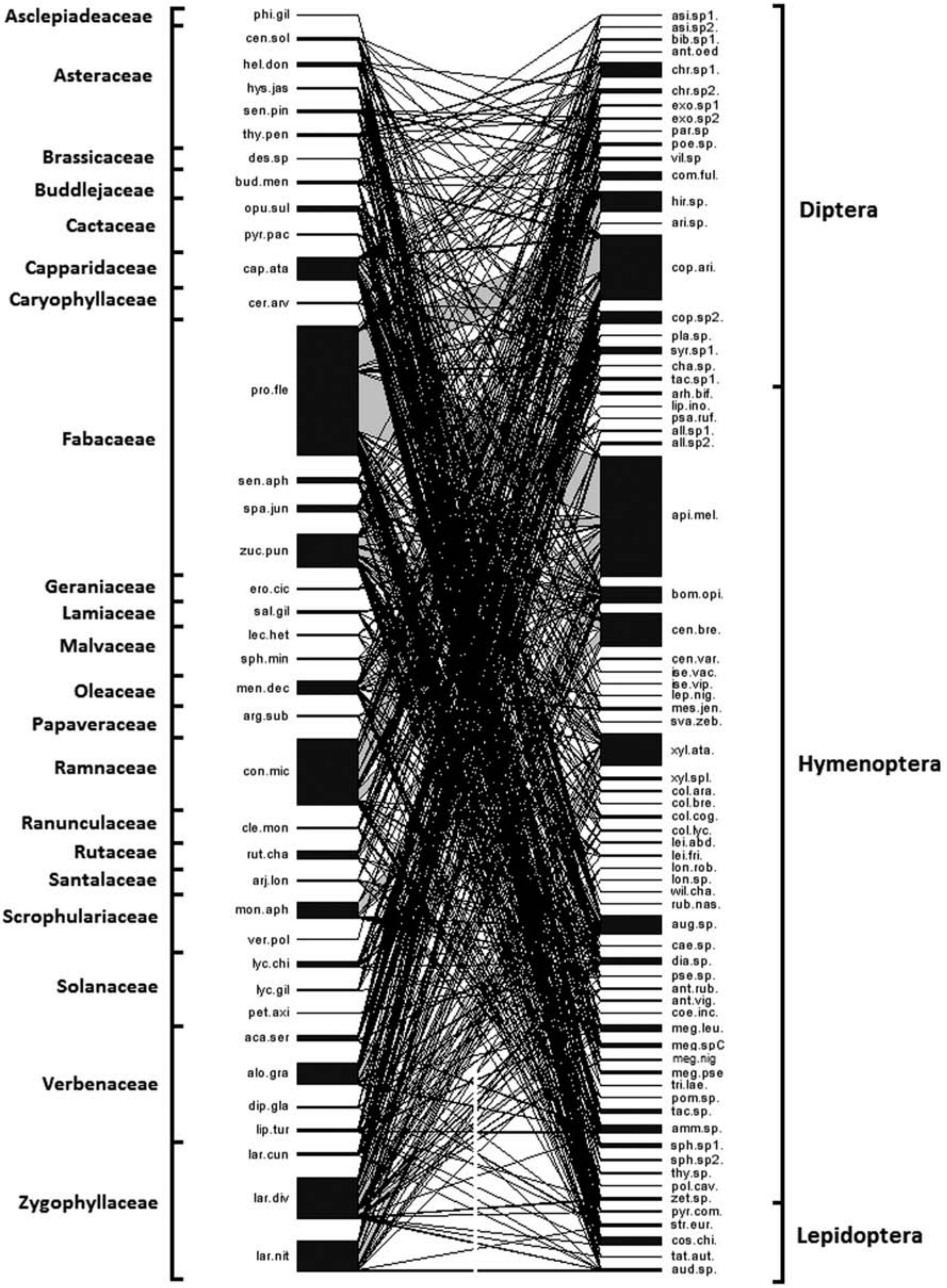

Figure 2. Plant–pollinator interaction network from our study site in Villavicencio Nature Reserve, Mendoza, Argentina. Data correspond to five flowering seasons, from 2006 to 2010. Plant species and families are on the left, and insect species and orders are on the right. Each bar corresponds to one species of plant or insect, identified with a six-letter code (see full names in Supplementary material Appendix 1 Table A2, A3). Bar width is proportional to the number of interactions observed for each species. Total number of interactions was 18 198.

is negligible compared to interspecific variation (Lomáscolo and Chacoff unpubl.). For herbaceous plants, where individuals are hard to identify, we selected nine flowers distributed widely per species. For flowers, we measured corolla depth as the distance from the tip of the petals to the base of the corolla, where we assumed that the nectaries resided (Supplementary material Appendix 1 Fig. A1). For species with open corollas, this measure equaled zero. Aperture was measured as the opening of the corolla at the point of inflection in the curvature of the corolla (Supplementary material Appendix 1 Fig. A1); in species with open corollas, aperture was equal to its diameter (i.e. the flower was completely open to pollinators).

Pollinator traits

All pollinators were insects. In most cases they were identified to species level or, when this was not possible, to family or genus level and then classified as morphotypes (Supplementary material Appendix 1 Table A3). Traits were measured in up to ten individuals of all the species or morphospecies recorded in the network (Supplementary material Appendix 1 Table A3). For most species we measured at least two individuals, with the exception of five species for which we had only one specimen in our collection. As with the plants, we decided to include those species in the analysis considering that interspecific variation in the traits measured is much higher than intraspecific variation (Chacoff and Castro-Urgal unpubl.) We measured a total of 294 individuals belonging to 71 species (Supplementary material Appendix 1 Table A3). Traits were measured with a graduated ocular. In each insect we measured body width as the maximum width of body or head (lateral body axis), and length of the sucking apparatus, which we called proboscis for all insect groups throughout the manuscript for simplicity (Supplementary material Appendix 1 Fig. A1). For Hymenoptera, length of the sucking apparatus was measured as the length of the prementum and glossa completely extended. For Diptera, total extended sucking apparatus length was measured after slightly pulling it out of the head and grasping the labella with fine forceps, to prevent retraction due to the contractile basal part. For Lepidoptera, we measured the total length of the unrolled proboscis.

Analysis of coevolution (H1)

Phylogenetic trees

The plant phylogeny was built using phylomatic (Webb and Donoghue 2005), based on the phylomatic tree R20120829 (Fig. 3). Further resolution of species in the family Verbenaceae was based on Marx et al. (2010), and that for the genus *Larrea* was based on Laport et al. (2012). The plant tree had 38 terminals. The insect phylogeny was built based on the tree of life web project (Maddison and Schulz 2007) (TOL) for both the more basal clades and most terminal relationships (Fig. 4). For specific groups we consulted the following sources: Hedtke et al. (2013) and Branstetter et al. (2017) for Apoidea; Branstetter et al. (2017) for Aculeata (stinging wasps); Ståhls et al. (2003) for Syrphidae; and Yeates (1994) for Bombilidae. The insect tree had 71 terminals. Trees built this way may lack accurate branch lengths and ultrametricity but they are extremely useful in a comparative context as they faithfully reflect the phylogenetic relationships of the species of interest and are amenable to comparative data analyses not limited by those requirements.

Reconstruction of evolutionary changes

On the phylogenetic tree of 38 plant species we mapped corolla length and aperture, and quantified the evolutionary change per node based on ancestral character state reconstructions using the software TNT (Goloboff and Catalano 2016) (Fig. 3A–B). This software is best suited for the analysis of quantitative continuous data in an explicit phylogenetic framework; the approach in TNT for mapping continuous characters is a natural extension of additive character optimization that proved accurate in recovering phylogenetic information from this type of characters (Goloboff et al. 2006). Here we concentrated in the pattern of coevolution for which the fast and demonstrably reliable reconstruction of ancestral states for continuous data was chosen over alternatives with more requirements. Because our phylogeny did not have branch lengths, we reconstructed character states based on parsimony, and not maximum likelihood. Maximum parsimony assumes that evolution occurs and that evolutionary changes can be reconstructed on a dichotomic branching pattern (i.e. a phylogenetic tree). Although generally assumed to require slow evolutionary rate to report reasonable results (Omland 1999), recent research shows that very high evolutionary rates of character change is as faithfully captured by parsimony reconstruction as small, even erratic changes (Moyers Arévalo et al. 2018). In addition, parsimony does not require specification of branch length and can successfully reconstruct evolutionary changes that allowed us to incorporate a wide diversity of taxa for which there was no published phylogeny with branch lengths available. The first approach used to quantify total phyletic change in each species was to consider only changes in the terminal branches; i.e. the difference between the observed value and the reconstructed ancestral value. However, to control for the fact that changes may have been accumulating from deeper times, we also analyzed the data considering changes occurring in successive older branches counting back from terminal branches (tree–down). Changes were tracked up to five nodes down but in no case phyletic changes accumulated for more than three nodes. Results were identical to those from the terminal branches and thus we only report results for the reconstruction based on terminal branches. We conducted the same search for evolutionary changes on the phylogenetic tree of the 71 pollinator species in the network, to record evolutionary changes in proboscis length and body width (Fig. 4A–B).

Tests of coevolution (H1)

Based on the network of plant–pollinator interactions collected in the area, we paired every plant and insect that

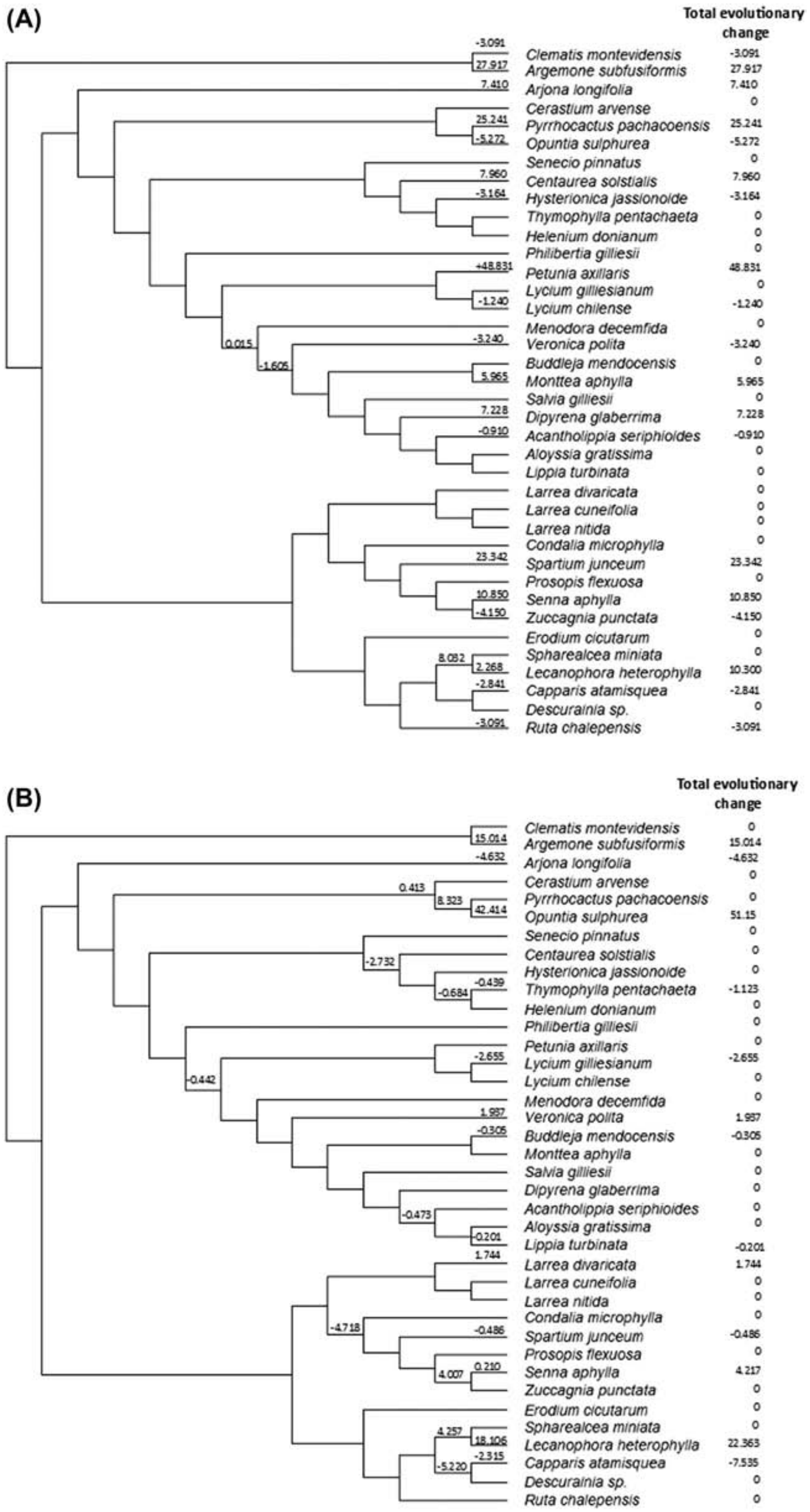

Figure 3. Phylogeny of the 38 plant species included in our study. The evolutionary change in corolla length (A) and in corolla aperture (B) as reconstructed following a maximum parsimony criterion in TNT are mapped and written on the tree. Total evolutionary change along the terminal branch was used in analyses. We also analyzed the data using the total change added along up to five consecutive nodes, counting back from terminal branches (column 'total evolutionary change'). Both analyses yielded identical results.

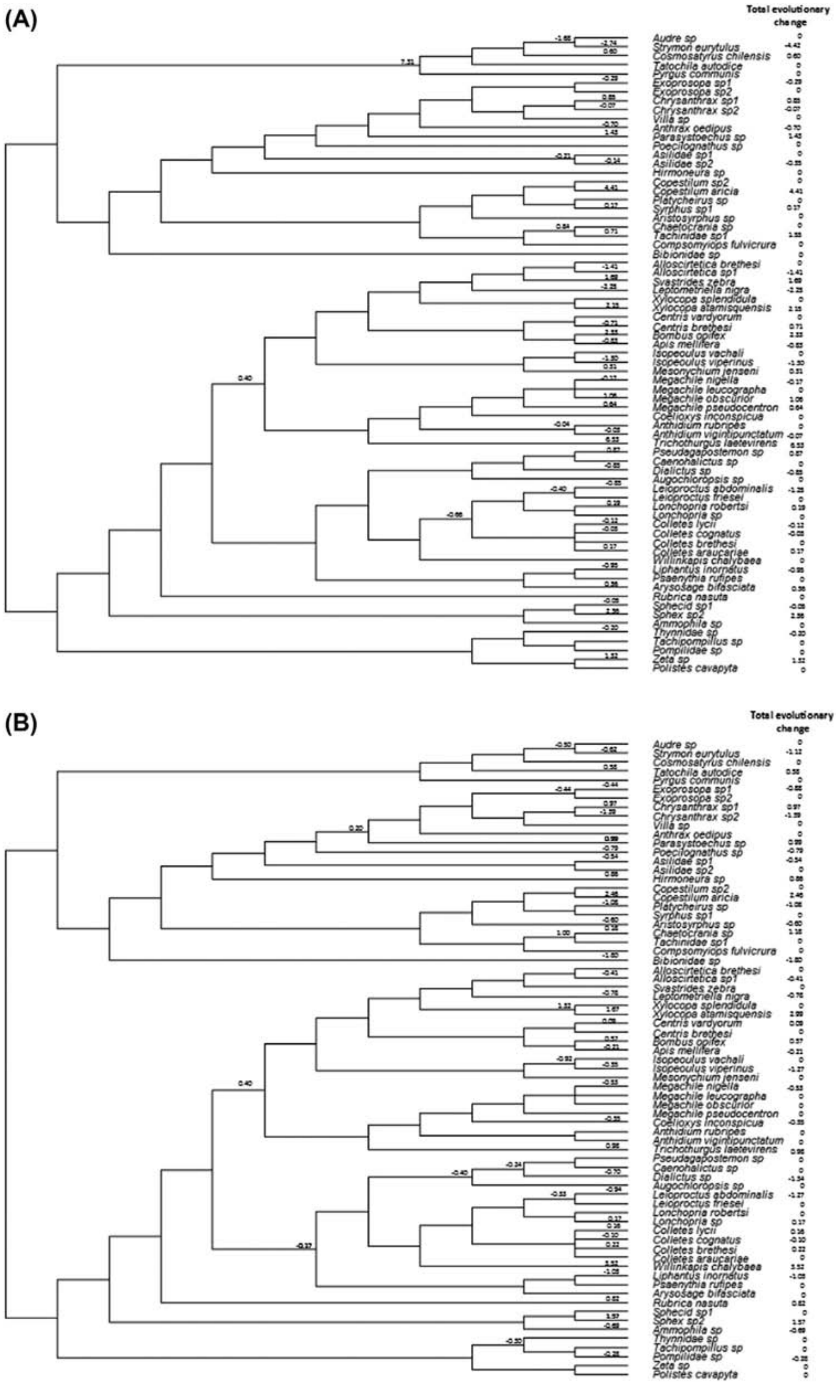

Figure 4. Phylogeny of the 71 insect species included in our study. The evolutionary change in proboscis length (A) and in body width (B) as reconstructed following a maximum parsimony criterion in TNT are mapped and written on the tree. Total evolutionary change along the terminal branch was used in analyses. We also analyzed the data using total change added along up to five consecutive nodes, counting back from terminal branches (column 'total evolutionary change'). Both analyses yielded identical results.

interacted. For each pair, we calculated the coevolutionary ratio, $cr = (\min(T_i/T_j,\ T_j/T_i))$, where T_i is the change in the terminal branch of the phylogeny in an insect trait (proboscis length or body width) and T_j is the change in the trait of the plant (corolla length or aperture) that interacts with the insect. The smallest number was set on top to avoid divisions by zero or by very small numbers. Results based on cumulative change were identical to those based on changes in terminal branches, so we only report the latter. Similar change in corresponding traits in both interaction partners tends to unity ($cr \rightarrow 1$) which we interpreted as a sign of coevolution. No change in both partners also tends to unity and was also considered as a sign of coevolution, as interaction partners may be maintaining their morphologies due to reciprocal selection. This decision was made under the assumption that morphologies that are favorable for both partners will be maintained unchanged by natural selection. This assumption renders our index of coevolution, *cr*, conservative, as random lack of change in the phylogeny, such as expected under no natural variation, would lead to a non-significant correlation with symmetry or frequency. Note that we did not distinguish the direction of the change, reflected by the sign (Fig. 3, 4) as we were not dealing with, nor did we try to draw conclusions about, the biological reasons for, or the outcome of, the coevolutionary process. Similar amount of phyletic change in interaction partners, even with different signs, represents coevolution. For example, changes with similar signs in both interaction partners may be due to a situation where plants with narrower corollas are less accessible to a certain, inefficient, pollinator species and, hence, the plant population will evolve towards narrower corollas. The inefficient pollinator in the hypothetical example, on the other hand, may be under selection to continue to exploit that plant as a resource and, hence, individuals with narrower thorax will be selectively favored and the pollinator population will evolve towards narrower thorax. Alternatively, changes with different signs in both interaction partners may result from a scenario where the plant's corolla may be a bit narrow for a highly efficient pollinator species that greatly depends on that plant for resources. Therefore, plants with slightly wider corollas will be selected for, as will pollinators with narrower thorax. Both plant and pollinator in the example are under reciprocal selective pressure to converge towards matching morphologies. In any of the cases described, regardless of the sign and the reason for change, interacting species are coevolving.

To test the first and second predictions of the first hypothesis, whether coevolution between plant and pollinator traits was greater in more frequent/symmetric interactions we performed three different tests.

1) First, we correlated frequency of interaction to *cr* for both pair of traits tested (proboscis versus corolla length, and body width versus corolla opening), using Spearman's rank correlations.
2) Second, because the measure of symmetry used is based on a measure of frequency of the interactions (see Network parameters of the main text), we wanted to control for the possibility that symmetry and frequency may be correlated. We performed a linear model where we wanted to see whether symmetry and frequency, or their interaction, predicted well *cr* for both pairs of traits tested (proboscis versus corolla length, and body width versus corolla opening). We did the same for the second prediction, regarding symmetry of interaction. The models were written as:

$$\mathrm{lm}_{\mathrm{length}} = cr_{\mathrm{length}} \sim \mathrm{symmetry} + \mathrm{frequency} \\ + \mathrm{symmetry} \times \mathrm{frequency}$$

$$\mathrm{lm}_{\mathrm{width}} = cr_{\mathrm{width}} \sim \mathrm{symmetry} + \mathrm{frequency} \\ + \mathrm{symmetry} \times \mathrm{frequency}$$

3) And third, we built a null model by doing permutations. First we divided the data into subsets defined by increasing thresholds of symmetry levels, and calculated the median *cr* for each subset. If symmetry of interaction strength was important for coevolution to occur, we expected a positive correlation between the value of symmetry (first column of Table 1) and the median value of *cr* for the different pairs of traits tested (corolla versus proboscis length, second column in Table 1, or corolla aperture and body width, third column in Table 1). Because the data were not independent, as each group of interactions with increasing symmetry level is a subset of the lower symmetry data set, we used permutation tests. The observed correlation coefficient, *r*, between level of symmetry and *cr*, was compared to a null distribution of correlation coefficients created by randomly permuting character states of interacting partners 999 times, calculating median *cr*, and running a correlation for each of the 999 permutations. Permutations also controlled for the probability of observing a significant correlation as an artifact of having interactions with unusually high levels of *cr* in the smaller subsets with higher symmetry or frequency. For the 999 correlation coefficients we calculated a 95% confidence interval to calculate the probability of observing our correlation coefficient by chance alone. This was repeated to test for the correlation between frequency level and median *cr*.

To do all the analyses that we report here, we included the supergeneralist alien species, *Apis mellifera*, as it has such a strong presence in the pollination network (it accounts for the highest number of interactions, Fig. 2, Supplementary material Appendix 1 Table A1). We also performed all analyses considering changes from the terminal branches only and considering continuous changes in up to five nodes. Analyses with or without *A. mellifera*, and with terminal or continuous changes yield identical results. Therefore, we report results including all species and continuous changes. There is one minor exception with the linear models, which is noted in Table 2.

Table 1. Data for analyses of coevolution between pollinating insects and their food plants. The value of frequency and symmetry is the threshold that defines each data subset, i.e. for the analysis of correlation between level of interaction frequency and coevolution (measured as the coevolutionary ratio, *cr*), we have 17 data subsets (each row of the table) where the first subset includes all the interactions in the network with frequency values less than, or equal to 0.1, the second subset includes all interactions with a level of frequency of 0.15 or less, the third 0.2, and so on, indicated in the column called Value for frequency or symmetry. The number of interactions included in each subset is indicated in the last column, No. of interactions included. The coevolutionary ratio, *cr*, is calculated as the ratio between evolutionary change in the plant's structure over evolutionary change in the insect structure (or the reverse ratio, always setting the lowest value in the numerator to avoid divisions by zero or very small numbers). The median across all interactions in each data subset is calculated and reported as the median *cr*. Then, to test the hypothesis that coevolution occurs most notably between the most frequent interactants a correlation is calculated between the level of frequency and the median *cr* for each pair of traits (corolla length versus proboscis length, and corolla aperture versus body width). Similarly, to test the hypothesis that coevolution occurs most notably between the most symmetric interactions, the same two correlations are calculated for symmetry levels. The results of such correlations, reported in the text and shown in Supplementary material Appendix 1 Fig. A2, show that coevolution seems to occur more readily in symmetric interactions, but not in the more frequent ones.

	Symmetry			Frequency		
Value for frequency/symmetry	Corolla versus proboscis length	Corolla aperture versus body width	No. of interactions included	Corolla versus proboscis length	Corolla aperture versus body width	No. of interactions included
	Median *cr*	Median *cr*		Median *cr*	Median *cr*	
0.1	0.024	0.122	342	5.88E-06	0.013	118
0.15	0.032	0.0854	305	0.026	4.76E-06	76
0.2	0.039	0.091	259	0.025	2.00E-03	68
0.25	0.031	0.125	226	0.026	1.75E-06	58
0.3	0.039	0.179	189	0.022	1.60E-06	48
0.35	0.042	0.12	165	0.068	0.014	37
0.4	0.052	0.12	145	0.049	0.015	32
0.45	0.067	0.149	136	0.072	0.031	24
0.5	0.065	0.269	118	0.072	0.031	22
0.55	0.065	0.269	106	0.114	0.039	17
0.6	0.042	0.291	89	0.114	0.017	13
0.65	0.036	0.291	81	5.88E-06	1	9
0.7	0.06	0.291	68	5.88E-06	1	7
0.75	0.116	0.269	56	5.88E-06	1	7
0.8	0.114	0.355	41	0.061	0.5	4
0.85	0.093	0.493	30	0.061	0.5	4
0.9	0.014	4.91E-01	21	0.061	0.5	4
0.95	0.411	0.751	10	0.061	0.5	4

Analysis of evolutionary stasis (H2)

For each species in the interaction network, we correlated the amount of evolutionary change, as measured by the reconstructed evolutionary changes on the phylogeny, with its degree of specialization (*d*) (Blüthgen et al. 2006). A positive and significant correlation would be interpreted as evidence that specialized organisms are under directional selection, while generalized organisms are under stasis due to multiple, perhaps conflicting, selection pressures.

Table 2. Results of the linear models using symmetry and frequency as predictor variables and 1) coevolution between proboscis and corolla length or 2) coevolution between body width and corolla aperture as dependent variables.

	Estimate	SE	t value	Pr(>|t|)
1) Model: lm = cr_{length} ~ symmetry + frequency + symmetry × frequency)				
Intercept	0.380	0.056	6.735	4.67e-11***
Symmetry	−0.074	0.075	−0.988	0.324
Frequency	−0.001	0.001	−1.215	0.225
Symmetry × Frequency	0.001	0.001	1.037	0.300
Multiple R^2: 0.006, p = 0.43				
2) Model: lm = cr_{width} ~ symmetry + frequency + symmetry × frequency				
Intercept	0.494	0.060	8.277	1.24e-15***
Symmetry	−0.157	0.079	−1.992	0.0469[*,‡]
Frequency	−0.001	0.001	−1.061	0.289
Symmetry × Frequency	0.001	0.001	0.815	0.416
Multiple R^2: 0.013, p = 0.10				

[‡] When the model is run including changes that occur in terminal changes, significance becomes marginal, as p = 0.098. *Significant at the alpha level < 0.05. ***Significant at the alpha level < 0.01.

Data deposition

Entomological and plant material identified were deposited in the entomological and botanical collections of IADIZA-CONICET.

Results

Interaction network

A total of nearly 210 sampling hours were completed for all sites, totaling 2508 censuses in the five sampling seasons, of which 511 were in 2006, 350 in 2007, 471 in 2008, 716 in 2009, and 460 for 2010. The number of sampling periods varied across plant species, sites, and years because species differed in their abundance, spatial distribution and duration of the flowering period. More details can be found in the original paper (Chacoff et al. 2012) and in the Supplementary material Appendix 1. The total number of interactions recorded was 18 198 (Supplementary material Appendix 1 Table A1).

Test of coevolution (H1)

Spearman correlations

For the test of the first prediction under H1, that coevolution was higher in the most frequent interactions, we did not find statistical support in the correlation between the coevolutionary ratio, *cr*, and frequency of interactions neither for coevolution between body width and corolla aperture ($\varrho = 0.009$, $p = 0.84$), nor between proboscis and corolla length ($\varrho = -0.02$, $p = 0.63$) (Fig. 5). Two of the interactions were much more frequent than the rest (*Prosopis flexuosa* with *Copestilum aricia* = 1418 times observed, and *P. flexuosa* with *Apis mellifera* = 2427 times observed) (Supplementary material Appendix 1 Table A1). We confirmed that the above results were not driven by those two outliers, as removing those interactions rendered the same qualitative results (frequency versus width, $\varrho = 0.0145$, $p = 0.750$; frequency versus length, $\varrho = -0.0143$, $p = 0.753$).

For the test of the second prediction under H1, that coevolution was higher in the most symmetric interactions, we found a significant positive correlation, albeit weak, between symmetry of interaction strength and coevolution between body width and corolla opening ($\varrho = 0.10$, $p = 0.033$). Symmetry, however, was not correlated with coevolution between proboscis and corolla length ($\varrho = 0.03$, $p = 0.48$).

Linear models

The results of these models are qualitatively similar to those found with the Spearman correlations we report in the main text, as we find that the only variable that significantly explains the coevolution between body width and corolla

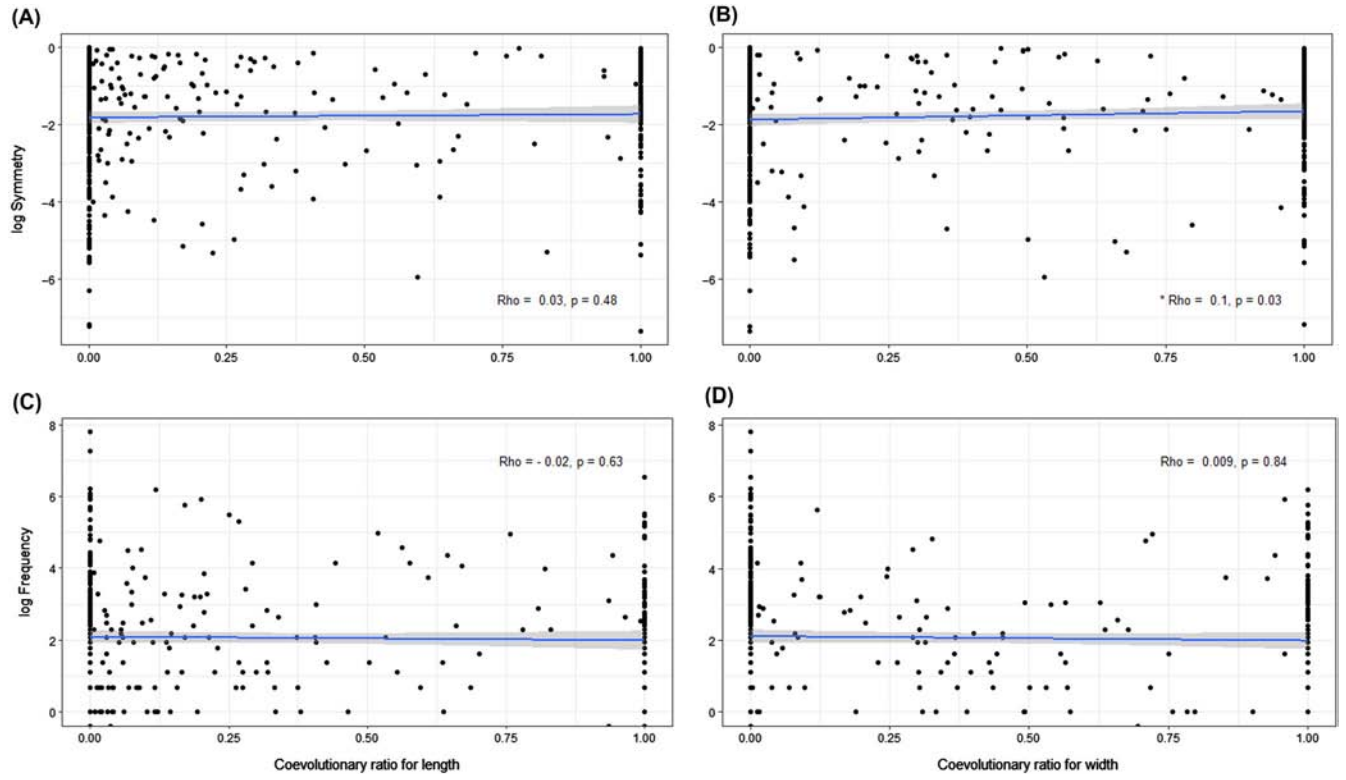

Figure 5. Results of the correlations to test whether coevolution occurs in relation to symmetry of interaction strength (A and B) or frequency of interaction (C and D). Subfigures A and C represent coevolution in proboscis and corolla length, while subfigures B and D represent coevolution in body width and corolla aperture. The correlation between symmetry of interaction strength with coevolution between corolla aperture and insect body width (B) is higher than expected by chance alone. We converted the y-axis to a log scale to visualize the data better.

aperture is symmetry. However, the R^2 is even lower than that for the non-parametric correlations reported in the main text and this is because the model includes frequency of interaction, which does not explain the variation in coevolution in either model (Table 1). Neither symmetry, nor frequency significantly explain coevolution between proboscis and corolla length.

Null model with permutations

We found a coupling of the reciprocal evolutionary change in pollinator and plant morphology in the most symmetric interactions, evidenced by a significant correlation between coevolution of body width and corolla opening, as measured by *cr*, with symmetry of interaction. The observed correlation was $r = 0.881$, which falls outside the 95% confidence interval of r *low* $= -0.151$ and r *high* $= 0.638$, generated by our simulations using random pairs of interactors. We did not find a significant correlation between coevolution and symmetry of interaction for corolla and proboscis length and frequency of interaction (observed $r = 0.533$, 95% confidence interval: r *low* $= -0.355$ and r *high* $= 0.543$), nor with either trait pair with frequency of interaction (corolla opening and body width observed $r = 0.694$, 95% confidence interval: r *low* $= 0.679$ and r *high* $= 0.710$; corolla and proboscis length observed $r = 0.253$, 95% confidence interval: r *low* $= -0.113$ and r *high* $= 0.598$) (Fig. 6, Table 1).

Test of evolutionary stasis (H2)

For the test of our second hypothesis, we found that the most specialized species do not show a greater amount of evolutionary change than do generalist species, as expected under directional selection, neither in plants (corolla length: $r = 0.08$, $p = 0.62$; corolla aperture: $r = 0.27$, $p = 0.10$), nor in pollinating insects (proboscis length: $r = -0.002$, $p = 0.99$; body width: $r = 0.065$, $p = 0.59$). In this case too, analyses excluding *A. mellifera* yielded identical results, so we report results with all species included.

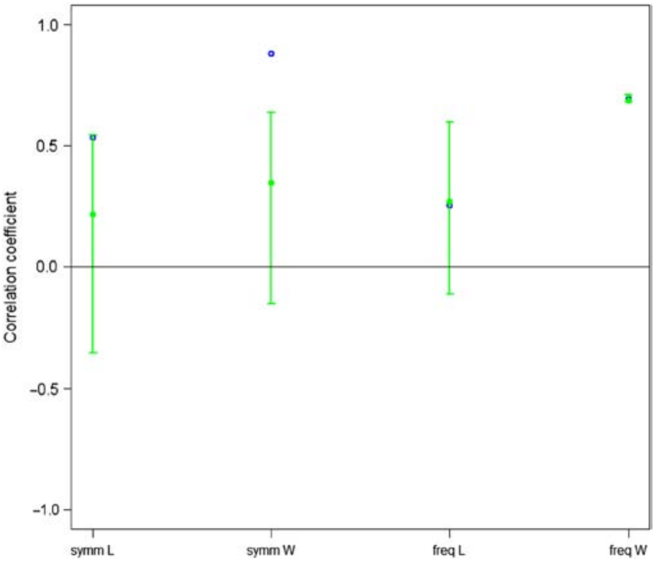

Figure 6. Results of the test of coevolution between corolla aperture of the flower and body width of the interacting insect (W), and between corolla and insect proboscis length (L) in relation to symmetry (symm) and frequency (freq). The test statistic, called the coevolutionary ratio (*cr*), was calculated as the ratio between evolutionary change in traits in pairs of interacting plant and insect species (largest number set as denominator). Blue points represent the observed correlation coefficient between symmetry or frequency levels and median value of *cr* for corolla length and proboscis length (symm L, freq L) and for corolla aperture and body width (symm W, freq W). Green circles represent the mean of 999 values of the correlation coefficients between the level of symmetry or frequency and the *cr*, calculated from random pairs of plants and pollinators. Error bars represent the 95% confidence interval. The correlation between levels of symmetry with coevolution is higher than expected by chance alone for corolla aperture and insect body width. However, this is not the case between frequency and coevolution for either pair of traits.

Discussion

The most important contribution of this study was the incorporation of testable hypotheses with specific predictions based on the structure of plant–animal interaction networks into the study of coevolution at the community level. Our novel approach, correlating evolutionary changes reconstructed based on empirical plant and insect trait data suggests that neither specialization, nor high frequency of interaction are a requisite for coevolution to occur between interaction partners.

We show that interaction symmetry may lead to morpho–functional coevolutionary matching regardless of frequency of interaction, as it seems to happen between body width and corolla aperture. We acknowledge that the variation explained is quite small, so we can only consider it a trend worth exploring further, perhaps with more species or more interactions, but especially so in an older and more stable environment, such as a tropical or subtropical forest, where specialization and symmetry of interactions may be higher and may vary more. However, we feel quite confident about the value of the trend found because results are quite robust to statistical analyses of very different nature, to the presence or absence of dominant species in the network, and to the amount of change considered in the analyses. More importantly, we think that we are showing a way to start dissecting the tremendous complexity in plant–animal interactions, in order to identify testable predictions about the evolutionary outcomes of interaction partners. We draw attention to the fact that not all coevolving symmetric interactions necessarily imply reciprocal specialization. Notably, most interactions showing highest symmetry are quite generalized, as they represent a low proportion of all the visits for interaction partners.

Our results suggest that, as long as reciprocal interaction strength is similar, even if similarly low, it may lead to similar levels of reciprocal evolution, i.e. coevolution, at least in some pairs of traits that mediate the interaction. In other words, we found that reciprocal interaction strengths of similar magnitudes, even if weak, are associated with similar morpho-functional evolutionary responses, i.e. coevolution. The difference between symmetrically weak and symmetrically strong interactions should be in the rate of coevolution, as the former should lead to a slower rate of coevolution than the latter. This prediction remains to be tested using dated phylogenies.

Invoking diffuse coevolution where multiple organisms interact may be an oversimplification of what may really be occurring. Instead, we should incorporate the structure of interactions, as we propose here, more specifically, which species within the group of interacting species may coevolve based on their symmetry of interaction. For coevolution to occur, interaction partners do not need to be reciprocally specialized. They do not even need to interact frequently, as long as they represent a similar proportion of each other's total interactions. Moreover, that proportion does not need to be particularly high. As an example, an insect that interacts very frequently with a specific plant may indeed exert a strong selective pressure on the plant, for instance, at the level of pollen transfer. Yet a similar, reciprocal effect of the plant on the insect will occur only if the proportions of the visits received or offered by both interaction partners to each other over the total number of interactions in which they are involved (i.e. interaction strength) are similar. The plant in the example may evolve in response to the frequently visiting insect but, our findings suggest, it will coevolve only with an insect with which it interacts with reciprocally similar interaction strength. Thus, we emphasize our prediction, which remains to be tested, that symmetrically weak interactions should lead to lower rates of coevolution than symmetrically strong ones. All of the expressed above agrees with our result that generalist species do not show greater evolutionary stasis (Janzen 1985) than the more specialized species, even though the former are subject to multiple, heterogeneous, and perhaps even contrary, selective pressures.

Another way to explain the coevolutionary matching of changing trajectories between pollinators and the plants they visit is that partners that have a pre-existing evolutionary trend to change in certain key morphologies that make them suitable to interact, may eventually encounter each other and become interaction partners in new environments. For example, insects that have a tendency to evolve narrower bodies might just find an adequate partner in a plant whose evolutionary trend is to narrow the corolla aperture. We call this process evolutionary fitting in analogy to the concept of ecological fitting proposed by Janzen (1980, 1985), whereby organisms newly colonizing an area use novel resources or interact with other species according to the suites of traits that they bring with them from their previous habitats. The fact that analyses considering changes that go deeper in time are similar to ones considering changes in terminal branches only, also points in the direction of potential evolutionary fitting. However, evolutionary fitting does not seem sufficient to explain the correlated evolution we report here between pollinator and plant traits. Even if a species with a historical evolutionary trend starts interacting with a new partner as, say, both species colonize a new habitat, our results suggest that only if this new pair of species interacts in a symmetrical manner will continue to change in a correlated manner, i.e. coevolve. Therefore, present-day interactions matter and they drive the matching evolutionary trend we found. Thus, symmetry of interaction is suggested here as, at least, an important catalyzer to spark coevolution between a plant and a pollinator. The association we found unveils the subtleties of evolution in noisy, multispecies networks, and points to the importance of the interaction network structure, especially symmetry of interaction strength, to understand coevolutionary dynamics within a community.

A potential caveat of studies like ours is that we analyze evolutionary change that started to happen perhaps thousands of years ago, and assume that such changes were effected by interactions that are similar to those we see today. It is reasonable to expect that the evolutionary change that we reconstruct may have been triggered by interaction partners that may be different from those we see today. However, because our results are similar when analyses are based on more recent changes, produced only along terminal branches, and when we go a bit further back in time including continuous changes up to five nodes back from terminal branches, this suggests that the changes in interaction partners may not be important enough to exert selective pressure that differ significantly from those we see today. In any case, we believe that the potential variation in interaction partners with time renders our analysis conservative, and that the significant coevolution that we see between body width and corolla aperture was identified despite possible historical changes in interaction partners, and not because of those changes.

Our results question the mechanisms invoked to date to predict and explain coevolution. Incorporating the structural features of plant–animal mutualistic networks into coevolutionary theory leads to different predictions from those of previous models of coevolution. Our analyses indicate that it is those predictions, but not those of previous theories, that are supported by the evidence in our study system. They also suggest that empirical evidence for coevolution was lacking because no previous studies examined predictions on the evolutionary change expected based on network structure. The scarcity of empirical evidence may also respond to the traditional focus on frequency of interactions alone, which may explain the component of fitness associated with pollen transfer in individual plant species (Vázquez et al. 2005, 2015), while symmetry is suggested here to be an important factor in morpho-functional coevolution. Beyond the long-known evidence of coevolution between extreme pairwise specialists, it seems that generalist species may also coevolve with their also generalist interaction partners, mediated, at least partly, by symmetry of interaction strength. In a natural world where non-reciprocal specialization is the norm, our results suggest that coevolution may be much more widespread and important than previously thought in shaping the morphology of organisms in a community of interacting organisms. Older environments, or environments that have been more stable and have a longer evolutionary history shared by members of the community than the Monte desert that we study here, may represent the ideal testing ground for our findings. The results of our study support the predictions of previous theoretical studies: that incorporating the complex structure of interactions will allow us to understand how the coevolutionary process at the community level proceeds. Testing our methods in other interaction networks, and performing empirical microevolutionary studies that quantify changes in plant or insect morphology are now needed to validate the proposed approach to study coevolution in communities of interacting mutualists. Considering the epigraph sentence by Schemske (1983) in a more general context, together with the results suggested by our study, mutualisms might be coevolving more than we thought, but we may have been looking with the wrong methods.

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Author contributions – SBL conceptualized ideas and methodology, collected data in the field and lab, designed and implemented computer code for statistical analysis, and wrote the first draft of the manuscript; DPV conceptualized ideas and methodology, collected data in the field and lab, designed and implemented computer code for statistical analysis, provided funding and contributed substantially manuscript revisions; NPG conceptualized ideas, designed and implemented computer code for statistical analysis and improved manuscript revisions; NPC conceptualized ideas and methodology, collected data in the field and lab and improved manuscript revisions; RCU helped to design methodology, collected data in the lab, and helped to improve manuscript revisions.

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Supplementary material (available online as Appendix oik-05960 at <www.oikosjournal.org/appendix/oik-05960>). Appendix 1.