

LETTER

The phylogenetic structure of plant–pollinator networks increases with habitat size and isolation

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Abstract

Similarity among species in traits related to ecological interactions is frequently associated with common ancestry. Thus, closely related species usually interact with ecologically similar partners, which can be reinforced by diverse co-evolutionary processes. The effect of habitat fragmentation on the phylogenetic signal in interspecific interactions and correspondence between plant and animal phylogenies is, however, unknown. Here, we address to what extent phylogenetic signal and co-phylogenetic congruence of plant–animal interactions depend on habitat size and isolation by analysing the phylogenetic structure of 12 pollination webs from isolated Pampean hills. Phylogenetic signal in interspecific interactions differed among webs, being stronger for flower-visiting insects than plants. Phylogenetic signal and overall co-phylogenetic congruence increased independently with hill size and isolation. We propose that habitat fragmentation would erode the phylogenetic structure of interaction webs. A decrease in phylogenetic signal and co-phylogenetic correspondence in plant–pollinator interactions could be associated with less reliable mutualism and erratic co-evolutionary change.

Keywords

Area effect, co-phylogenetic correspondence, habitat islands, isolation, mutualistic networks, Pampas, phylogenetic structure, pollination webs.

Ecology Letters (2016) **19**: 29–36

INTRODUCTION

Habitat fragmentation can drive the direct extinction not only of species, but also of the interspecific interactions that shape the web of life (Bascompte & Jordano 2007; Aizen *et al.* 2012). In turn, interaction loss causes the disruption of diverse ecological processes, which can further affect both short-term species survivorship (e.g. Pauw 2007) and long-term evolutionary change (e.g. Galetti *et al.* 2013). Although consideration of evolutionary and co-evolutionary processes adds an important dimension to the values of conservation (Crandall *et al.* 2000; Moritz 2002), their study in the context of meta-communities persisting in fragmented habitats is still in its infancy (Peralta *et al.* 2015). We propose that the assessment of the phylogenetic structure of interaction webs like plant–pollinator networks, across habitat islands, can provide information on what landscape factors and habitat characteristics contribute to the preservation of long-term (co)evolutionary processes.

Phenotypic traits shaping plant–animal interactions, particularly plant–animal mutualisms, are often structured phylogenetically. As a consequence, plant or animal species sharing common ancestry – and thus similar traits – tend to interact with largely overlapping ecological assemblages of animal or plant species, respectively, with matching traits (Rezende *et al.*

2007; Gómez *et al.* 2010). This type of phylogenetic structure based on a total or partial phenotypic matching between interacting partners can result from one-on-one and even multi-specific co-evolutionary processes (Marussich & Machado 2007; Segraves 2010; Guimarães *et al.* 2011). Similarly, these different modes of co-evolution, i.e. pair-wise and multi-species, will strengthen the correspondence between plant and animal phylogenies (e.g. Legendre *et al.* 2002). In addition, trait matching between species that did not co-evolve, but co-evolved elsewhere with close relatives of the species with which they interact at present may also contribute to maintain and reinforce the phylogenetic structure of plant and animal interactions (e.g. Pearse *et al.* 2013). Therefore, we interpret the presence of phylogenetic structure in mutualistic webs as evidence of temporally persistent interactions between pairs or (somewhat shifting) sets of partner species with co-adapted traits, and its absence as evidence of less reliable mutualism (i.e. mutualistic interactions not sustained over time) leading to erratic selective pressures.

Both the phylogenetic signal in ecological interactions at each trophic level, as well as the overall plant–animal phylogenetic congruence can be eroded under a scenario of habitat fragmentation for at least two reasons. First, plant and animal populations trapped in small habitat islands are more prone to random extinction due to demographic and genetic

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bottlenecks (Young *et al.* 1996; Tschardtke *et al.* 2002). Increasing random population extinction would, in turn, decrease the strength of the phylogenetic signal in assemblage composition, because this factor would reduce the deterministic role of trait matching in shaping plant–pollinator interactions (Stang *et al.* 2006; Schleuning *et al.* 2015). Second, meta-community dynamics – characteristic of fragmented habitats – implies not only the random extinction of small local populations, but also the colonisation of a focal habitat island by random samples of non-resident and, probably, transient species from nearby habitat islands (Leibold *et al.* 2004). These transient species are expected to establish rather facultative interactions, particularly with generalist species (Aizen *et al.* 2012), which would also weaken the phylogenetic signal in ecological interactions and the degree of phylogenetic match between plants and animals. Therefore, the overall phylogenetic and co-phylogenetic structure of pollination webs should be better preserved in large and isolated habitat islands than in small and central ones, which are subjected to higher species and interaction turnover (Jamoneau *et al.* 2012).

Here, we explore the role of habitat size and isolation on the phylogenetic signal and co-phylogenetic congruence of plant–pollinator interactions by analysing 12 pollination webs from the ‘sierras’ of the central Argentine Pampas (Sabatino *et al.* 2010; Aizen *et al.* 2012). These ancient hills, which range from tens to thousands of hectares, can be considered as ‘habitat islands’ as they were connected by a matrix of natural grassland prior to European colonisation, but are now surrounded by a matrix devoted to intensive agriculture and isolated from each other by distances ranging from a few hundred metres to several kilometres (Fig. 1). Because of their relatively recent isolation and because they remain mostly untilled, these sierras still preserve many floristic elements that

were formerly common in the surrounding plains and elsewhere in southern South America, including several species from lineages of Gondwanan origin (Cabrera 1994) (see also Supporting Materials and Methods, Landscape’s human transformation).

Previous studies in this system (Sabatino *et al.* 2010; Aizen *et al.* 2012; Gilarranz *et al.* 2015) showed that trends in number of species and interactions, as well as in overall network architecture (i.e. nestedness) could be predicted based on meta-community principles (Leibold *et al.* 2004). Here, we move one step forward and ask whether a meta-community dynamics could also induce changes in the phylogenetic and co-phylogenetic structure of these pollination webs. In particular, on the basis of the hypotheses stated above, we test the predictions that both phylogenetic signal and co-phylogenetic congruence in interactions between plants and their flower visitors increase with habitat area and isolation. We found support for these expectations, which also suggests that the process of habitat fragmentation has community-wide implications for both the dynamics of mutualisms and the integrity of co-evolutionary processes.

MATERIALS AND METHODS

Study system and dataset

We surveyed pollination networks from 12 out of a total of 18 sierras located between Mar del Plata (37°58' S, 57°35' W) and Balcarce (37°50' S, 58°15' W), Buenos Aires Province, Argentina (data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.cr3ft>). These sierras are part of the Tandilian orographic system dating back to the lower Paleozoic, a system which comprises about 24 isolated ortho-quartzitic hills up to *c.* 500 m in altitude jutting out from the loessic (Quaternary) Pampas’ plains, lying in the southeast of the ‘Pampeano Austral’ biogeographic district (Cabrera 1994). The 12 study sierras represent a wide range in size (from 13 to 2100 ha; Table S1) and isolation (between 0.8 and 9.1 km from their nearest neighbour), which is typical of the entire orographic system. The climate of the region is temperate with warm summers (mean January temperature 20.8 °C) and mild winters (mean July temperature 5.2 °C).

The sierras have rich vegetation characterised by a gentle rocky basal slope dominated by shrubs, herbs and geophytes, a barely vegetated steep scarp and a flat top with a mosaic of exposed bedrock and loessic patches dominated by grasses. The most abundant and diverse plant families are insect pollinated (i.e. Asteraceae, Apiaceae, Fabaceae and Scrophulariaceae). Despite intensive apiculture with European honey bees (*Apis mellifera*) in the region, the sierras support a rich flower-visitor community that comprises mainly insects (Hymenoptera, followed by Diptera, Coleoptera and Lepidoptera) and one species of vertebrate, the hummingbird *Chlorostilbon aureoventris* (Aizen *et al.* 2012). The matrix that nowadays surrounds the sierras is an intensively managed agroecosystem devoted principally to the cultivation of soybean (about 70% of the agriculture area). However, other crops such as sunflower, wheat, corn, potato and canola are still cultivated in the region. Cattle roamed freely and fires were set frequently



Figure 1 The study hilly landscape of the Argentine Pampas. The photograph shows ‘sierras’ (a and c) and the surrounding agricultural matrix (b). The conical sierra at the back (a) is ‘Amarante’, a hill of 190 ha and 361 m a.s.l., as seen from ‘La Chata’, a hill of 229 ha and 357 m a.s.l., which is 2.6 km away. The terrain in the foreground (c) at La Chata is the type of habitat in which we surveyed plants and flower visitors.

to promote vegetation re-growth in all the study sierras except for Difuntito, a small-fenced hill (Table S1). Cattle had been excluded and fire suppressed at Difuntito for at least the preceding 18 years (Sabatino *et al.* 2010).

Field work was conducted during the 2007–2008 Austral flowering season (October–April). On each sierra, we delimited and sampled an area of 0.5 ha on the north-facing slope, about 200 m from the edge of the nearest agricultural field. This sun-exposed slope exhibits the highest plant diversity among the sierra habitats. Also, while our protocol avoided edges, proximity to the agricultural fields increased the chance to sample plant and pollinator species that used to be common, or at least present, in the surrounding matrix before agriculturalisation. Within each 0.5 ha area, we set two parallel 100-m transects, 50 m apart and along each transect we established five permanent 1-m radius plots *c.* 25 m apart (i.e. 10 plots per sierra). Within each plot, we identified all plants in flower and recorded all flower visitors that contacted floral sexual organs during a 15-min period. All plots from a given sierra were sampled consecutively between 09:00 and 18:00 h and each sierra was sampled an average of 10 times throughout the flowering season, once every 2 weeks, recording a total of 13 174 flower visitors during 318 h of observation distributed over 127 days. All flower visitors were morphotyped and identified with the aid of a reference collection and specialist help at least to the family level. We could identify to species or genus 52 or 80% of all flower visitors respectively. Neither the total number of flower visitors recorded in each sierra (a measure of sampling effort) nor the incidence of alien species (*c.* 10% of all species) was influenced by sierra size or isolation (Sabatino *et al.* 2010).

Sierra area and connectivity

The 18 sierras from our study area, including the 12 sierras surveyed, were digitalised from a Google-Earth image. We measured the area of each sierra to the nearest hectare. Then, we constructed a spatial network connecting the sierras. Two patches (in this case two sierras) were connected if the lineal distance between them (i.e. edge to edge) was smaller than a threshold distance. From this spatial network map, we estimated the betweenness centrality of each patch as a direct measure of connectivity or an inverse measure of isolation. Patch betweenness centrality quantifies how well a given habitat patch is connected to other patches by taking into account the spatial configuration of the entire patch network. For a focal habitat patch, patch betweenness centrality is proportional to the number of all possible paths connecting all pairs of habitat patches which pass through that patch (Freeman 1977). This inverse measure of isolation, which is based on graph theory, suits to studies concerned with meta-population and meta-community dynamics (Urban & Keitt 2001; Gilarranz *et al.* 2015). We chose a threshold distance of 12 km, a value that maximised the association (i.e. the coefficient of determination) between phylogenetic structure and centrality (see Supporting Materials and Methods, Threshold distance), at least for the two of the three focal phylogenetic correspondence variables for which centrality had a significant effect (Table S2). This threshold distance

also maximised the association between nestedness – an important structural network property – and centrality (Gilarranz *et al.* 2015). From a biological perspective, this threshold is just above the maximum foraging distances known for most bees (Greenleaf *et al.* 2007). We considered the paths connecting all the 18 sierras in the landscape to estimate the centrality (hereafter connectivity) of the 12 study sierras. There was no evidence that the (log) area of a sierra and its connectivity were significantly associated ($r = 0.167$, $n = 12$, $P = 0.60$), so both factors estimate independent aspects of the study habitat islands.

Phylogenies and phylogenetic distances

Phylogenetic distances between all pairs of plant species and flower-visiting insect species were calculated from reconstructed phylogenies. Phylogenetic relations among all plant species recorded in the 12 sierras (i.e. a total of 96 species) were inferred using the Phylomatic online tool (Webb & Donoghue 2005), selecting the R20120829 source tree as the master phylogeny. We further resolved the phylogenetic relations for plant species in polytomic families using the most updated intrafamily phylogenies when available (see Supporting Materials and Methods, Plant and animal phylogenies). Phylogenetic relations among all flower-visiting animal species (i.e. a total of 171 species) were reconstructed by assembling the information contained in different published sources. We excluded the one observed vertebrate species, the hummingbird *Chlorostilbon aureoventris*, from the final animal phylogeny because of the long time of divergence between vertebrates and insects (Blair 2009) and because this bird was censused only once (Aizen *et al.* 2012). The phylogenetic relations among the main groups of insects were extracted from Wiegmann *et al.* (2009). We then added resolved topologies for each group at the tips of this template phylogeny from different source phylogenies (see Supporting Materials and Methods, Plant and animal phylogenies). On the basis of the time of divergence of major clades for plants and insects, we estimated branch lengths using the *bladj* command in Phylocom (Webb *et al.* 2008). Thus, the resulting trees were ultrametric with time-calibrated branch lengths (Figs S1 and S2). We then obtained matrices of pair-wise phylogenetic distances among all plant and animal species pairs using the *phydist* command in Phylocom (Webb *et al.* 2008).

To account for the effect of the incomplete resolution of the trees, we applied an alternative procedure of phylogenetic reconstruction that simultaneously resolves polytomies and adjusts branch lengths using an evolutionary constant rate birth–death model (see Supporting Materials and Methods, Plant and animal phylogenies). Mantel correlations between matrices of pair-wise phylogenetic distances constructed with the *bladj* and this alternative procedures were extremely high, $r = 0.995$ (95% CI = 0.994–0.996) and $r = 0.996$ (95% CI = 0.995–0.997) for plants and insects respectively. This almost perfect congruence implies that polytomies (which were mostly present at terminal nodes) as well as uncertainty in the time of divergence of some nodes had minimal influence on overall phylogenetic structure.

Ecological distances

Following Rezende *et al.* (2007) and Elias *et al.* (2013), we used $1-S$ as an estimate of ecological distances, where S is the Jaccard index of similarity obtained from qualitative interaction matrices (Legendre & Legendre 1998). Accordingly, the ecological similarity between two species, i and j , is defined as $S(i, j) = a/(b+c-a)$, where a represents the number of interacting species shared between species i and j , and b and c the total number of species interacting with species i and j respectively. The ecological distance between two plant (animal) species ranges from 0, when they interact with the same animal (plant) species assemblage, to 1, when they interact with completely different species assemblages. Ecological distances for all pairs of plants and animals were estimated from the synthetic 96×171 species interaction meta-web and from the interaction matrix of each sierra separately.

Phylogenetic signal in species assemblages

We estimated the extent to which phylogenetic proximity is associated with similarity in the composition of the interacting species assemblage for the meta-web and the interaction matrix of each sierra separately. To this aim, we compared phylogenetic and ecological distance matrices (Rezende *et al.* 2007; Elias *et al.* 2013) using Mantel tests performed with the package *vegan* v. 2.0-10 (Dixon 2003) for R (R Core Team 2014). Because differences in the number of interacting species composing each species' assemblage (i.e. species degree) affect Jaccard similarity, we performed partial Mantel tests controlling for differences in species richness. Degree distance matrices were calculated from the absolute difference in the number of interacting animal (plant) species for all pairs of plant (animal) species (Rezende *et al.* 2007). Therefore, this test assesses whether phylogeny affects the identity of the interaction partners independently of differences in the number of interacting species, which can be unduly influenced by differences in sampling effort (Vázquez & Aizen 2003).

Plant–animal phylogenetic congruence

We used the ParaFit co-phylogenetic analysis (Legendre *et al.* 2002), as implemented in the package *ape* v. 3.1-2 (Paradis *et al.* 2004) for R (R Core Team 2014), to assess the degree of correspondence between plant and animal phylogenetic trees, for both the meta-web and the interaction web of each sierra (Fig. S3). This analysis is based on the transformation of plant and animal phylogenetic trees into matrices of principal coordinates. The ParaFit global estimate is the sum of squares of all the values of $d_{i,j}$ in a matrix D , which is the result of $D = P \cdot I^T \cdot A$, where P is the plant phylogenetic distance matrix (with principal coordinates in rows), A represents the animal phylogenetic distance matrix (with principal coordinates in columns) and I^T is the transposed plant–animal binary interaction matrix.

Statistical analyses

For significance testing of both partial Mantel correlation coefficients and ParaFit global estimates, we conducted

permutations of the plant–pollinator interaction matrices. We preferred this method rather than permuting either distance matrices (Mantel correlations) or species phylogeny matrices (ParaFit estimates). On the basis of null model 1 of Vázquez & Aizen (2003), we used an algorithm, implemented in R (R Core Team 2014), which shuffled the observed number of interaction links among the cells of a given matrix with the only restriction that each species had at least one interaction. The advantages of this procedure is the preservation of matrix size, while avoiding misestimated type I errors associated with the permutation of distance matrices in Mantel correlation tests (Harmon & Glor 2010). Each estimate was compared with a distribution of expected values generated from 1000 randomisations of the synthetic plant–pollinator interaction matrix (i.e. the meta-web) and each sierra's interaction matrix.

To assess and compare the strength of Mantel associations and co-phylogenetic signals among binary matrices of different shape and completeness, observed estimates were z -transformed according to $(x - \bar{x})/SD$, where \bar{x} and SD are the mean and standard deviations across the 1000 randomisations (Elias *et al.* 2013). Because ParaFit estimates tended to differ by orders of magnitude and have a highly right-skewed distribution, they were log-transformed before calculation of z -scores. We evaluated differences in the strength of the plant vs. animal phylogenetic signal (i.e. the z -transformed Mantel correlations) across the 12 sierras by means of a paired t -test. Finally, we analysed the effect of (log) sierra area and connectivity on z -scores with a linear multiple regression model.

To discard any confounding effect associated with differences in web size or phylogenetic diversity among sierras, we included, alternatively, the total (log) number of species (i.e. plants and animals), the (log) number of interaction links of the plant–pollinator web sampled in each sierra and the (log) Faith's phylogenetic diversity (i.e. the sum of the lengths of all phylogeny's branches; Faith 1992) as predictive variables in the above regression models. All analyses between each response variable and sierra area and connectivity yielded the same directional trends when absolute measurements, rather than z -scores, were used (results not shown). Spatial autocorrelation of residuals from the above models was addressed using Moran's I estimates for increasing distance categories (Fortin *et al.* 2002; see Supporting Materials and Methods, Spatial autocorrelation).

Finally, we explored whether patterns of decreasing phylogenetic structure in plant–pollinator interaction with decreasing sierra area and increasing connectivity could be linked to a loss of the phylogenetic signal in specialisation and/or loss of phylogenetic diversity. To analyse the first possibility, we considered the number of interaction links per plant or animal species (i.e. species degree) from the meta-web, corrected by total observation frequency, as a measure of specialisation/generalisation. To estimate this parameter, we used the residuals of linear regressions between the (log) number of interaction links and the (log) number of observations with intercepts forced to zero (Vázquez & Aizen 2003), considering plants and flower visitors separately. Then, we estimated, for both the meta-phylogenies and each sierra's phylogenies, the amount of phylogenetic signal in the adjusted number of interaction links by means of the K statistic (i.e. the amount

of signal of the real data, expressed as a fraction of that expected based on a Brownian model of trait evolution) as detailed in Rezende *et al.* (2007). To analyse the second possibility, following Eiserhardt *et al.* (2015), we compared the observed lengths of the plant and animal phylogenies of each sierra (i.e. Faith's phylogenetic diversity) with the lengths of 1000 trees that were sub-sampled randomly from the plant and animal meta-phylogenies, keeping the number of species equal to those in the observed trees. For comparisons across sierras, both the K statistic and phylogenetic diversity deficit were transformed as z -scores.

RESULTS

Plant–pollinator interactions in the study sierras were phylogenetically structured. Analysis of the synthetic interaction meta-web revealed that closely related plants tended to interact with more ecologically similar sets of animal species than less related plant species ($z = 5.07$, $P < 0.001$). Similarly, closely related animals tended to interact with more similar sets of plant species than less related animal species ($z = 7.24$, $P < 0.001$). Analysis of these two phylogenetic signals on species assemblages across the 12 sierras showed not only significant co-variation between them (Fig. 2a), but also that the animal phylogeny was more strongly associated with the identity of interactive plant mutualists than vice versa (paired t -test: $t_{11} = 5.16$, $P < 0.0005$). Overall, there was some co-phylogenetic congruence between the synthetic plant and animal meta-phylogenies ($z = 5.84$, $P < 0.001$), which, at the level of individual sierras, was associated with both plant and animal phylogenetic effects on the similarity of the interacting animal and plant assemblages respectively (Fig. 2b and c).

The strength of all the above three estimates of phylogenetic structure varied among sierras and was associated with either sierra area or connectivity. First, the association between animal pair-wise phylogenetic distances and plant pair-wise ecological distances departed from null expectations in 10 of the 12 individual pollination webs. The strength of this phylogenetic effect increased significantly with sierra area (Fig. 3, Table S3). Second, the association between plant pair-wise

phylogenetic distances and animal pair-wise ecological distances departed from null expectations in four of the 12 pollination webs. The strength of this phylogenetic effect decreased with sierra connectivity (Fig. 3, Table S3). Third, the congruence between the plant and animal phylogenies was weak in general, exceeding null expectations in only three of the 12 individual pollination webs (Fig. 4). However, this co-phylogenetic congruence became stronger as both sierra area and isolation increased (Fig. 4, Table S4). The relation between co-phylogenetic congruence and area changed from marginal to highly significant after excluding Difuntito, a well-preserved small sierra with an unexpectedly high number of species and interactions (Table S1). However, Difuntito did not influence the direction and magnitude of any of the reported trends to any large extent (Figs 3 and 4). Factors associated with network size, phylogenetic diversity or any other spatially autocorrelated factor did not confound the effects of sierra area and connectivity on the strength of the web phylogenetic structure (Tables S5–S12). Also, despite an overall significant phylogenetic signal in the degree of specialisation (i.e. the adjusted number of links; $K = 0.301$, $P = 0.008$ for the plant meta-phylogeny, and $K = 0.522$, $P < 0.001$ for the animal meta-phylogeny), there was no evidence of a significant decrease in either the phylogenetic signal in specialisation or phylogenetic diversity with decreasing sierra area or increasing connectivity (Figs S4 and S5, Tables S13–S14).

DISCUSSION

Overall, the most phylogenetically structured pollination webs were found in large and relatively isolated sierras, supporting expectations based on meta-community principles. Underlying these results are the well-sustained assumptions that (1) some degree of phenotypic matching between co-occurring plant and pollinator species is needed for interaction establishment and functional efficiency (Stang *et al.* 2006; Schleuning *et al.* 2015), and (2) phenotypic matching involves traits that are phylogenetically conserved (Rezende *et al.* 2007; Gómez *et al.* 2010). Therefore, to the extent that phylogenetic structure of mutualistic interactions reflects co-evolution, we predict that

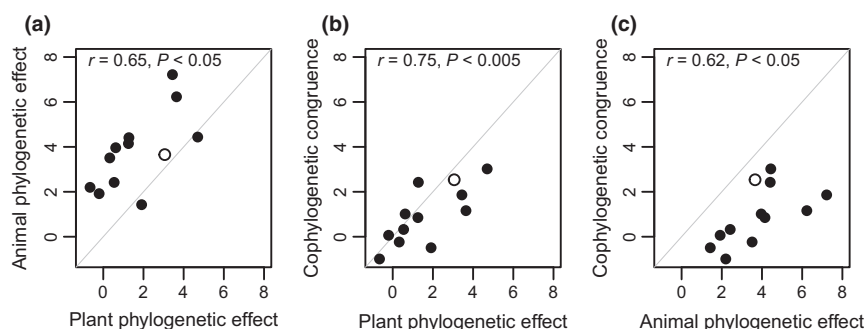


Figure 2 Pearson's correlations between the three estimates (z -scores) of phylogenetic structure of plant–pollinator interactions across the 12 sierras. (a) Plant vs. animal phylogenetic effect (i.e. Mantel correlations between phylogenetic relatedness and ecological similarity), (b) co-phylogenetic congruence (i.e. ParaFit index) vs. plant phylogenetic effect and (c) co-phylogenetic congruence vs. animal phylogenetic effect. The open circle in each panel represents Difuntito, a well-preserved small sierra with an unexpectedly high number of species and interactions (Table S1). The grey line of intercept = 0 and slope = 1 is provided as reference.

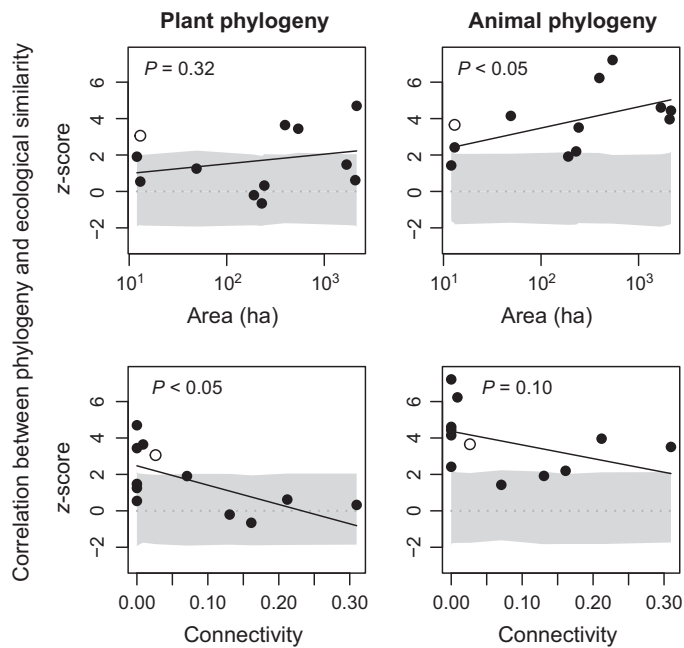


Figure 3 Correlation between phylogenetic relatedness and ecological similarity of interacting species as a function of sierra area and connectivity. Connectivity is measured as betweenness centrality, an inverse measure of patch isolation. A partial Mantel correlation (z -score) between phylogenetic and ecological distance matrices was estimated for each pollination web after accounting for differences in the number of interacting species composing each species' assemblage (i.e. species degree) and plotted against sierra area (upper panels) and connectivity (lower panels). The grey zone is the region delimited by the 2.5 and 97.5 percentiles from the random distributions of z -scores. The open circle in each panel represents the phylogenetic correspondence estimated for a web sampled at Difuntito, a well-preserved small sierra with an unexpectedly high number of species and interactions (Table S1). The corresponding partial regression linear equation is depicted in each graph (see Table S3).

the transformation of continuous habitats into a network of remnant habitat patches will jeopardise co-adaptation.

According to our conceptual framework, species abundance, which increases with habitat size, represents a key factor underlying phylogenetic structure of interactions. Although phenotypic matching indicates the potential for the establishment of an effective interaction (Stang *et al.* 2006; Schleuning *et al.* 2015), species abundance can explain interaction persistence in time and space (Aizen *et al.* 2012; Carstensen *et al.* 2014) and, therefore, the potential for co-adaptation. As a consequence, increasing random extinctions of small populations of well-matched partner species occurring in small fragments would not only decrease resemblance in species assemblages between closely related interacting partners (Figs 3 and 4), but also by disassembling trait matching, could decrease the fitness of the surviving interaction partners.

Species colonisation, which increases with habitat connectivity, is the second factor proposed to affect the amount of underlying phylogenetic structure of interactions in fragmented habitats. Central habitat islands are by definition 'stepping stones' of a higher number of dispersal routes than peripheral, isolated islands (Urban & Keitt 2001). Although populations of resident species in well-connected habitat

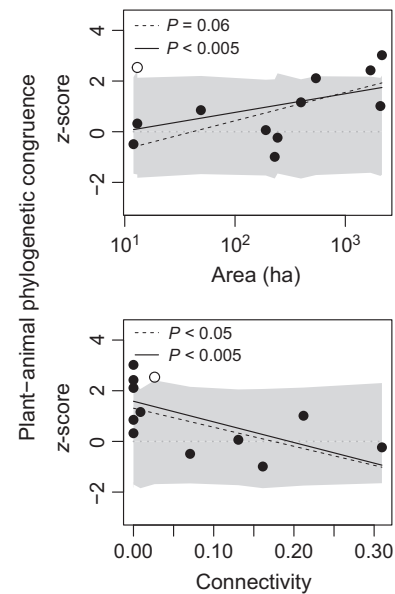


Figure 4 Congruence between the plant and animal phylogenies as a function of sierra area and connectivity. Connectivity is measured as betweenness centrality, an inverse measure of patch isolation. The degree of congruence between the plant and animal phylogenies (here measured with the z -transformed ParaFit index) was estimated for each pollination web and plotted against sierra area (upper panel) and connectivity (lower panel). The grey zone is the region delimited by the 2.5 and 97.5 percentiles from the random distributions of z -scores. The open circle in each panel represents the phylogenetic correspondence estimated for the web sampled at Difuntito, a well-preserved small sierra with an unexpectedly high number of species and interactions (Table S1). Partial regression linear equations are depicted in each graph including all estimates (continuous line) and after excluding the estimate for Difuntito (dashed line) (see Table S4).

patches could benefit from an enhanced rescue effect, populations of non-resident species are also more likely to colonise these patches (Leibold *et al.* 2004). These newcomers could establish ephemeral and probably loose phenotypically matched interactions with generalists (Aizen *et al.* 2012), increasing, as previously reported, the nested structure of a pollination web (Gilarranz *et al.* 2015), but blurring, as found here, its phylogenetic structure (Figs 3 and 4). As it is predicted for species losing rare but efficient mutualists in small habitat islands, a fitness cost is also expected for these transient species from interacting with ill-matched partners.

Our framework clearly favours a view of random species extinction and colonisation affecting not only network architecture, as reported in Gilarranz *et al.* (2015), but also phylogenetic structure (Figs 3 and 4). However, deterministic, phylogenetic-related factors associated with habitat patch size and connectivity could provide alternative explanations for the phylogenetic patterns we found. This could occur if lineages of either plant or pollinator specialists are differentially pruned in small habitat patches, or if lineages of generalists proliferate in more connected patches. Both processes would predict a decrease in the phylogenetic signal in the number of interaction links per species, and of phylogenetic diversity compared with random expectations (e.g. Rezende *et al.* 2007). However, despite significant phylogenetic signals in

both plant and pollinator specialisation, none of these patterns were supported by our data (Tables S13 and S14). This result does not necessarily undermine the importance of particular life-history species traits as determinants of interaction disruption (Aizen *et al.* 2012), but it stresses the role of a meta-community dynamics influencing phylogenetic structure.

Although both the plant and animal phylogenetic effects on the resemblance of their respective animal and plant species assemblages showed similar trends (Fig. 3), the effect of animal phylogeny increased significantly with sierra area, whereas the effect of plant phylogeny increased significantly with sierra isolation. Higher average mobility and shorter generation times of insects than plants (Menéndez 2007) could be at the core of these somewhat contrasting results. The animal phylogenetic effect on plant assemblage composition might decrease in small habitat islands if insect populations were more vulnerable to local extinction than plant populations, whereas insects' higher vagility could decrease the plant phylogenetic effect on flower-visitor assemblage composition in more central habitats. Beyond speculation, our results also showed, as it has been previously reported (Rezende *et al.* 2007), that the animal phylogeny is more strongly associated with the identity of interactive mutualists across pollination webs than vice versa. This stronger animal than plant phylogenetic effect could relate, among other factors, to (1) a longer evolutionary history of insects than flowering plants (Hedges & Kumar 2009), (2) convergent character evolution in unrelated plant lineages as a response to the selection pressure from a suite of phylogenetically related pollinators (i.e. the floral syndrome hypothesis; Fenster *et al.* 2004) and (3) the exploitation of different resources (e.g. pollen vs. nectar) from a wide range of taxonomically diverse plant species (Engel & Dingemans-Bakels 1980). Whatever the ultimate cause or causes behind these differences, both plant and animal phylogenetic effects on the identity of interaction partners could be contributing to the co-phylogenetic structure of plant–pollinator interactions (Fig 2b and c).

The phylogenetic congruence between plants and animals reported here is basically determined by a correspondence at internal rather than terminal nodes (see the comparison of polytomic and fully resolved phylogenies in phylogenies and phylogenetic distances), which concurs with the view that plant–pollinator interactions are commonly shaped by co-evolutionary processes within species-rich assemblages of low specificity. According to this perspective, phylogenetic structure of plant–animal interactions is mostly determined by a correspondence at high taxonomical (i.e. suprageneric) levels, which means that guilds (i.e. functional groups) of closely related and unrelated species, rather than single species, exert symmetric or asymmetric selection pressures on each other (Fenster *et al.* 2004; Lunau 2004; Strauss & Irwin 2004). Despite its multi-specific nature, this co-evolutionary process is key in the preservation of evolutionary history, increasing species adaptation (Galetti *et al.* 2013) and long-term biodiversity maintenance (Thompson 1999). Our results suggest that this co-evolutionary process, and the co-phylogenetic pattern it generates, is eroded under scenarios of habitat fragmentation that increase species turnover (e.g. a network of small but connected habitat patches), resulting in more erratic selection forces that could

lead to an evolutionary increase in generalisation (Fenster *et al.* 2004). Accordingly, co-evolutionary processes moulding well-matched plant–animal mutualisms would be better preserved in large but relatively isolated habitat islands, expanding to the level of webs of interactive mutualists Mayr's popularised proposal that speciation principally occurs in allopatry (Mayr 1976). Of course, in conservation terms the benefits of patch connectivity for biodiversity maintenance (Leibold *et al.* 2004; Gilarranz *et al.* 2015) will have to be balanced against the potential costs of co-adaptation loss.

Most natural and semi-natural habitats are now fragmented to different degrees. Habitat fragmentation has been shown to be one of the most important drivers of the disruption of different types of interspecific interactions, principally of plant–pollinator interactions (Aguilar *et al.* 2006), which can represent a proximate cause of species extinctions (Sabatino *et al.* 2010). Here, we provide the first empirical demonstration that this general loss of biodiversity is accompanied by a loss of phylogenetic structure in pollination mutualistic networks. Erosion of this phylogenetic structure can in turn indicate degradation of co-evolutionary processes, which maintain and boost biodiversity on Earth (Thompson 1999). Our results suggest that these processes will benefit from setting aside and preserving large expanses of habitat and isolating them from much exterior influence.

ACKNOWLEDGEMENTS

The authors thank S. Nuismer, A. Pauw and three anonymous reviewers for useful comments and suggestions, A. Sáez and D. Porrini for field assistance, V. Izpizua and M. Nuciari for help in plant identification and J. Farina and A. Roig-Alsina for help in identifying insects. This study was funded in part by the National Institute of Agricultural Technology (INTA), Balcarce (PNECO1302), the Argentina National Research Council for Research (CONICET) (PIP 01623), the National Fund for Research (PICT 01300), the National University of Comahue (B152/04) and the European Research Council (through an Advanced Grant to JB). M.A.A., G.G. and M.S. are career researchers of CONICET.

AUTHORSHIP

MAA conceived the study with input from all authors, performed the analyses, and wrote the first draft of the manuscript; MS collected data; GG and MV built the phylogenies and all authors contributed substantially to the final manuscript.

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Editor, Jennifer Dunne

Manuscript received 15 July 2015

First decision made 30 September 2015

Manuscript accepted 2 October 2015