

# Influence of plant–pollinator interactions on the assembly of plant and hummingbird communities

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## Summary

**1.** Understanding how ecological processes structure species assemblages is a central issue in community ecology. While the influence of plant–pollinator interactions on each other's evolution is well recognized, their role in the assembly of interdependent communities of plants and pollinators is still unclear.

**2.** Using data from seven communities of hummingbirds and plants that they pollinate from two tropical rain forest types (lowland and montane), we evaluated phylogenetic relationships and signal of functional traits, over space and time, to test predictions on the main processes (environmental filtering, facilitation or competition) that are driving these hummingbird–plant assemblages.

**3.** Our findings suggest that the main processes driving these assemblages varied between hummingbirds and plants and between habitats, and even among communities at the same habitat. The non-conserved floral trait and the phylogenetic patterns (even or random) give support to the hypothesis of facilitation or competition as processes regulating the composition of plant assemblages. Moreover, the positive relationship between fitness and flowering synchrony suggests facilitation as the most important mechanism for montane plant communities. Distinctively, for lowland plant communities, the combination of non-conserved traits and clustered phylogenetic patterns may be a result of either adaptive radiation or biotic filtering driven by a particular pollinator species that plays a main role as plant community organizer. Lastly, evidence of trait conservatism, together with clustered or even phylogenetic patterns, suggests that facilitation or competition may drive the assembly of montane hummingbird communities, despite the predominance of random phylogenetic patterns.

**4. Synthesis.** Overall, we present a pathway to identify central ecological processes that may drive the assembly of plant–pollinator communities. We show that different processes related with pollination that vary in space and time may contribute to the assembly of the interdependent tropical communities of plants and pollinators. These findings highlight the importance of considering ecological interactions when evaluating community assembly processes.

**Key-words:** community ecology, competition, facilitation, functional ecology, mutualism, ornithophily, phylogenetic structure, pollination

## Introduction

Understanding which factors determine species coexistence in communities is a fundamental question in ecology and evolutionary biology. Historically, it has been debated whether the assembly of communities is regulated by deterministic (e.g. competitive exclusion), neutral (i.e. species are ecologically equivalent) or historical (e.g. patterns of speciation and dispersal) evolutionary processes (see Cavender-Bares *et al.*

2009). In addition, current ecological perspectives highlight that species coexistence in communities may depend on how biotic interactions, environmental filtering and neutral drift in abundance balance each other, affecting species assemblages (Webb *et al.* 2002; Baraloto *et al.* 2012). Several studies have used phylogenetic and functional trait approaches to examine processes that may influence the assembly of plant communities (Kraft & Ackerly 2010; Baraloto *et al.* 2012; Yang *et al.* 2014). Similar approaches have been applied in the study of animal communities (e.g. Ingram & Shurin 2009; Gómez *et al.* 2010). However, most of these studies with plant communities, for instance, focused on the vegetative

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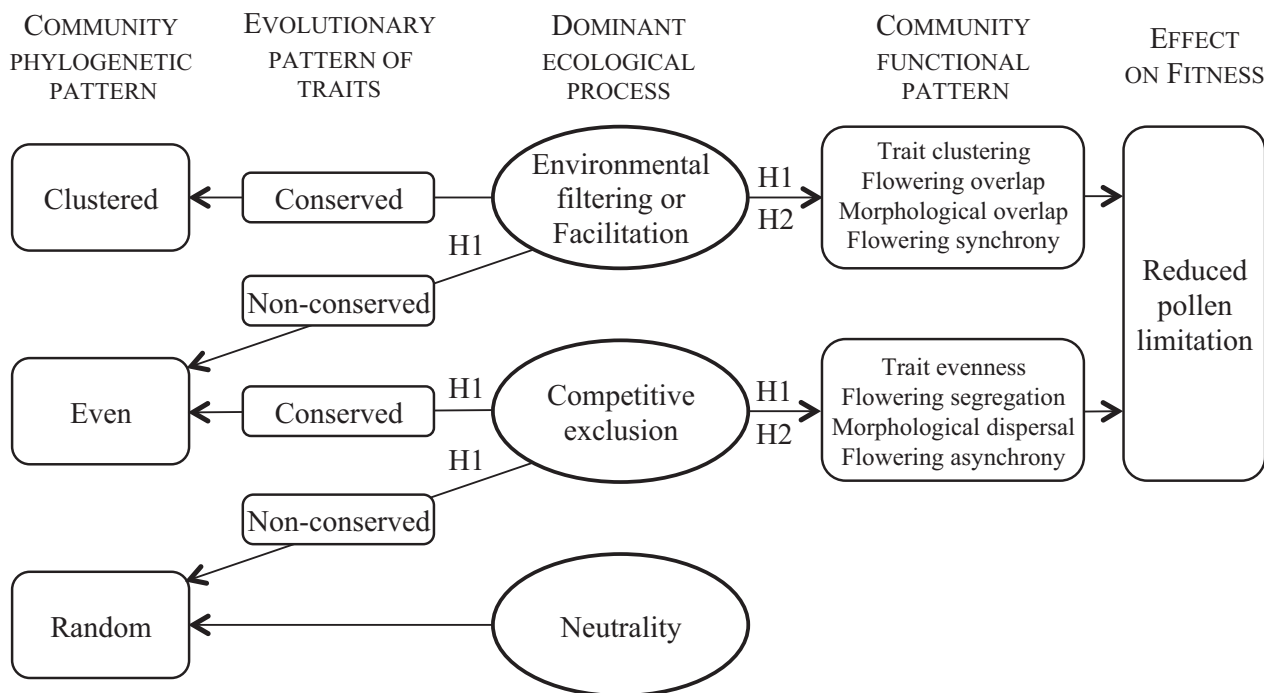
(ecophysiological) traits, neglecting indirect interactions among plants, such as those mediated by pollinators (but see Armbruster 1995; Sargent & Ackerly 2008; Pellissier, Alvarez & Guisan 2012). Interactions between plants and pollinators can be a major evolutionary force underlying the success and diversification of flowering plants (e.g. Kay *et al.* 2005). Likewise, plants may influence pollinator diversification (e.g. McLeish, Van Noort & Tolley 2010). Moreover, plant–pollinator interactions entail many consequences on the evolution and ecology of both plants and animals (Mitchell *et al.* 2009; Vázquez *et al.* 2009b). Hence, a dynamic interplay between ecology and evolution is expected within communities, although it deserves further demonstration (Johnson & Stinchcombe 2007). Therefore, to better understand the evolutionary and ecological consequences of the current interdependent assemblages of plants and pollinators using phylogenetic and functional approaches, it is necessary to evaluate whether traits that influence plant–pollinator interactions are phylogenetically conserved or not.

The set of floral features of each plant species (such as flower shape, dimension, colour, odour, type of reward, anthesis dynamics and phenology) influences which and how animals may explore the flowers, and hence the success of the pollination process. Indeed, many plant species are pollen limited and, due to their direct effects on the quality and quantity of seeds produced (Aizen & Harder 2007), pollination levels may influence plant species' recruitment, relative abundance and persistence within a community (Ashman *et al.* 2004). In an evolutionary perspective, differences in the composition of the assemblage of the most effective pollinators among different regions may drive floral divergence, whereas if the most effective pollinator species is shared by different plant species, convergence of floral traits is expected (Fenster *et al.* 2004; Rosas-Guerrero *et al.* 2014). However, important ecological processes (e.g. environmental filtering, facilitation, competition) will interfere on how a particular assemblage of pollinators will affect plant fitness, and hence plant community structure and dynamics. Specifically, environmental filtering (*sensu stricto*) refers to abiotic factors that prevent the establishment or persistence of species in a particular site (Kraft *et al.* 2015), while biotic factors such as the local pollinator assemblage may prevent the establishment and/or persistence of a plant species (or lineage) in a community (Sargent & Ackerly 2008; Chalcoff, Aizen & Ezcurra 2012), particularly in the absence of a particular pollinator species. Moreover, the capabilities and preferences of floral visitors and interspecific behavioural interactions act as regulators of pollinator activity, affecting the degree of specialization of the systems and the plant fitness (see Mitchell *et al.* 2009). For instance, co-flowering plants sharing pollinators may experience positive outcomes on the plant fitness, whereby the presence of a plant attracts flower visitors to a given region increasing pollination of neighbouring plants (facilitation), or negative effects, whereby the presence of an attractive plant species reduces visitation to neighbouring plants (competition) (e.g. Hegland, Grytnes & Totland 2009). Although an amount of studies on pollination of plant

assemblages has interpreted their findings in the light of niche and neutral theories (e.g. Heithaus 1974; Feinsinger 1987; Vázquez, Chalcoff, and Cagnolo 2009a; McEwen & Vamosi 2010; Hegland & Totland 2012), the role of environmental filtering and competitive and facilitative processes via shared pollinators in the assembly of plant communities is still unclear (Armbruster 1995; Sargent & Ackerly 2008).

The action of environmental filtering and competitive and facilitative processes over ecological and evolutionary time-scales can result in distinct combinations of morpho-physio-phenological features that indirectly impact individual fitness (i.e. functional traits, *sensu* Violle *et al.* 2007) among communities (Webb *et al.* 2002). Moreover, functional trait distribution throughout the phylogeny of a particular assemblage of species is one of the cornerstones of community assembly theory. Indeed, Sargent & Ackerly (2008) highlight a set of assembly rules based on plant ecophysiological traits to morphological pollination-related traits. Following their framework, and assuming a relatively high level of specialization, when either facilitation or environmental filtering is the dominant process driving community assembly, it is expected that plant species within a given region will have more similar pollinator syndromes (i.e. combination of floral features associated with a pollinator, *sensu* Faegri & van der Pijl 1979). In this way, if traits are phylogenetically conserved, communities would consist of closely related plants, and floral trait distribution throughout the phylogenetic tree would be clustered (Fig. 1). If traits are not phylogenetically conserved and instead evolution was convergent, communities would consist of distantly related plants, and traits would be evenly distributed throughout the phylogenetic tree. On the other hand, when pollinator-mediated competition is the main process driving plant species relations, the establishment of distantly related plants that use different pollinator resources in the same region might be favoured if traits are conserved. In this situation, we expect an even distribution of floral traits across the phylogenetic tree (Fig. 1). However, if traits are not conserved, a random phylogenetic pattern of plant species with divergent pollinators would be expected (Fig. 1). Sargent & Ackerly (2008) provide some examples of these scenarios, but a more comprehensive empirical support still lacks.

In turn, morpho-physiological traits (such as body weight, wing shape, and bill length and curvature) determine flight ability and foraging efficiency of hummingbird species (Graham *et al.* 2009), and these traits affect dispersal capability of hummingbirds inhabiting a certain geographic region (Brown & Bowers 1985). Moreover, hummingbird species abundances and niche overlap are regulated by the availability of plant resources in Neotropical forests (Feinsinger & Colwell 1978; Kodric-Brown *et al.* 1984), and ecological interactions among hummingbirds are constrained by resource accessibility (Vizentin-Bugoni, Maruyama & Sazima 2014). From an evolutionary perspective, evidence of phylogenetic signal in hummingbird–plant interactions was detected in several communities (Martín González *et al.* 2015). Therefore, we may draw up expectations based on assembly rules for



**Fig. 1.** Conceptual framework of the effect of dominant ecological processes related to plant–pollinator interactions on phylogenetic and functional patterns of plant–pollinator community assembly. Boxes in the 1st column indicate the expected taxa distribution of local communities based on the species pool. Boxes in the 4th column indicate the expected patterns of plant functional diversity associated with functional traits within plant assemblages. The same predictions outlined in the first four columns are valid for the assembly of pollinator communities, associated with functional traits such as feeding apparatus and body mass. Lastly, in the 5th column, an overall reduction in pollen limitation in the plant community is expected depending on the functional diversity pattern resulting from distinct ecological processes. Expanded from frameworks by Webb *et al.* (2002), Sargent & Ackerly (2008) and Mitchell *et al.* (2009).

hummingbird assemblages that are similar to the expectations outlined above using plant traits (Fig. 1). For instance, if competition mediated by flower resources is the main process that drives hummingbird species relationships in a certain community and their functional traits are conserved, we expect an even phylogenetic pattern of hummingbird species (Fig. 1). Here, we evaluate whether the structure of functional traits related to plant–pollinator interactions and the phylogenetic relationships can predict the dominant ecological processes acting on community assembly of hummingbirds and plants in the Atlantic rain forest of south-eastern Brazil.

Hummingbird-pollinated plant species are spread out over many Neotropical families. While hummingbirds can visit many plants with non-ornithophilous flowers (e.g. Dalsgaard *et al.* 2008), they most fit on a well-defined ornithophilous syndrome in rain forests (Stiles 1985), in which tubular, odourless and reddish flowers with dilute nectar are broad convergent traits (Faegri & van der Pijl 1979; Rosas-Guerrero *et al.* 2014). However, ornithophilous flowers differ in traits such as corolla size and exposition of reproductive parts (Buzato, Sazima & Sazima 2000), and nectar production (McDade & Weeks 2004). Such variability in flower traits may lead to some level of specialization in plant–hummingbird relationships, especially in more diverse plant communities such as the Brazilian Atlantic rain forest (Dalsgaard *et al.* 2011; Maglianesi *et al.* 2014; Vizentin-Bugoni, Maruyama &

Sazima 2014). Indeed, morpho-physiological and behavioural characteristics vary greatly among sympatric species of hummingbirds, although they are more similar within their major clades (Feinsinger & Colwell 1978; Graham *et al.* 2009). Given that plant species have some level of pollinator specialization (Dalsgaard *et al.* 2008; Maglianesi *et al.* 2014; Vizentin-Bugoni, Maruyama & Sazima 2014), we expect that the great variability of hummingbirds and plants even within the same biome (e.g. lowland and montane communities in the Atlantic rain forest, Buzato, Sazima & Sazima 2000) is partially explained by differences in functional traits that regulate interactions between them. Therefore, we expect that hummingbirds and plants will have an even or random phylogenetic pattern within habitats, while distribution pattern of functional traits throughout the phylogenetic tree will indicate convergent evolution for plants and conservatism for hummingbirds (hypothesis 1, Fig. 1).

The idea that plants pollinated by hummingbirds flower sequentially within a community was proposed in the first comprehensive studies in Neotropical forests (e.g. Stiles 1978). Several subsequent studies found similar phenological dynamics (Wolf, Stiles & Hainsworth 1976; Kodric-Brown *et al.* 1984; Stiles 1985; Sazima, Buzato & Sazima 1995; Buzato, Sazima & Sazima 2000; Aizen & Vázquez 2006) despite some variation on the overall quantity of flowers (Aizen & Rovere 2010). This flowering pattern could result from diffuse co-evolution of hummingbirds and plants shaped

by the needs of continual resources for hummingbirds (Stiles 1978, 1985). Such diffuse co-evolution could result from pollinator-mediated competition among plant species, so that the temporal segregation of flowering peaks among species with similar floral traits would lead to positive effects on plant fitness (Waser 1978; Mitchell *et al.* 2009). Therefore, we expect to find evidence of competitive interactions among plant species with similar floral traits as being a main ecological process in the studied communities. Such effect of competition would translate into a temporally segregated pattern of flowering and floral traits being evenly distributed over time; while if facilitation is the main ecological process, flowering overlap will be higher (hypothesis 2, Fig. 1). However, the evolutionary lability of flowering phenology is subject to certain unavoidable constraints (Forrest & Miller-Rushing 2010; Du *et al.* 2015), and hummingbird–plant communities harbour dozens of plant species, many of them visited by the same species of hummingbirds (Buzato, Sazima & Sazima 2000). In this context, some degree of flowering overlapping will certainly occur. As interspecific competition reduces plant fitness (Aizen & Vázquez 2006; Aizen & Rovere 2010), if competition is the main ecological process, the lower the morphological overlap and flowering synchrony among co-flowering species, the higher will be the fitness. By contrast, we expect plant fitness will increase with increased morphological overlap and flowering synchrony if facilitation is the main ecological process (hypothesis 3, Fig. 1).

## Materials and methods

### STUDY SYSTEM

To evaluate the variability in the phylogenetic and functional trait structure of hummingbird–plant communities, we gathered information from several studies conducted in the Atlantic rain forest of south-eastern Brazil, which presented data of species identity and floral traits. We reviewed published studies primarily using the data base ‘Institute for Scientific Information Web of Science®’, with the

following keyword combination: ‘hummingbird\* AND atlantic forest OR hummingbird\* AND atlantic rainforest’. Doctoral theses from personal library collections of the authors were added. This led to a total of seven hummingbird–plant communities, which were described in six studies (Table 1). These studies covered a range of habitats, from lowland (0–100 m a.s.l.) to montane (850–1600 m a.s.l.) rain forests, and comprised information on a total of 134 plant species (plant species pool), mostly (ca. 70%) represented by species of Acanthaceae, Bromeliaceae, Gesneriaceae, and Rubiaceae, and 20 hummingbird species (hummingbird species pool), more represented by species of Emeralds (Trochilini) (40%) and Hermits (Phaethornithinae) (25%).

For plant species, two floral traits were considered in this study: effective corolla length, that is the measure from the base of the nectar chambers to the distal portion of the flower which determines how far the head of a feeding bird could fit into a flower (*sensu* Wolf, Stiles & Hainsworth 1976); and stigma length, that is the measure from the base of the flower to the distal position of the stigma of the flower which determines the extension of pollen deposition in the hummingbird body (Fig. S1, Supporting Information). We chose these two traits because they are highly variable among the species, and affect the way the hummingbirds access floral resources and interfere with pollen deposition.

For effective corolla length, information was gathered for plant species present in all studied communities. For 17.2% (23 of 134) of the species, effective corolla length was not described in the primary data sources of ornithophilous communities. In these cases, we estimated effective corolla length from measurements taken from digitalized herbarium sheets that are available online in virtual collections (*speciesLink* – <http://splink.cria.org.br> and *JBRJ* – <http://www.jbrj.gov.br/jabot>) (15 species), or from personal field notes (M. Wolowski and P.J. Bergamo) for seven species and from taxonomic description of one species (*Nidularium bicolor*, Leme 2000). When multiple measurements were available for a given species, we calculated the average of these measurements for the species. Information on stigma length (mean and standard deviation) was only obtained for one of the studied plant communities (montane forest at Itatiaia – ITA). These data came from personal field notes (M.B.F. Canela; M. Wolowski) except for one species (*Psittacanthus brasiliensis*), for which stigma length information was extracted from its taxonomic description (Eichler 1868).

**Table 1.** Set of hummingbird–plant communities used in the data analyses. All communities are from the Atlantic rain forest of south-eastern Brazil

Community	Number of plant species; hummingbird species	Altitude (m a.s.l.)	Latitude	Longitude	References
Lowland rain forest					
Caraguatatuba (CAG)	38; 11	50–100	23°35' S	45°20' W	Buzato, Sazima & Sazima (2000)
Carlos Botelho (CBO)	44; 13	100	24°00' S	47°45' W	Rocca-de-Andrade (2006)
			24°15' S	48°10' W	
Picinguaba (PIC)	21; 13	10	23°20' S	44°48' W	Araujo (1996)
			23°22' S	44°52' W	
Montane rain forest					
Campos do Jordão (CJO)	25; 6	1400–1600	22°44' S	45°35' W	Buzato, Sazima & Sazima (2000)
Cunha (CUN)	31; 8	1000–1100	23°10' S	44°55' W	Buzato, Sazima & Sazima (2000)
Itatiaia (ITA)	36; 5	1000–1100	22°27' S	44°36' W	Canela (2006), Wolowski, Ashman & Freitas (2013a)
Santa Virginia (SVG)	42; 9	850–1100	23°17' S	45°03' W	Vizentin-Bugoni, Maruyama & Sazima (2014)
			23°24' S	45°11' W	



We also obtained qualitative data of flowering phenology from the primary data sources for the flowering activity of each species per census of a year-period in each plant community. For the ITA community, we also had access to quantitative data of flowering phenology from January 2010 to December 2011. Data were sampled in a total of 157 circular plots with 5 m radius distributed over seven transects delimited by trails or roads (total length of 5 km), which are characterized by significant presence of ornithophilous plant species (*sensu* Faegri & van der Pijl 1979). Transects were distant from each other by 50 m to 1 km. Plots were permanent and distant from each other by  $25 \pm 5$  m along transects; the amount of sample units per transect was defined by the extent of each transect. Total area sampled was 1.24 ha. On each month, we counted the number of floral buds and open flowers per individual plant per species with naked eye or with binoculars aid in each plot. We counted the number of inflorescences when the direct count of flowers was not possible (three species) due to the large number of flowers. In such cases, the average number of flowers per inflorescence was determined to generate an estimate of flowers per plant. Thus, we obtained the total number of flowers (floral buds and open flowers) per species per month for the whole sampled area, which resulted in 24 'temporal hummingbird-plant communities', that is sets of plant species that may share pollinators at a given moment.

For hummingbird species, we considered two traits that are highly variable among the species and are directly related to the foraging behaviour: bill length, which reflects the accessibility to floral resources, and body mass, which reflects flying ability. For both traits, the values for each species were obtained from Grantsau (1989).

#### PHYLOGENETIC AND FUNCTIONAL STRUCTURE ACROSS SPACE

We evaluated spatial variance of the phylogenetic structure of hummingbird and plant communities. For this, we first built a phylogenetic tree hypothesis for the plant species pool using the angiosperm APG III (APG 2009) consensus tree (R20120829), available in the website <https://github.com/camwebb/tree-of-trees/tree/master/megatrees>, using Phylomatic (Webb & Donoghue 2005). Then, we resolved the relationships within Bromeliaceae assuming monophyly for subfamilies and polytomies for genera because interfamily relationships are well-established, while intrafamily are not (Givnish *et al.* 2011). Next, we calibrated branch lengths using the branch length adjuster function (BLADJ) from Phylocom 4.2 (Webb, Ackerly & Kembel 2011). For this, we used ages of clade divergence according to Bell, Soltis & Soltis (2010), which is based on the minimum age of clade divergence from Wikström, Savolainen & Chase (2001).

Then, for each of the seven plant communities, we calculated two measures of phylogenetic distance: mean phylogenetic distance (MPD) and mean nearest taxon distance (MNTD), and associated standard deviations with 'comstruct' function in Phylocom 4.2 (Webb, Ackerly & Kembel 2011). To evaluate whether MPD and MNTD differed significantly from what would be expected by chance, we compared these values with MPD and MNTD values obtained from randomly generated null communities for each of the seven communities. We used the independent swap algorithm (Gotelli 2000) as the null model because it maintains species occurrence frequency and sample species richness per site and allows to consider abundance. To calculate *P*-values, we used the rank of observed MPD/MNTD values relative to the values in the null communities. These same decisions were made for all similar analyses of phylogenetic and functional structures. We also calculated the two measures of standardized effect size of phylogenetic community structure: net

relatedness index (NRI) and nearest taxon index (NTI), which describe the difference between average phylogenetic distances in the observed and null communities, standardized by the standard deviation of phylogenetic distances in the null communities. NRI and NTI were calculated as  $-1 \times \text{SES.MPD}$  and  $-1 \times \text{SES.MNTD}$ , respectively. NRI and NTI values significantly greater than what would be expected by chance indicate that the structure of the communities is phylogenetically clustered and significant negative values indicate phylogenetic evenness. The NRI is a measure sensitive to phylogeny-wide patterns, while NTI is a measure sensitive to patterns at the terminals of the phylogeny (Webb 2000; Kraft *et al.* 2007). MNTD values may be biased by the amount of polytomies at the terminals of the phylogeny, which may reduce the power to detect non-random community structure. Thus, we interpreted results primarily based on MPD, but also present MNTD results in the Supporting Information.

For the hummingbird communities, we built a phylogenetic tree hypothesis for the species pool using the time-calibrated phylogeny from McGuire *et al.* (2014). For this, we pruned the species that were not represented in the hummingbird species pool with APE (Paradis, Claude & Strimmer 2004) in R (R Core Team 2014). Because *Phaethornis squalidus* was the only species from the species pool that was not represented in the phylogeny from McGuire *et al.* (2014), we assumed a polytomy for the three *Phaethornis* species. Then, we calculated MPD, MNTD, associated standard deviations, and NRI and NTI for each of the seven hummingbird communities with PICANTE (Kembel *et al.* 2010) in R (R Core Team 2014) using a phylogenetic distance matrix using the functions 'ses.mpd' and 'ses.mntd'.

To evaluate whether there was a phylogenetic signal for effective corolla length in the plant species pool and for bill length and body mass in the hummingbird species pool, we calculated the *K* statistic (Blomberg, Garland Jr & Ives 2003). *K* measures the amount of phylogenetic signal for continuous-valued characters and was calculated based on variance of phylogenetically independent contrasts relative to tip shuffling randomization with Phytools (Revell 2012). Moreover, we ran a quantitative trait simulation on phylogenies under a Brownian motion null model to assess whether *K*-value depart from Brownian motion evolution with Phytools (Revell 2012). Values of *K* non-significantly different from 1 indicate a Brownian motion evolution and some degree of phylogenetic signal or conservatism, while values of *K* non-significantly different from 0 indicate lack of phylogenetic signal.

To assess whether hummingbird-plant communities are structured according to their functional traits, we first calculated mean difference in traits between species [similar to MPD, hereafter Mean Trait Distance (MTD)] and mean minimum difference in traits between species [similar to MNTD, hereafter Mean Minimum Trait Distance (MNTD)]. We made these calculations with Picante (Kembel *et al.* 2010) based on the distance matrix for each trait (i.e. effective corolla length for the species in the plant species pool and bill length and body mass for the species in the hummingbird species pool).

#### FUNCTIONAL STRUCTURE OVER TIME

We evaluated whether, as a strategy to avoid competition for pollinators, flowering was segregated within the communities of hummingbird-pollinated plants (i.e. if flowering overlap was minimized). To do that, we used null models to test whether flowering overlap between species was significantly less than what would be expected by random, using the software TIMEOVERLAP (Castro-Arellano *et al.* 2010). We used quantitative or qualitative data of flowering phenology from each community for each 12-month time period (Table S3) to create

matrices with information on the presence of flowering or amount of resource offered by a given species (i.e. the total number of flowers). We used the default number of interactions of 10 000 of the software. For a one-tailed test, we calculated  $P$ -value as the proportion of randomizations that resulted in an overlap value that was equal to or lower than the observed overlap value. We used both Pianka (Pianka 1973) and Czechanowski (Feinsinger, Spears & Poole 1981) indices to test whether overlap was significantly less than what would be expected by chance (i.e.  $P$ -value < 0.05).

Then, we used null models to evaluate whether the temporal dispersion of floral traits within the ITA community was higher or lower than what would be expected by chance over time. To do this, each set of plant species in bloom in a given month was considered a distinct temporal hummingbird–plant community, leading to a total of 24 temporal hummingbird–plant communities. For each of these communities, we calculated the measures of MTD and MMTD for the focal floral trait (effective corolla length and stigma length) using distance matrix for these traits with PICANTE (Kembel *et al.* 2010) in R (R Core Team 2014) and considering floral abundance, that is the number of flowering individuals per month.

#### PLANT FITNESS, MORPHOLOGICAL OVERLAP AND FLOWERING SYNCHRONY

To evaluate whether the overlap on floral morphology and flowering synchrony explains fitness differences among co-flowering species, we estimated pollen limitation for each species from the ITA community. Pollen limitation – the reduction in the reproductive success due to inadequate pollen deposition in the stigma (*sensu* Ashman *et al.* 2004) – is a proxy for reduction in plant fitness. It was quantified as the difference in fruit set after manual cross-pollinations and pollinations in natural conditions, so that as pollen limitation increases fitness decreases (Wolowski, Ashman & Freitas 2013a). Morphological overlap of each species was estimated based on the amount of overlap of stigma length of the species with the remaining co-flowering species in the community. For this, we calculated the absolute amount of overlap between the 95% confidence interval of stigma length of each pair of species whose flowering overlapped. Then, overall morphological overlap for a given plant species was calculated as the sum of the amount of overlaps of the stigma length of the species with the other co-flowering species. Flowering synchrony was measured based on the index by Freitas & Bolmgren (2008), which takes into account

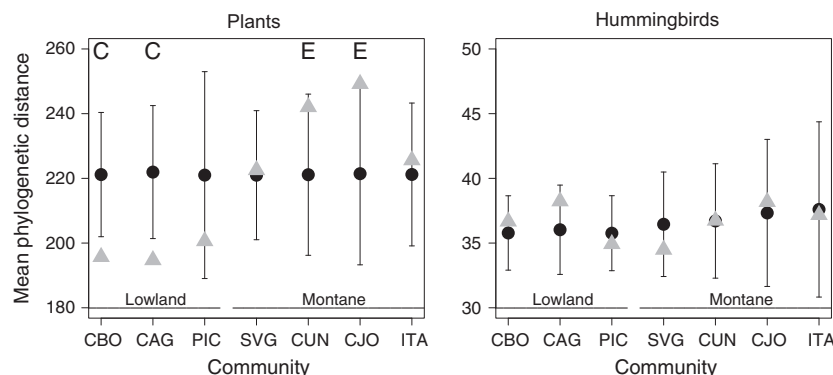
the relative number of flowers produced per species per measured census (month here), that is the quantitative co-occurrence of flowering among species.

We evaluated whether pollen limitation was affected by morphological overlap and flowering synchrony using phylogenetic generalized least-squares regression that takes into account the phylogenetic relations between species using CAPER package (Orme 2012) in R (R Core Team 2014). For that, we built a second phylogenetic tree hypothesis based solely on the species present in the ITA community, following the same procedure described above for the species pool. Pollen limitation and flowering synchrony were log transformed, and residuals analysis was conducted to verify that model assumptions (normal distribution and homoscedasticity) were met. We performed model selection considering morphological overlap, flowering synchrony and the interaction between predictors. Predictors were not correlated (Pearson's correlation = 0.07;  $P$ -value = 0.79). We selected the model with the lowest Akaike information criteria (AIC) and Bayesian information criteria (BIC) values as the best model. We considered that positive relationships between morphological or phenological overlap and pollen limitation indicate competition as the dominant process in the community while negative relationships point to facilitation. Lack of significant relationships were interpreted as neutral process with caution, depending on graphic evaluation of results and potential lack of statistical power (Fig. S2).

## Results

#### PHYLOGENETIC AND FUNCTIONAL STRUCTURE ACROSS SPACE

Our results show that hummingbird–plant communities from different habitats have distinct phylogenetic structure patterns (Fig. 2, Table S1). More specifically, for the plant communities, when considering the mean phylogenetic distance (MPD), two lowland communities were more clustered than what would be expected by chance (CAG and CBO), and the third lowland community (PIC) did not differ from random. For montane communities, two were more even than what would be expected by chance (CJO and CUN), while two did not differ from random (SVG and ITA, Fig. 2). The



**Fig. 2.** Phylogenetic structure of seven communities of plants and hummingbirds in the Atlantic rain forest of south-eastern Brazil. The observed values (grey triangles) of mean phylogenetic distance are compared against the values obtained from randomly generated null communities (mean and 95% confidence intervals presented in black). Values significantly lower than random values indicate phylogenetic clustering (C), while values higher than random indicate phylogenetic evenness (E). Communities abbreviations are as follows: CBO, Carlos Botelho; CAG, Caraguatatuba; PIC, Picinguaba; SVG, Santa Virginia; CUN, Cunha; CJO, Campos do Jordão; ITA, Itatiaia. Statistical details are presented in Table S1.

differences between lowland and montane habitats were less marked when considering mean nearest taxon distance (MNTD), but a third montane community (ITA) was more even than what be expected by chance for this metric (Table S1). For all hummingbird communities, phylogenetic structure patterns did not differ from random for MPD (Fig. 2, Table S1). However, two montane communities had phylogenetic patterns that differ from random for MNTD, indicating evenness (CUN) or clustering pattern (ITA, marginal  $P$ -value) (Table S1).

We detected no phylogenetic signal for effective corolla length in the plant species pool ( $K = 0.110$ ,  $P$ -value = 0.09, Fig. S3). On the other hand, we detected phylogenetic signals for bill length ( $K = 1.126$ ,  $P$ -value = 0.006, Fig. S4) and for body mass ( $K = 0.747$ ,  $P$ -value = 0.05, Fig. S4) in the hummingbird species pool. However, in these cases,  $K$  values were indistinguishable from one ( $P$ -value = 0.71 and  $P$ -value = 0.24, respectively), suggesting trait conservatism. Patterns of functional trait structure were similar for all communities, with no indication that effective corolla length, bill length or body mass were clustered or even distributed within communities (i.e. distribution of these traits did not differ from random, Fig. S5, Table S2).

The combination of trait not conserved and even phylogenetic pattern in three montane plant communities (considering both phylogenetic distance measures, Table S1) suggests that environmental filtering or facilitation is the dominant process in the assembly of these communities (Fig. 1). For these communities, patterns were consistent when considering in the analysis the whole plant species pool or only the montane plants as the species pool. On the other hand, the combination of non-conserved trait with the random phylogenetic structure found in two plant communities (PIC, SVG) suggests that competition is the dominant process in the assembly of these communities. For two montane hummingbird communities, we found trait conservatism and clustered phylogenetic pattern in ITA, suggesting environmental filtering or facilitation, and trait conservatism and even phylogenetic pattern in CUN, suggesting competition (Table S1). Finally, there are no predictions based on our framework (Fig. 1) for some cases: the combination of non-conserved trait and clustered phylogenetic pattern found for two lowland plant communities and trait conservatism and random phylogenetic pattern in most hummingbird communities.

#### FUNCTIONAL STRUCTURE OVER TIME

Our results do not show clear support for the role of competition in structuring flowering and reproductive plant traits. The patterns of flowering phenology structure of most communities did not differ from random (65.6%), followed by flowering overlap (27.3%) (Table S3). We did however get partial support for flowering segregation, that is that plants flowered sequentially (Fig. 3), as significant effects were detected with Czechanowski index within a year-period in the ITA community. Moreover, we found that floral traits (effective corolla length and stigma length) of the species in bloom were

randomly, rather than evenly dispersed within each time period (Fig. S6). Only occasionally, species with different corolla lengths were evenly represented (1 of 24 months) and stigma length organized in clusters (2 of 24) within the temporal communities (Fig. S6).

#### PLANT FITNESS, MORPHOLOGICAL OVERLAP AND FLOWERING SYNCHRONY

Species which had higher flowering synchrony (here, the quantitative co-occurrence of flowers) with the remaining ornithophilous species present in the ITA plant community had lower levels of pollen limitation (Fig. 4). This indicates the prevalence of facilitative, rather than competitive, processes among co-flowering plants in this community (see Fig. 1). No phylogenetic signal was detected in this model ( $\lambda = 0$ ,  $P$ -value = 1). In contrast, morphological overlap was not related with pollen limitation (Table 2), reflecting neither a positive (facilitation) nor a negative (competition) effect of this trait on the fitness within this community.

#### Discussion

While the role of plant–pollinator interactions in the evolution of plants and hummingbirds is well recognized, it is still unclear how variable are the ecological processes that drive the composition of plant–pollinator assemblages across different habitats and over time. Our data suggest that deterministic (competition vs. facilitation) and evolutionary processes may play an important role in the assembly of hummingbird–plant communities from the Atlantic rain forest in Brazil. Moreover, we show that the effect of those processes in plant fitness can vary over time. We discuss our findings and their implications for the study of evolutionary and ecologic processes operating on community assembly.

#### ECOLOGICAL PROCESSES DRIVING COMMUNITY ASSEMBLY VARIED BETWEEN HUMMINGBIRDS AND PLANTS AND ACROSS HABITATS

Our findings suggest that processes driving plant community assembly in the lowland are not the same as those in the montane Atlantic rain forest. For instance, evidence of environmental filtering or facilitation was restricted to montane plant communities (three of four communities), while competition was evidenced for both lowland and montane communities. Moreover, there was no phylogenetic signal for floral trait. Due to the amount of unresolved polytomies within plant families, especially in the richest family Bromeliaceae, phylogenetic signal may have been overestimated (Blomberg, Garland Jr & Ives 2003). A more confident estimate for the phylogenetic signal (i.e. resolution of large polytomies) could be obtained by reconstructing molecular phylogeny with the species from the communities. Nevertheless, unresolved polytomies are an issue in all our study sites, and despite those, we found clear differences between montane and lowland communities.

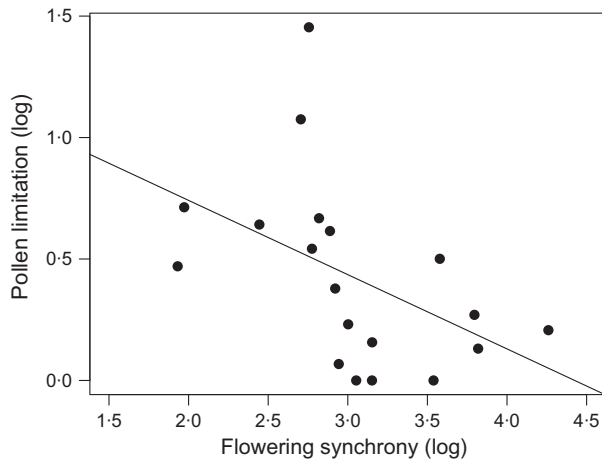
Species	2010												2011											
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
<i>Nidularium itatiaiae</i> L.B.Sm.																								
<i>Salvia sellowiana</i> Benth.																								
<i>Vriesea gradata</i> (Baker) Mez																								
<i>Stromanthe thalia</i> (Vell.) J.M.A.Braga																								
<i>Odontonema barbelerioides</i> (Nees) Kuntze																								
<i>Pitcairnia flammea</i> Lindl.																								
<i>Elleanthus brasiliensis</i> (Lindl.) Rchb.f.																								
<i>Nematanthus crassifolius</i> (Schott) Wiehler																								
<i>Staurogyne itatiaiae</i> (Wawra) Leonard																								
<i>Vriesea carinata</i> Wawra																								
<i>Sinningia cooperi</i> (Paxton) Wiehler																								
<i>Manettia mitis</i> (Vell.) K.Schum.																								
<i>Nematanthus fornx</i> (Vell.) Chautems																								
<i>Justicia sebastianopolitana</i> Profice																								
<i>Tillandsia tenuifolia</i> L.																								
<i>Sinningia gigantifolia</i> Chautems																								
<i>Nematanthus lanceolatus</i> (Poir.) Chautems																								
<i>Quesnelia augusto-coburgii</i> Wawra																								
<i>Billbergia distachia</i> (Vell.) Mez																								
<i>Aechmea vanhoutteana</i> (Van Houtte) Mez																								
<i>Tillandsia geminiflora</i> Brongn.																								
<i>Pyrostegia venusta</i> (Ker Gawl.) Miers																								
<i>Velloziella dracocephaloides</i> (Vell.) Baill.																								
<i>Tillandsia stricta</i> Sol.																								
<i>Mendoncia velloziana</i> Mart.																								
<i>Aechmea nudicaulis</i> (L.) Griseb.																								
<i>Billbergia vittata</i> Brong																								
<i>Nidularium bicolor</i> (E.Pereira) Leme																								
<i>Psittacanthus brasiliensis</i> (Desr.) G.Don																								
<i>Vriesea penduliflora</i> L.B.Sm.																								
Number of taxa	25												30											
Pianka index	0.164 (0.086)												0.176 (0.074)											
Czechanowski index	0.125 (0.071)												0.139 (0.024)											

**Fig. 3.** Flowering phenology structure of the hummingbird-pollinated plant community from Itatiaia, south-eastern Brazil, measured using Pianka and Czechanowski indices with null models in TimeOverlap (Castro-Arellano *et al.* 2010) for each year (2010 and 2011). Number of taxa represents the number of species observed in flowering during each year. Pianka and Czechanowski indices values are the observed overlap value of the flowering phenology of the community. *P*-values (between parenthesis) are the proportion of randomizations that resulted in overlap values equal to or less than the observed overlap value.

The fact that we only found phylogenetic clustering for lowland plant communities could be related to some peculiarities of hummingbird-pollinated plant communities in this habitat that were not included in our analyses (e.g. floral traits related to resource availability). For instance, the saw-billed hermit (*Ramphodon naevius*, Phaethornithinae) occurs

exclusively in the lowland Atlantic rain forest; it is the largest hummingbird of the Atlantic rain forest and acts as 'community organizer', which explores and dominates the richest resources in these communities (Sazima, Buzato & Sazima 1995). The prevalence of this high-demanding and long-billed hermit hummingbird in lowland communities may favour





**Fig. 4.** Negative relationship between pollen limitation and flowering synchrony evaluated with phylogenetic generalized least-squares regression. Statistical details are provided in Table 2.

**Table 2.** Effect of morphological overlap (MO) and flowering synchrony (FS) on pollen limitation. The data were analysed using phylogenetic generalized least-squares regression, and the best model (indicated in bold) was selected as the one with lowest Akaike information criteria (AIC) and Bayesian information criteria (BIC). Information on degrees of freedom (d.f.) is also provided

Predictors' variables	Multiple $R^2$ (Adjusted $R^2$ )	d.f.	AIC	BIC
<b>FS</b>	<b>0.211 (0.175)</b>	<b>17</b>	<b>15.9</b>	<b>17.8</b>
FS + MO	0.226 (0.129)	16	17.7	20.6
FS $\times$ MO	0.232 (0.079)	15	19.6	23.4
MO	0.003 (−0.056)	17	20.6	22.5
Null model			18.6	19.6

plant lineages with high resource availability (e.g. floral display and nectar production) in detriment of low-rewarding groups. Thus, the dominance of the saw-billed hermit in the lowland forests may result in a biotic filter (*sensu* Chalcoff, Aizen & Ezcurra 2012), an equivalent of environmental filtering that would favour species from lineages with nectar-rich flowers in the community assembly, explaining the phylogenetic clustering that we found in two lowland plant communities. Indeed, the two lowland plant communities where clustering was detected have many species (65.7% of the lowland pool of species) from large and highly diverse clades as the monocots in the Atlantic rain forest. Several species in this clade tend to occur together in lowland rain forest communities (e.g. species of Poales and Zingiberales), leading to a trend of treewide phylogenetic clustering of co-occurring species in those communities. Similarly, species from species-rich families (e.g. Bromeliaceae and Heliconiaceae) commonly occurred together in this habitat (Buzato, Sazima & Sazima 2000). Moreover, higher nectar production is common in lineages of Zingiberales (e.g. Heliconiaceae and Costaceae, Ornelas *et al.* 2007) that are more representative in lowland communities, in contrast to poor-nectar production recorded in Lamiales (e.g. Acanthaceae, Bignoniaceae, Gesneriaceae,

Lamiaceae and Scrophulariaceae, Ornelas *et al.* 2007), which are more represented in the montane communities. Therefore, our data suggest that filtering driven by a high resource-demanding species may be a main ecological process driving the assembly in these lowland plant communities. Finally, we have no initial prediction (Fig. 1) that can be related with the combination of non-conserved trait and clustered phylogenetic pattern that was found for two of three lowland plant communities. However, this pattern is consistent with evolutionary change in traits associated with niche occupancy and adaptive radiation (Emerson & Gillespie 2008), which corresponds to classic ideas about evolution of ornithophilous plants in lowland Neotropical forests (see Kay *et al.* 2005) as well as the occurrence of many closely related species of monocots in the Atlantic rain forest.

In the montane Atlantic rain forest assemblages, there is less species bearing high-resource flowers, leading to higher overlapping in resource use by hummingbirds than in lowland communities (i.e. more generalized interactions, Sazima, Buzato & Sazima 1995, 1996; Buzato, Sazima & Sazima 2000). When combined with the evidence of non-conserved floral trait and even phylogenetic pattern (likely due to the high frequency of species from families that are widely scattered across phylogeny, that is Alstroemeriaceae, Lamiaceae and Malvaceae), such higher overlap in resource use supports the idea that environmental filtering (e.g. the capability to persist under montane climatic conditions) or facilitation (e.g. a particular set of common ornithophilous traits that maximize facilitative interactions) is more critical for the community assembly than competitive exclusion in the montane Atlantic rain forest (after Sargent & Ackerly 2008). Shifts in species composition due to environmental changes with increasing altitude are well known for several taxonomic and functional groups in the Neotropics as a whole (Antonelli *et al.* 2009; Graham *et al.* 2009; Antonelli & Sanmartín 2011; Maglianesi *et al.* 2014) as well as for Atlantic rain forest communities (e.g. Oliveira-Filho & Fontes 2000; Menini Neto, Forzza & Zappi 2009). In any case, it is more likely that facilitative interactions and stable coexistence among species explain such higher overlap in resource use by hummingbirds in those montane habitats. Because functional structure of the traits measured in this study did not contribute to clarify this hypothesis, we could be missing other relevant traits for interactions with hummingbirds, notably nectar volume and concentration, which are recognized to influence hummingbird–plant interactions (Temeles & Kress 2003; Dalsgaard *et al.* 2009).

Similar to plant communities, we gathered evidence that different ecological processes may structure the assembly of hummingbird communities even in the same habitat depending on the phylogenetic pattern. This differs from the divergence of ecological processes structuring lowland and highland hummingbird communities in the Andes (Graham *et al.* 2009), but it conforms to the predominance of low proportion of smaller ranged hummingbird species and high variation on degree of ecological specialization in the communities of the Atlantic rain forest (Sonne *et al.* 2016). A possible explanation for the occurrence of distinct ecological

processes is that montane climate conditions in this biome are not harsh enough to restrict the establishment of hummingbird species. Thus, it is likely that, similarly to what was suggested for the montane plant communities, facilitation and competition also play a role in these communities. Hence, facilitation and competition seem to be the proximate processes structuring these plant–pollinator assemblages of the Atlantic rain forest, which highlights the role of biotic interactions and ecological interactions on community assembly. Future studies should incorporate other community parameters (e.g. species richness and abundance) and plant–pollinator interactions aspects (e.g. network specialization) to understand what explains relative importance of each of these processes on community assembly.

#### PREDOMINANCE OF RANDOM FUNCTIONAL STRUCTURE OVER TIME

We got limited support for our hypothesis of competitive interactions shaping flowering phenology among plant species. Random and overlap flowering patterns were widely predominant as flowering segregation was detected only in 1 year for one montane community (Itatiaia). Moreover, the temporal distribution pattern of reproductive traits (effective corolla length and stigma length) in this montane community was randomly dispersed rather than evenly over time. Following the principle of limiting similarity (MacArthur & Levins 1967), if floral traits are phylogenetically conserved, synchronous flowering plants pollinated by the same functional group may lead to diversification of floral traits (Sargent & Ackerly 2008; Pellissier, Alvarez & Guisan 2012) to avoid interspecific pollen transfer (Mitchell *et al.* 2009). However, there was no phylogenetic signal for floral traits in roughly 95% of the temporal sets of species analysed in this community. Therefore, against our initial expectations, these results suggest the lack of a predominant ecological process, that is competition or facilitation, as determinants of flowering and floral trait distribution over time. However, it is also possible that the apparently random flowering and floral trait distribution patterns result from a balance between competitive and facilitative interactions, an underlying problem of assembly approaches (Purves & Pacala 2005). Indeed, while some studies assume that either competition or facilitation is the main pattern among co-flowering plants in a given community (e.g. Gumbert, Kunze & Chittka 1999; Feldman, Morris & Wilson 2004), in reality several studies show that within a given plant community there is a mix of competition and facilitative effects associated with pollination (Bartomeus, Vilà & Santamaría 2008; Vilà *et al.* 2009). Such a balance between positive and negative effects has been detected in other communities (Hegland, Grytnes & Totland 2009; Hegland & Totland 2012). This more complex organization is particularly expected in tropical communities, which typically have several hummingbird species differing in their morphology and foraging behaviour and dozens of ornithophilous species with varying nectar production and accessibility to floral visitors (Kodric-Brown *et al.* 1984).

#### FACILITATION IS EVIDENCED BY HIGHER FITNESS IN SYNCHRONY PLANT SPECIES

As expected, flowering synchrony (i.e. a measure of quantitative flowering overlap among species), explained differences in fitness (i.e. fecundity) among the ornithophilous species in the Itatiaia montane community. The fact that pollen limitation decreased with increased flowering synchrony among species is consistent with predictions of facilitative effects, and contrary to our expectations of interspecific competition (after Aizen & Vázquez 2006; Aizen & Rovere 2010). Such findings are in accordance with phylogenetic structure results (hypothesis 1), which also support the idea of facilitative processes in montane communities as a whole.

The influence of phenology is highly context-dependent and reproductive advantage due to synchronous flowering greatly varies across species (Munguía-Rosas *et al.* 2011). While in some cases, plant species that are less attractive to pollinators may benefit from co-flowering with highly attractive species (e.g. Lopezaraiza-Mikel *et al.* 2007), in other situations a high value of flowering synchrony can lead to a high deposition of heterospecific pollen, and reduction in fecundity (Morales & Traveset 2008). As we did not find a differential distribution of stigma length among months, and there is high pollinator overlapping for ornithophilous plants, there are at least two explanations for the negative correlation between synchrony and pollen limitation in this community. First, the higher homospecific pollen deposition due to the higher pollinator visitation overpasses the negative effects of pollen mixture on plant fitness (Feinsinger 1987). Indeed, previous studies involving relatively generalized plant–pollinator relationships showed improved rates of shared-pollinator visitation in neighbouring species due to the presence of very attractive plants (Moeller 2004; Hegland, Grytnes & Totland 2009; Carvalheiro *et al.* 2014), that may alleviate pollen limitation (Ashman *et al.* 2004; but see Hegland & Totland 2008). Alternatively, heterospecific pollen deposition may have positive effects, as illustrated by increased outcrossing in *Mimulus guttatus* (Arceo-Gómez & Ashman 2014). Most hummingbird-pollinated plants are self-compatible (Wolowski *et al.* 2013b), and such a positive effect would happen in the case that for several species in the Itatiaia community, self-pollen is less competitive than outcross-pollen with higher heterospecific pollen deposition (see Ashman & Arceo-Gómez 2013).

In contrast to phenology, morphological overlap among co-flowering species did not predict pollen limitation in the Itatiaia community. Overall, these results may indicate that plant–hummingbird interactions in this community are more regulated by population dynamics' features that determine the availability of resource to hummingbirds (e.g. local abundance and flowering synchrony with the interspecific neighbourhood), than by specific floral traits subjected to phenotypic selection mediated by pollinators, such as floral dimensions. A similar idea was previously suggested for the community organization of hummingbird-pollinated plants in Central America rain forests (Feinsinger 1987). Thus, floral

morphology of ornithophilous plants does not impose strong restrictions to visits by different hummingbirds, excepting in the cases of mismatch between the shape and size of corollas and hummingbirds' bill (Maglianesi *et al.* 2014; Vizentin-Bugoni, Maruyama & Sazima 2014).

As reproductive phenology responds to shifts in temperature and moisture (e.g. Sherry *et al.* 2007), the effects of phenology role in community assembly here presented are crucial to predict how communities will respond to disturbances and global climate changes. Moreover, our results highlight that flowering intensity (i.e. flower availability), a variable that was included in our measure of synchrony, may be more critical to plant fitness than overall flowering time length, although studies on climate regulation of reproductive phenology concentrate on the latter (see Freitas & Bolmgren 2008; Pires, Silva & Freitas 2014).

## Conclusions

In this study, we provide a comprehensive test to predict the assembly of tropical communities based on hypotheses and analyses that considered phylogenetic structure patterns in concert with trait-based and fitness-based patterns of plant–hummingbird interactions at the community level. Our results provide evidence of lack of phylogenetic signal in nectar accessibility (i.e. effective corolla length) in the plant species pool, an underlying assumption of pollination syndromes (Fenster *et al.* 2004). Furthermore, the main ecological processes driving hummingbird-pollinated plant assemblages seem to differ among habitats that occur at different elevations: environmental filtering or facilitation in montane communities, while for lowland communities, biotic interactions mediated by a large rich-resource requiring hummingbird species and adaptive radiations are the most likely driving processes. Such substantial spatial and temporal variation among ornithophilous plant assemblages is expected in complex systems such as tropical forests. Overall, phylogenetic, functional and plant fitness data indicated that facilitation of pollination among co-flowering plants is a more important process than competition during community assembly. Finally, our results suggest that facilitation and competition act in the assembly of hummingbird communities even in the same habitat, indicating that different ecological processes may drive the assembly of the interdependent communities of plants and pollinators.

Further studies are needed to evaluate how generalized are such patterns in hummingbird–plant communities. Our study supports that current perspectives based on approaches that merge phylogenetic and functional ecology are a promising avenue to address the relevance of the plant sexual reproduction and mutualistic interactions in the dynamics of communities, helping predict species assembly processes.

## Authors' contributions

M.W. and L.F. conceived the ideas, designed methodology, collected the data and led the writing of the manuscript. All authors analysed the data, contributed critically to the drafts and gave final approval for publication.

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## Data accessibility

Data of hummingbird and plant species occurrence, functional traits, plant fitness, morphological overlap, flowering synchrony and flowering phenology are deposited in the Dryad Digital Repository <http://doi.org/10.5061/dryad.fn921> (Wolowski, Carvalheiro & Freitas 2016).

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Measures of phylogenetic structure of hummingbird-plant communities.

**Table S2.** Measures of trait-based structure of hummingbird-plant communities.

**Table S3.** Flowering phenology structure of hummingbird-pollinated plant communities.

**Fig. S1.** Floral scheme.

**Fig. S2.** Residuals analysis of the phylogenetic generalized least-squares regression.

**Fig. S3.** Distribution of effective corolla length across the phylogenetic tree of hummingbird-pollinated plants.

**Fig. S4.** Distribution of bill length and body mass across the phylogenetic tree of hummingbirds.

**Fig. S5.** Functional trait-based structure of hummingbird-plant communities.

**Fig. S6.** Floral trait-based structure of temporal hummingbird-pollinated plant communities.