

Beyond neutral and forbidden links: morphological matches and the assembly of mutualistic hawkmoth–plant networks

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Summary

1. A major challenge in evolutionary ecology is to understand how co-evolutionary processes shape patterns of interactions between species at community level. Pollination of flowers with long corolla tubes by long-tongued hawkmoths has been invoked as a showcase model of co-evolution. Recently, optimal foraging models have predicted that there might be a close association between mouthparts' length and the corolla depth of the visited flowers, thus favouring trait convergence and specialization at community level.

2. Here, we assessed whether hawkmoths more frequently pollinate plants with floral tube lengths similar to their proboscis lengths (morphological match hypothesis) against abundance-based processes (neutral hypothesis) and ecological trait mismatches constraints (forbidden links hypothesis), and how these processes structure hawkmoth–plant mutualistic networks from five communities in four biogeographical regions of South America.

3. We found convergence in morphological traits across the five communities and that the distribution of morphological differences between hawkmoths and plants is consistent with expectations under the morphological match hypothesis in three of the five communities. In the two remaining communities, which are ecotones between two distinct biogeographical areas, interactions are better predicted by the neutral hypothesis.

4. Our findings are consistent with the idea that diffuse co-evolution drives the evolution of extremely long proboscises and flower tubes, and highlight the importance of morphological traits, beyond the forbidden links hypothesis, in structuring interactions between mutualistic partners, revealing that the role of niche-based processes can be much more complex than previously known.

Key-words: co-evolution, morphological forbidden link hypothesis, morphological match hypothesis, neutral hypothesis, plant–pollinator networks

Introduction

A major challenge in evolutionary ecology is to understand how co-evolutionary processes shape patterns of interactions in biological communities (Guimarães,

Jordano & Thompson 2011). At the community level, reciprocal selection between free-living species could favour convergence and specialization on a core set of mutualistic traits (Thompson 2005). In the last two decades, different network approaches have generated important insights into the organization of plant–pollinator interactions at the community level, for example the nested and modular organization, the pervasive

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asymmetry in species dependencies and strength of interactions between partners (Bascompte *et al.* 2003; Vázquez & Aizen 2004; Bascompte, Jordano & Olesen 2006; Olesen *et al.* 2007; Vázquez, Chacoff & Cagnolo 2009b; Jordano 2010). A number of factors have been proposed to explain these network patterns, such as species abundance, complementarity in spatio-temporal distribution, phylogenetic relationships and matching of phenotypic traits of interacting species. However, their relative importance continues to be debated (Stang *et al.* 2009; Vázquez *et al.* 2009a; Maruyama *et al.* 2014; Vizentin-Bugoni, Maruyama & Sazima 2014).

Two main hypotheses, related to species abundance and flower–pollinator trait-matching, have been postulated as the main factors modulating the occurrence of plant–pollinator interactions. On the one hand, the ‘neutral hypothesis’ postulates that individuals interact randomly. Hence, network interaction patterns are mainly dependent on species abundances; that is, abundant species interact more frequently and with more species than rare species (Dupont, Hansen & Olesen 2003; Ollerton *et al.* 2003; Vázquez 2005; Vázquez *et al.* 2009a). On the other hand, the ‘forbidden links hypothesis’, also known as ‘barrier models’, postulates that interactions are constrained by morphological plant–pollinator trait-matching and/or phenological coupling between mutualistic partners (Santamaría & Rodríguez-Gironés 2007; Olesen *et al.* 2011; Maruyama *et al.* 2014; Vizentin-Bugoni, Maruyama & Sazima 2014). Specifically for flower–pollinator morphological matching, this hypothesis assumes that interactions occur only if the length of the pollinator’s mouthparts is equal to or greater than flower length (Dupont, Hansen & Olesen 2003; Jordano, Bascompte & Olesen 2003; Vázquez 2005; Stang *et al.* 2009; Vizentin-Bugoni, Maruyama & Sazima 2014). Thus, morphological forbidden links are represented by the impossibility of interactions between pollinator species with short mouthparts and long-flowered plant species. Although the forbidden link hypothesis associated with species morphology has frequently been invoked (Jordano, Bascompte & Olesen 2003), few studies have actually tested whether it plays a more important role than other determinants, for example species abundance, in determining patterns of interaction networks (*e.g.* Stang *et al.* 2009; Maruyama *et al.* 2014; Vizentin-Bugoni, Maruyama & Sazima 2014).

Finally, optimal foraging models assume that for every species there is a trade-off between the suitability of a resource and its availability. These models predict that there is a close association between mouthparts’ length and the corolla depth of the visited flowers, thus favouring trait convergence and specialization at community level. They assume that for every species there is a trade-off between the suitability of a resource and availability, have predicted that there might be a close association between mouthparts’ length and the corolla depth of the visited flowers (Rodríguez-Gironés & Santamaría 2006, 2007; Rodríguez-Gironés & Llandres 2008). According to

this hypothesis, resource competition may trigger the co-evolution of long mouthparts and deep flower tubes depending on the context of a given community. Thus, a given pollinator could be either a poor or an effective pollinator of a particular flower type depending on the distributions of corolla tube depths and mouthpart lengths in the community. Because reciprocal selection acting on mutualistic partners favours convergence and specialization (Thompson 2005; Rodríguez-Gironés & Santamaría 2010), the distribution of characters involved in trait-matching, such as mouthparts and flower lengths, should converge at the community level (see Johnson & Raguso 2016). These patterns have been observed in hawkmoth–plant communities in tropical regions (Agosta & Janzen 2005; Martins & Johnson 2013), but have not been analysed from a network perspective.

In this context, we here evaluate a third (but not mutually exclusive) ‘Morphological match hypothesis’, in which the probability of an interaction depends on the frequency of all possible differences between mouthpart and flower lengths in a given community. Under this hypothesis, hawkmoths, especially those long-tongued species, should preferentially visit flowers similar in length to their proboscises (Agosta & Janzen 2005; Rodríguez-Gironés & Llandres 2008). Previous studies have shown that hawkmoth-pollinated species with extremely long corolla tubes offer larger amounts of nectar than those species with short corolla tubes (Haber & Frankie 1989; Martins & Johnson 2013; Johnson & Raguso 2016). Thus, if flowers were too short, the energetic reward would be insufficient to compensate for the high foraging costs of long-tongued hawkmoths. In addition, by foraging at long-tubed flowers, the long-tongued hawkmoths are released from competition with all other short-tongued flower visitors (Agosta & Janzen 2005). On the other hand, if flowers are too long, a situation arises similar to that under the forbidden links hypothesis; that is, short-tongued pollinators cannot access the nectar and may learn to avoid such flowers (Balkenius, Kelber & Balkenius 2004).

Since the seminal publication of the subject by Darwin (1862), pollination of long flowers by long-tongued nocturnal hawkmoths (Lepidoptera: Sphingidae) has been invoked as a showcase model of co-evolution because this interaction implies reciprocal selection between proboscis and flower tube length for successful pollination (Darwin 1862; Nilsson *et al.* 1987; Arditti *et al.* 2012). However, diffuse community level rather than paired co-evolutionary processes are supposed to drive the evolution of proboscis and flower lengths, because one-to-one interactions are rare in plant–pollinator systems and, when multiple species interact, selection pressures imposed by one species are not independent of the selection pressures imposed by a second species (Nilsson *et al.* 1987; Haber & Frankie 1989; Hougen-Eitzman & Rausher 1994; Agosta & Janzen 2005; Moré *et al.* 2012; Martins & Johnson 2013). Here, we analysed the role of the neutral, forbidden links and morphological match hypotheses in structuring

mutualistic hawkmoth–plant networks. To this end, we collected data from five communities in four South American biogeographical regions including a wide range of proboscis and flower tube lengths. Then, we tested whether the pattern of morphological differences between proboscises and flowers in each community departed from expectations under the neutral, forbidden links or morphological match hypotheses. Evidence supporting the morphological match hypothesis would provide insights into how diffuse co-evolution shapes interaction patterns in specialized plant–pollinator networks.

Materials and methods

STUDY AREA AND HAWKMOTH–PLANT NETWORKS

Field work was carried out in five communities in South America (Fig. S1, Supporting information) corresponding to four different biogeographical regions: Atlantic Rain forest (AF), Cerrado (CE), transition zone between western Chaco woodland and Yungas montane rain forest (CY1 and CY2) and Chaco montane dry woodland (CM). In each community, we determined the hawkmoth assemblage by sampling all the individuals attracted to vertical sheet light traps (480 h in AF, 168 h in CE, 20 h in CY1, 24 h in CY2 and 80 h in CM). Hawkmoth nomenclature and classification follows Kitching (2015). Captured hawkmoths were kept in individual entomological envelopes to avoid pollen contamination among individuals. For general details of the moth collecting method, see Amorim, Wyatt & Sazima (2014) for AF, Amorim *et al.* (2009) for CE and Moré (2008) for CE, CY1 and CY2 communities. Hawkmoths are present throughout the year in the Brazilian communities (see Amorim *et al.* 2009; Amorim, Wyatt & Sazima 2014) while in Argentina they show a marked seasonality flying mostly from October to March (Moré 2008). Thus, we sampled monthly in AF and CE and during spring and summer in CY1, CY2 and CM communities. Sampling effort was greater in Brazilian (AF and CE) than in Argentine (CM, CY1 and CY2) communities because the flowering period extends throughout the year and hawkmoth species richness was higher (Moré, Kitching & Cocucci 2005; Amorim *et al.* 2009). Captured hawkmoths in each community ranged from 321 to 577 individuals with a mean of 388. Rarefaction species and interactions curves were performed using the iNEXT package (Chao *et al.* 2014; Hsieh, Ma & Chao 2015) in R software (R Development Core Team 2014). We carefully inspected captured hawkmoths under a stereo microscope to locate pollen loads and identified pollen types by comparison with reference samples taken from flowering plants in each community (Kislev, Kraviz & Lorch 1972; Haber & Frankie 1989) or with pollen identification guides (Markgraf & D'Antoni 1978). This animal-centred survey allows exposure of an important number of interactions that would be undetected in a plant-centred approach (Bosch *et al.* 2009; Chacoff *et al.* 2012). This method is especially appropriate for our system because it allows for recording of actual hawkmoth visits to a given plant species (Alarcón, Davidowitz & Bronstein 2008). In addition, hawkmoth–plant interactions usually have low frequencies (see Oliveira, Gibbs & Barbosa 2004; Amorim, Wyatt & Sazima 2014) and are difficult to record as they occur at night. Also, hawkmoths are long-distance flying insects with flight capacity of over 15 km (see Amorim, Wyatt & Sazima 2014),

thus making inappropriate the use of independent estimations of plant abundances. Hence, in order to build the quantitative interaction matrices, we assigned to each cell the number of individual hawkmoths carrying pollen from a given plant species. Individuals not carrying pollen were not included in the interaction matrices but were included in trait distribution analyses. We excluded from matrices those pollen types belonging to anemophilous or non-nectariferous species because these pollen grains could become passively attached to hawkmoths' bodies when resting or flying near these plants (Alarcón, Davidowitz & Bronstein 2008). Excluded pollen types represent less than 1% of the total interactions recorded.

FLOWER AND PROBOSCIS LENGTHS

We considered flower tube length as the main constraint determining nectar accessibility to hawkmoths (Haber & Frankie 1989; Martins & Johnson 2013; Amorim, Wyatt & Sazima 2014; Johnson & Raguso 2016). We measured hawkmoth proboscis lengths (HPLs) and effective flower lengths (EFLs) with a digital calliper (0.1 mm accuracy). EFL was measured in ten plants per species when their abundances allowed it. We measured EFL as corolla tube length in tubular and salverform flowers or as stamen length in brush-type and funnel-shape flowers. In the case of unidentified pollen types, we assumed an effective flower length of 15 mm because we previously identified most plant species present in each community with typical moth-pollinated flowers, and all species have flower tube lengths longer than 15 mm (Amorim 2008, 2012; Moré 2008).

DISTRIBUTION OF MORPHOLOGICAL TRAIT-MATCHING IN HAWKMOTH–PLANT NETWORKS

We assumed that co-evolutionary processes favour convergence between proboscis and flower lengths at the community level, so we used the morphological difference between HPL and EFL as a continuous variable to assess trait-matching in hawkmoth–plant networks. We estimated the weighted mean and standard deviation of the observed morphological differences for each community. To estimate these parameters, all possible pairwise differences were weighted by their respective interaction frequency. For example, in the CM community, the morphological difference between *Lintneria maura* (Burmeister, 1879) and *Cestrum parqui* Benth. was 28.91 mm, and there were seven interactions between these species. When an interaction between two species did not occur, the morphological difference was not taken into account for parameter estimation. We followed this approach to maintain the connectance value of each network and to avoid disproportional influence of rare hawkmoth species in the mean value (see Hypothesis testing below).

HYPOTHESES TESTING

For each community, we simulated the distributions of morphological differences under the three hypotheses. These simulations considered all the possible pairwise morphological differences and the expected frequency of interactions under a given hypothesis, that is neutral, forbidden links and morphological match hypotheses (Fig. 1). Under the neutral hypothesis, the individuals of the community interact according to their abundances (Vázquez *et al.* 2007). Thus, the weighted mean and

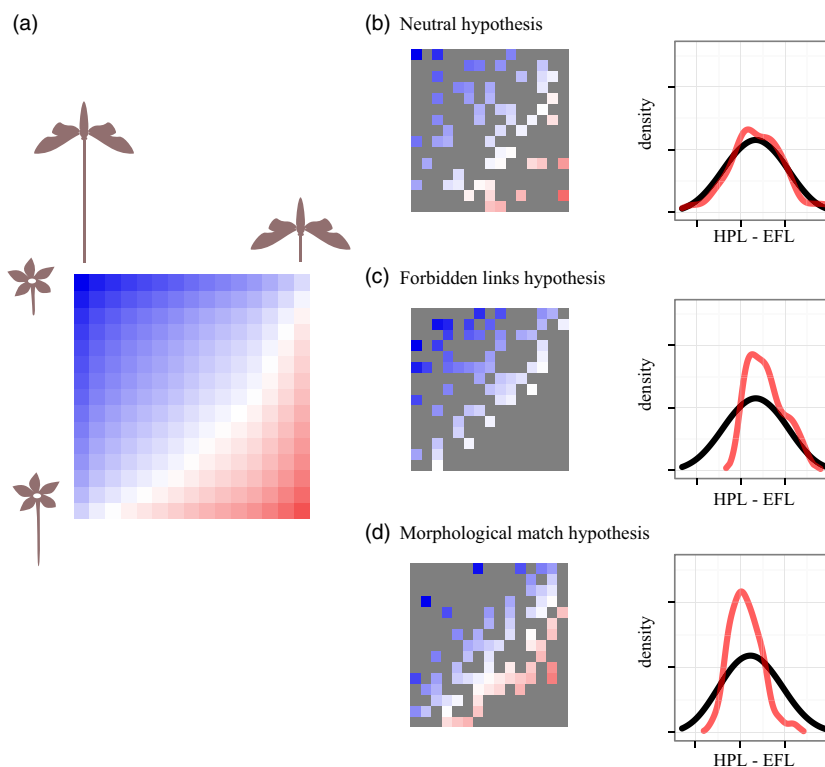


Fig. 1. Possible scenarios of trait-matching as determinant of the species assembly in hawkmoth–plant networks. (a) Idealized matrix representing all possible pairwise differences in trait-matching between hawkmoth proboscis length (HPL) and effective flower length (EFL). Pollinator and plant species are sorted according to their average HPL and EFL, respectively. Blue cells indicate positive differences (HPL > EFL), red cells indicate negative differences (HPL < EFL) and white cells indicate equal lengths. (b–d) Hypotheses concerning the influence of trait-matching on plant–pollinator assemblages. Left panel: simulated interaction matrices under each hypothesis; cell colours correspond to the descriptions above, and grey cells indicate non-existing interactions. Right panel: Distribution of all possible pairwise trait-matching differences (HPL – EFL, black line) and of the predicted combinations (red line) under each hypothesis. (b) Neutral hypothesis, where interactions are independent of trait-matching. Notice that under this hypothesis distribution parameters (mean and standard deviation) must be the same for both distributions. (c) Forbidden links hypothesis, where interactions occurred only if HPL is equal to or greater than EFL. Notice the absence of interactions below the diagonal of the interaction matrix and that distribution parameters differ. (d) Morphological match hypothesis, where the probability of occurrence of an interaction depends on the frequency of possible pairwise differences between HPL and EFL. Notice the absence of interactions both above and below the diagonal and that both distributions have the same mean but differ in standard deviation.

standard deviation of observed differences should not differ from those of all possible pairwise differences, simply because under neutral hypothesis interactions occur at random and their frequency depends on species abundances (Fig. 1b). Under the forbidden links hypothesis (Fig. 1c), hawkmoths interact with flowers only when morphological difference is equal to or greater than zero, that is HPL is equal or longer than EFL (Dupont, Hansen & Olesen 2003; Jordano, Bascompte & Olesen 2003; Stang *et al.* 2009; Maruyama *et al.* 2014; Vizentin-Bugoni, Maruyama & Sazima 2014). Thus, the weighted mean of morphological differences should be greater than the mean of all possible pairwise differences, since the absence of negative morphological differences corresponds to forbidden interactions. Likewise, the standard deviation should be smaller (Fig. 1c). Finally, under the morphological match hypothesis, the weighted mean of morphological differences should be the same as the mean of all possible pairwise differences, but the standard deviation should be smaller, because those interactions involving both extremes, positive or negative morphological differences, should not occur.

Simulations were performed using the original and modified versions of the *vaznull* function of the BIPARTITE v. 2.04 package

(Vázquez *et al.* 2007; Dormann, Gruber & Fründ 2008) in R software (R Development Core Team 2014). To test the neutral hypothesis we used the original *vaznull* function. In the case of the forbidden links hypothesis, we used a modified version of the *vaznull* function (see Data S1) in which we multiplied the binary matrix that assigns interactions according to species abundances by a second matrix in which possible links were assigned a value of one and forbidden links of zero, in order to constrain the probability of occurrence of a particular interaction, so that

$$p_{ij} = \begin{cases} 1 & \text{for } (\text{HPL}_j - \text{EFL}_i) \geq 0 \\ 0 & \text{else,} \end{cases} \quad \text{eqn 1}$$

where p_{ij} is the probability of occurrence of a given interaction, taking into account both proboscis length (HPL) and flower length (EFL).

Finally, in the morphological match hypothesis, we multiplied the binary matrix by an interaction probability matrix. In each cell of this matrix, the probability (p_{ij}) of any given interaction depends on the absolute morphological difference value (HPL–EFL) and the maximum absolute difference such that

$$p_{ij} = 1 - \frac{|\text{HPL}_j - \text{EFL}_i|}{\max |\text{HPL}_j - \text{EFL}_i|}, \quad \text{eqn 2}$$

thus, when the difference attains its maximum value, the probability of occurrence is zero, and when the difference is zero (*i.e.* perfect match), the probability of occurrence is one. In all simulations, the marginal totals and the connectance of the observed matrix were preserved.

Finally, for each community, we tested whether the observed weighted mean and standard deviation of morphological differences lay within the respective 95% confidence intervals obtained from 5000 simulations runs under the three hypotheses.

Results

NETWORK DESCRIPTION AND TRAIT DISTRIBUTIONS

We recorded an average of 23 plant species (ranging from 17 in CY1 up to 34 in AF) pollinated by an average of 23 hawkmoth species (ranging from 8 in CM up to 40 in CE) per community. The more representative

hawkmoth-pollinated plant families were Fabaceae ($n = 9$), Rubiaceae ($n = 8$), Apocynaceae ($n = 6$) and Solanaceae ($n = 6$). A total of 353 interactions were observed in average per community, ranging from 270 in AF to 429 in CM (Tables S1–S3). Rarefaction analyses of light trapped hawkmoths showed a tendency to stabilization indicating an adequate sampling effort. Rarefaction curves of interactions also tended to stabilization in three of the five communities (CM, CY1 and CY2, with values of sample coverage of 0.90, 0.92 and 0.90 respectively), while in the other two more diverse and larger ones (CE and AF), sampled interactions involved 0.70 and 0.56 of sample coverage, respectively (Fig. S2).

Community mean HPL ranged from 43.24 to 52.89 mm, and mean EFL ranged from 31.26 to 35.48 mm (Fig. 2). Mean HPL in each community was consistently greater than the respective EFL. Frequency distributions of HPL and EFL in each community were in general right-skewed, ranging from 0.89 to 1.75 in flower length and from 0.04 to 1.41 in proboscis length (Fig. 2).

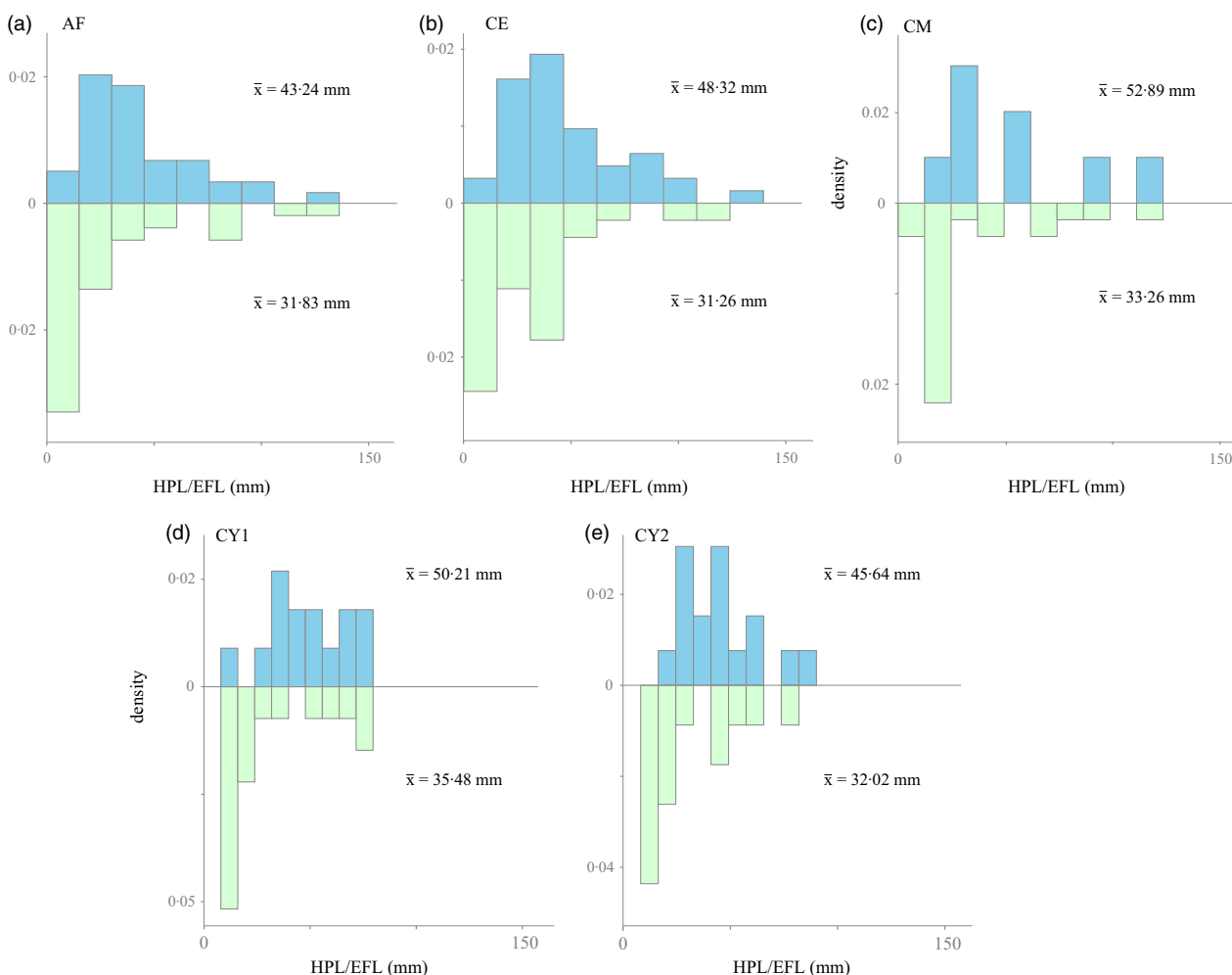


Fig. 2. Trait distributions per community. Hawkmoth proboscis length (HPL) distribution of captured individuals using light traps (upper histograms). Effective flower length (EFL) distributions (lower histograms) of plants species. a) Atlantic Rain forest (AF), b) Cerrado (CE), c) Chaco Montane dry woodland (CM), d) Chaco–Yungas transition 1 (CY1) and e) Chaco–Yungas transition 2 (CY2).

Mean weighted morphological difference per community (\pm SD) were -2.06 ± 34.33 mm in AF, 12.27 ± 33.77 mm in CE, 5.75 ± 32.1 mm in CM, 19.33 ± 26.11 in CY1 and 18.99 ± 25.72 in CY2 (red lines in Fig. 3). Additionally, the distributions of both the observed morphological differences and all possible pairwise differences are shown in Fig. S3.

HYPOTHESIS TESTING

In three communities (CM, CE and AF), the observed values of both mean and standard deviation of morphological differences supported the morphological match hypothesis (Fig. 3a–c, centre and right panels). In those communities the mean difference is low, fitting with the predictions of either the neutral or morphological match hypotheses. Standard deviations were lower than predicted under the neutral hypothesis and interactions involving extreme positive or negative differences rarely occurred.

In the other two communities (CY1 and CY2) both mean and standard deviations were not consistent with the morphological match hypothesis (Fig. 3d–e, centre and right panels). Observed means lay within the predictions of the neutral hypothesis. In community CY2, the observed standard deviations were significantly lower than expected under the neutral hypothesis. Inspection of the observed morphological differences showed that these two communities are characterized by higher frequency of interactions where $HPL > EFL$ (Fig. S3).

Discussion

Co-evolutionary selection is a key process that shapes trait distributions, interaction patterns and even the network structure of free-living mutualists across landscapes (Thompson 2005; Nuismer, Jordano & Bascompte 2012). Here we found right-skewed proboscis and flower length distributions in five communities from four contrasting biogeographical areas in South America. These patterns are similar to those observed in other hawkmoth–plant communities from Costa Rica and Madagascar (Agosta & Janzen 2005). Hence, convergence in morphological traits across landscapes supports the idea that diffuse co-evolution is the process driving the evolution of plant–pollinator traits within communities (Agosta & Janzen 2005; Vázquez 2005; Rodríguez-Gironés & Llandres 2008; Pauw, Stofberg & Waterman 2009). Our findings highlight the importance of morphological traits in structuring plant–pollinator interactions reported for other specialized systems such as plant–hummingbird communities (Maglianesi *et al.* 2014; Maruyama *et al.* 2014; Vizentin-Bugoni, Maruyama & Sazima 2014).

However, beyond the agreement that niche-based processes are important in structuring plant–pollinator networks, we also showed that for three of the five studied communities, the distribution of morphological differences

between plant and pollinator interacting traits is consistent with the expectations under the morphological match hypothesis. These results suggest that hawkmoths, despite being able to access the flowers over a broader spectrum of plants in the communities, may preferentially visit those species in which flowers are similar in length to their proboscises. In these three communities, long-tongued hawkmoths are much less abundant than their short-tongued counterparts (Moré, Sérsic & Cocucci 2007; Amorim *et al.* 2009; Amorim, Wyatt & Sazima 2014) making nectar access in short-tubed flowers less profitable than in long-tubed flowers. On the other hand, the two communities where the interaction patterns were not consistent with the morphological match hypothesis presented similar abundances of long- and short-tongued hawkmoths (Fig. 2), leading to a higher frequency of opportunistic interactions between long-tongued hawkmoths and short-tube flowers (Fig. S3). The high abundance of long-tongued hawkmoth individuals may lead to competition for the relatively scarce long-tubed flowers, and shifts to other nectar sources. In addition, some of the long-tubed flowers in these communities (e.g. *Echinopsis ancistrophora* Speg.) bloom synchronously and thus are available only a few days per season (Schlumpberger *et al.* 2009).

Even though, it is worth mentioning that our animal-centred approach may lead to an underestimation of neutral-based processes, since the nature of our system makes independent sampling of plant abundance unfeasible. However, our findings are consistent with the predictions of optimal foraging models, which propose that resource competition between short- and long-tongued floral visitors leads to resource partitioning and triggers the co-evolution or adjustment between flower and proboscis lengths (Rodríguez-Gironés & Santamaría 2006, 2007; Rodríguez-Gironés & Llandres 2008). Additionally, as long-tongued hawkmoths require higher amounts of energy to sustain the cost of foraging (Heinrich 1983; Agosta & Janzen 2005), long-tubed flowers represent the best foraging choices for long-tongued hawkmoths because they commonly offer larger amounts of nectar and reduce competition from short-tongued counterparts (Haber & Frankie 1989; Martins & Johnson 2013; Johnson & Raguso 2016; F.W. Amorim unpublished results, see also Ornelas *et al.* 2007 for a similar pattern in hummingbird-pollinated plants).

Although we did not test if phenology determined temporal matches among interacting species, resulting in forbidden links (Olesen *et al.* 2011; Maruyama *et al.* 2014; Vizentin-Bugoni, Maruyama & Sazima 2014), our work highlights that hawkmoths more frequently visit plants with floral tube lengths similar to their proboscis in most of the studied communities. In this scenario, our study supports the hypothesis that diffuse co-evolution could be the process driving the adjustment of hawkmoth–plant morphological traits across different environments. Probably, behavioural decisions driven by optimal foraging strategy and other niche-based processes are more impor-

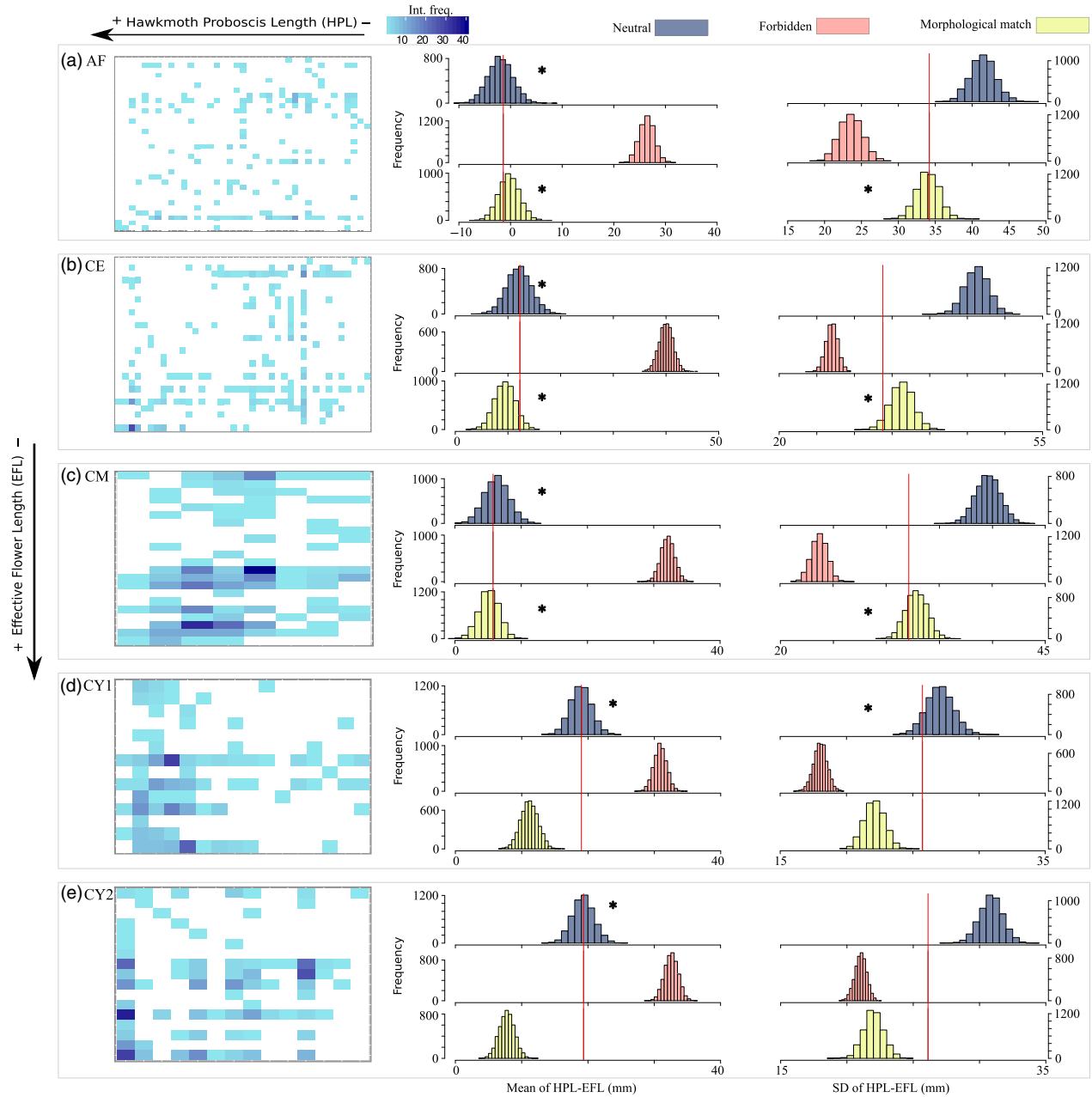


Fig. 3. Observed hawkmoth–plant networks of five communities and parameter simulations for the three hypotheses of interaction occurrence. The five communities are arranged in rows. Columns, from left to right, show the observed interaction networks, and the frequency distributions of mean and SD of trait-matching. In the observed hawkmoth–plant networks, hawkmoth species are sorted in columns from left to right by decreasing HPL. Plant species are sorted in rows, from top to bottom, by increasing EFL. Cell colour intensity corresponds to interaction frequency as indicated in the scale at the top of the figure. The centre and right panels show the frequency distributions of 5000 simulated mismatch means and standard deviations, respectively, under the neutral (blue), forbidden links (pale red) and morphological match (yellow) hypotheses. Vertical red lines correspond to the observed mismatch distribution parameters and asterisk indicate if observed value laid within the predictions of the different hypotheses. Studied communities, a) Atlantic Rain forest (AF), b) Cerrado (CE), c) Chaco Montane dry woodland (CM), d) Chaco–Yungas transition 1 (CY1) and e) Chaco–Yungas transition 2 (CY2).

tant than simple abundance or morphological barriers in defining interactions and network structure. Nevertheless, the approach of morphological match does not exclude other factors, such as phenology, from contributing towards explaining the observed patterns of interactions. Other flower traits such as flower scent, visual signals and

nectar energetic content, may also influence the interaction pattern between mutualistic species (Gómez, Perfectti & Lorite 2015; Larue, Raguso & Junker 2016). Future studies will help to clarify the relative contributions to and importance of these traits for the structure and assembly of hawkmoth–plant communities.

Our results highlight the role that co-evolutionary dynamics may play in shaping trait distribution and interaction patterns in mutualistic systems in different ecological areas of South America. Hawkmoth–plant networks, a system in which interactions are mainly constrained by morphological matches, differ from generalist plant–pollinator networks, where interactions could be affected by multidimensional traits (e.g. flower architecture, flower signals, flower rewards and pollinator behaviour). While we have found that the morphological match hypothesis is not the only mechanism to explain patterns of hawkmoth–plant interactions, additional studies are needed to evaluate whether this hypothesis is applicable to others mutualistic networks, both specialized and generalist.

Acknowledgements

We thank Luciano Cagnolo, Pietro Maruyama, Jeferson Vizentin-Bugoni, Ignasi Bartomeus and two anonymous reviewers for valuable suggestions on previous versions of the manuscript. F.W.A. also thanks the professors and colleagues of São Paulo School on Ecological Networks (funded by FAPESP) for early insights on the discussions presented in the manuscript. F.D.S. doctoral scholarship is funded by the Agencia Nacional de Promoción Científica y Tecnológica (PICT-2011-0837 to Alicia Noemí Sérsic, M.M. and María Cristina Acosta). M.M., S.B.V. and A.A.C. also acknowledge the assistance of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Universidad Nacional de Córdoba, both of which support the research facilities and Agencia Nacional de Promoción Científica y Tecnológica (PICT-2012-1553 to Andrea Cocucci) for funding. This research is part of F.W.A.'s MSc and PhD studies supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Instituto de Biologia of Universidade Federal de Uberlândia, and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Process Number 2007/58666-3) as part of the Thematic Project Functional Gradient (Process Number 03/12595-7), within the BIOTA/FAPESP Program. M.S. acknowledges the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Grant 303084/2011-1).

Data accessibility

Raw data of interaction networks, hawkmoth proboscis lengths and effective flower lengths are available from the Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.qn83r> (Sazatornil *et al.* 2016).

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Received 29 October 2015; accepted 22 February 2016

Handling Editor: Daniel Stouffer

Supporting Information

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

Figure S1. Studied hawkmoth-plant networks.

Figure S2. Rarefactions curves of species and interaction number.

Figure S3. Distribution of morphological differences in the studied communities.

Table S1. Hawkmoth species.

Table S2. Plant species.

Table S3. Summary of the studied networks.

Data S1. R code to perform the simulations.