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# The integration of alien plants in mutualistic plant–hummingbird networks across the Americas: the importance of species traits and insularity

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## ABSTRACT

**Aim** To investigate the role of alien plants in mutualistic plant–hummingbird networks, assessing the importance of species traits, floral abundance and insularity on alien plant integration.

**Location** Mainland and insular Americas.

**Methods** We used species-level network indices to assess the role of alien plants in 21 quantitative plant–hummingbird networks where alien plants occur. We then evaluated whether plant traits, including previous adaptations to bird pollination, and insularity predict these network roles. Additionally, for a subset of networks for which floral abundance data were available, we tested whether this relates to network roles. Finally, we tested the association between hummingbird traits and the probability of interaction with alien plants across the networks.

**Results** Within the 21 networks, we identified 32 alien plant species and 352 native plant species. On average, alien plant species attracted more hummingbird species (i.e. aliens had a higher degree) and had a higher proportion of interactions across their hummingbird visitors than native plants (i.e. aliens had a higher species strength). At the same time, an average alien plant was visited more exclusively by certain hummingbird species (i.e. had a higher level of complementary specialization). Large alien plants and those occurring on islands had more evenly distributed interactions, thereby acting as connectors. Other evaluated plant traits and floral abundance were unimportant predictors of network roles. Short-billed hummingbirds had higher probability of including alien plants in their interactions than long-billed species.

**Main conclusions** Once incorporated into plant–hummingbird networks, alien plants appear strongly integrated and, thus, may have a large influence on network dynamics. Plant traits and floral abundance were generally poor predictors of how well alien species are integrated. Short-billed hummingbirds, often characterized as functionally generalized pollinators, facilitate the integration of alien plants. Our results show that plant–hummingbird networks are open for invasion.

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## Keywords

abundance, exotic plants, generalization, invasion biology, network roles, ornithophily, pollination, specialization.

## INTRODUCTION

Alien species may become invasive and are a major threat to biodiversity and ecosystem functioning, including key ecosystem services such as pollination (Colautti & MacIsaac, 2004; Gurevitch & Padilla, 2004; Pyšek *et al.*, 2004; Morales & Traveset, 2009; Simberloff *et al.*, 2013). The successful establishment of alien plant species might be contingent on the acquisition of mutualistic partners, for example pollinators, outside their native range (Richardson *et al.*, 2000; Bufford & Daehler, 2014; Traveset & Richardson, 2014). Under such a scenario, alien plants may compete for pollinators and decrease the fitness of native plants, for instance, by offering greater quantities of floral rewards and thereby decreasing the attractiveness of native flowers (Chittka & Schürkens, 2001; Morales & Traveset, 2009). Conversely, alien plants could also benefit native plants by increasing the overall availability of floral resources, thereby increasing pollinator abundance and activity on native plants (Bjerknes *et al.*, 2007; Lopezaraiza-Mikel *et al.*, 2007; Bartomeus *et al.*, 2008). Thus, alien plants' ability to establish, and their effect on the pollination of native plants, may depend on their floral traits and the community context (Bjerknes *et al.*, 2007; Morales & Traveset, 2009; Gibson *et al.*, 2012; Simberloff *et al.*, 2013).

In order to understand the potential impacts of alien species on ecosystems, it is therefore important to characterize the community-wide roles of these plants (Davis *et al.*, 2011). One approach to doing this is to use ecological interaction network analyses to conduct community-wide studies identifying and describing the interactions between organisms. Several studies have used such an approach to investigate the role of alien plants in plant–pollinator communities (Memmott & Waser, 2002; Olesen *et al.*, 2002; Aizen *et al.*, 2008; Vilà *et al.*, 2009; Albrecht *et al.*, 2014; Stouffer *et al.*, 2014; Traveset & Richardson, 2014). However, most of these studies have considered either temperate systems, which predominantly consist of functionally generalized insect pollinators (e.g. Aizen *et al.*, 2008; Bartomeus *et al.*, 2008), or focus on generalized island communities where the impact of invasive species might be most severe (e.g. Olesen *et al.*, 2002; Traveset *et al.*, 2013; Traveset & Richardson, 2014; but see Kaiser-Bunbury *et al.*, 2011). As an interaction network's stability may be more sensitive to the integration of alien species in specialized than in generalized systems (Kaiser-Bunbury *et al.*, 2011), studies on specialized systems and over large geographical scales can contribute to our understanding of the general effects of alien species.

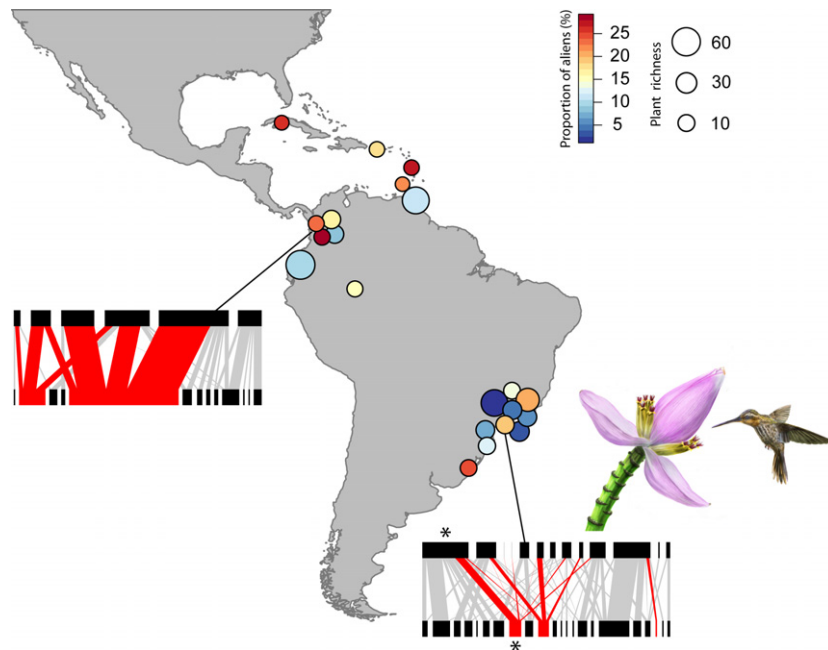
One such potential model system is the interaction networks between plants and hummingbirds across the Americas, which range from relatively specialized to generalized networks and include both mainland and insular environments (Stiles, 1981; Dalsgaard *et al.*, 2011; Martín González *et al.*, 2015). Hummingbirds are the most functionally specialized group of nectar-feeding birds and the most important vertebrate pollinators in the Americas (Stiles, 1981; Bawa, 1990; Cronk & Ojeda, 2008). As specific floral phenotypes are often associated with hummingbird pollination (Cronk & Ojeda, 2008; Ferreira *et al.*, 2016), it could be expected that alien plants lacking a shared evolutionary history with hummingbirds would not be readily incorporated as important species in those networks (Richardson *et al.*, 2000; Aizen *et al.*, 2008). Conversely, Old World plants with convergent adaptations to bird pollination, notably to sunbirds and honeyeaters in Africa and Southeast Asia (Cronk & Ojeda, 2008; Fleming & Muchhala, 2008; Ollerton *et al.*, 2012; Janeček *et al.*, 2015), could be well integrated in novel plant–hummingbird communities in the Americas – at least more than alien plant species not previously pollinated by birds (see Johnson & Raguso, 2016; for examples between specialized flowers and long-tongued hawkmoths).

Given the increasing concerns over the effects of alien species on ecosystems (Davis *et al.*, 2011; Richardson & Ricciardi, 2013; Simberloff *et al.*, 2013), community-wide studies on the role of alien plants across large geographical gradients could provide new insights into their potential threats to biodiversity. Here, we characterize the role of alien plants in 21 quantitative plant–hummingbird networks distributed broadly across the Neotropics, including both mainland and island environments (Fig. 1). We asked three questions: (1) whether an average alien plant is topologically more important than a native species, i.e. whether alien plants have a disproportionate large effect on plant–hummingbird networks; (2) whether alien plant traits, such as pre-adaptation to bird pollination in combination with the geographical setting of the network, i.e. insularity, affect the integration of plants into networks; (3) whether hummingbirds with short bills, often characterized as functionally more generalized species, facilitate the integration of alien plant species into networks.

## METHODS

### Plant–hummingbird networks and alien plants classification

In order to investigate the role of alien plant species in pollination networks, we compiled plant–hummingbird networks



**Figure 1** Distribution of 21 Neotropical plant–hummingbird networks containing alien plant species. Circle size represents the total number of plant species in each network; colours indicate the proportion of alien plants in each network. Note that some points have been slightly moved to avoid overlap. Two network representations illustrate how alien plants are integrated into the networks (top network, Colombian Andes, Snow & Snow, 1980; bottom network, Brazilian Atlantic Rainforest, Maruyama *et al.*, 2015). Top and bottom rectangles denote hummingbirds and plants, respectively. Alien plants and their interactions are marked in red. The illustration depicts one such interaction from the bottom network, between the Saw-billed Hermit *Ramphodon naevius* and the Flowering banana *Musa ornata* originally from Southeast Asia (credit: Pedro Lorenzo).

in which exotic plant species could be confidently identified (Fig. 1). For this, we used an established database on quantitative plant–hummingbird interaction networks (see Dalsgaard *et al.*, 2011 and Martín González *et al.*, 2015 for previous versions of the database, updated details in Tables S1–S3). We only considered legitimate interactions here, in which a hummingbird was observed contacting the reproductive structures of the flowers and with potential for pollination. For each network, plants were classified as either native or alien – taking into account the locality of a given network and the plant distribution range according to openly available databases, notably Tropicos (<http://www.tropicos.org/>), GRIN Taxonomy for Plants for North America (<http://www.ars-grin.gov/>), Flora of the West Indies for the Caribbean (<http://botany.si.edu/antilles/WestIndies/query.cfm>), Brazilian Flora Checklist for networks from Brazil (<http://floradobrasil.jbrj.gov.br/>) and The Plant List (<http://www.theplantlist.org/>). Plant names used here followed The Plant List database. A total of 75 (19%) plant occurrences in the networks were not identified to species level, but to genus or family level only (Table S2); for these, we adopted a conservative approach of only attributing ‘alien’ status if the genus/family at the given locality was identified as alien in the databases. We note, however, that excluding these species did not affect the comparison between native and alien plants. Because the geographical origin of some plants is poorly known, the classification of these can be imprecise

(Pyšek *et al.*, 2004), and the use of a single general database has been argued for in order to standardize possible bias (Stouffer *et al.*, 2014). However, our dataset is composed primarily of networks from the Neotropical region, which has relatively poor historical species records compared to North America and Europe (Pyšek *et al.*, 2004). Because even for well-recorded regions these general databases can fail to successfully classify species (see Stouffer *et al.*, 2014), we preferred to use regional databases, which rely on local plant specialists, for example the Brazilian Flora Checklist. Whenever conflicts among databases appeared, or we were unsure of the classification, we contacted experts with working experience on the flora of the specific region (listed in the Acknowledgements). We refer to the plants considered here solely as alien, because to define these as invasive requires more than distributional information, for example ecological and demographic parameters that we currently lack (Colautti & MacIsaac, 2004). Moreover, all hummingbirds were considered as natives.

### Species-level network metrics

For each plant–hummingbird community, interactions were summarized as a bipartite matrix, with each cell filled with the frequency of the pairwise interaction between a plant and a hummingbird species. The role of each plant species within the networks was described by five distinct species-level

network indices that capture distinct topological properties of a species: (1) the degree of a species ( $k_i$ ) is computed as the number of partners a given species  $i$  is linked to in the network; (2) species strength ( $s_i$ ) is the sum of dependencies across all interaction partners of a given species  $i$ ; dependency is calculated as the proportion of interactions performed by species  $i$  to a specific partner (Bascompte *et al.*, 2006); (3) complementary specialization ( $d'_i$ ) quantifies how interaction frequencies of a given species deviate in relation to the availability of interaction partners in the network, defined by their marginal totals; the higher the value of  $d'$ , the more exclusive are the interactions of the species in relation to the other species in the network (Blüthgen *et al.*, 2006). In addition, we calculated the level of quantitative modularity of each network, i.e. formation of distinct sub-communities within an ecological network, characterized by high within-module prevalence over between-module interactions (Dormann & Strauss, 2014). For each network, we estimated the module conformation using the QUANBiMO algorithm with the number of Markov chain Monte Carlo (MCMC) moves to yield no improvement before the algorithm stops set to  $10^7$  steps (Dormann & Strauss, 2014). From the module conformation with the highest modularity after 20 independent runs for each network (as in Maruyama *et al.*, 2014), we calculated two species-level network indices: (4) between-module connectivity  $c$  and (5) within-module connectivity  $z$ . Whereas  $c_i$  describes how evenly the interactions of species  $i$  are distributed across modules in the network,  $z_i$  quantifies the importance of a given species  $i$  within its module (Dormann & Strauss, 2014). Species-level network indices showed a positive correlation in some cases, indicating that species with high values for a given index tended to also have high values for another index (Table S4). The correlation was especially high between degree and species strength (Pearson's  $r = 0.68$ ; Table S4) and between species strength and within-module connectivity,  $z$  (Pearson's  $r = 0.70$ ; Table S4). However, these species-level network indices complement each other and we therefore used all five indices when comparing alien vs. native plants. In order to compare the five species-level network indices across different networks, we transformed all indices to  $z$ -scores, i.e. indices were standardized within each network by subtracting the mean value of each group (plants or hummingbirds) and dividing the results by its standard deviation (as in Vidal *et al.*, 2014). Calculations of species-level network indices were conducted with the bipartite package (Dormann *et al.*, 2008) in R (R Development Core Team 2016).

### Question 1: Are alien plants topologically more important than native plants in the networks?

To test whether alien plant species differed from native species, we used a null model to contrast the observed difference in means of the species-level network indices between native and alien plants to the differences in the means calculated from randomizations shuffling the alien or native status of

the plants (the proportion of alien/natives was fixed; Vidal *et al.*, 2014). The significance ( $P$ -value) was obtained by dividing the number of times the absolute differences generated from 1,000 randomizations were equal or larger than the observed difference in the means by the number of randomizations (Manly, 1997). Whenever a plant species occurred in more than a single network (74 species, 19.3% of all plants), the average for each of the standardized indices was calculated and used for the null model analysis. We note that with the exception of the degree ( $k$ ) which becomes non-significant, results were qualitatively similar if we consider the instances in which the same species occurred in different networks as distinct samples. Thus, we kept the same approach adopted in Vidal *et al.* (2014). To quantify the magnitude of the difference between native and alien plant species, we calculated Cohen's  $d$  effect size as the standardized mean difference between the indices of each group, i.e. the difference between means divided by the standard deviation of the respective index for all plants (Nakagawa & Cuthill, 2007; Sullivan & Feinn, 2012). For example, an effect size of around 0.5 is considered a medium effect, meaning that an average alien plant species has a higher index value than 69% of the natives (Nakagawa & Cuthill, 2007; Sullivan & Feinn, 2012).

### Question 2: Do plant traits and insularity affect the network roles of alien plants?

For all alien plants identified in the 21 networks, we classified the species according to traits we hypothesized as relevant for their role in the networks. Trait information was gathered from the original sources of the network data (Table S1), as well as by a follow-up literature search using Google Scholar<sup>®</sup> with the species name as the search term (a list of the data sources is found in Data S1). All alien plants were classified according to (1) the size of the plant, which potentially reflects their floral display (i.e. large or small, the former including trees and large herbs such as bananas, and the latter including shrubs, climbers and small herbs); (2) flower type (tubular, brush or other), (3) the length of the floral corolla or equivalent structures restricting the access to pollinator (mm) and (4) whether or not they are bird-pollinated in their native range (Tables S5 & S6). To determine the latter, we used references from the plant–hummingbird network database as well as field-based studies on the floral morphology and pollination biology of the plants, including information on the associated floral visitors and pollinators (Tables S5 & S6). Additionally, we classified whether an alien plant occurred on an island or on mainland communities. As we were only able to evaluate alien plant traits, and not the traits of the native plants, we asked whether particular characteristics of the aliens influence its integration into the networks.

We evaluated how plant traits and insularity are related to plant species-level network indices with linear mixed-effects models (LMM) using the lme4 package (Bates *et al.*, 2014)

in R (R Development Core Team 2014). We used the plant traits (i.e. size, flower type, flower length and previous association to bird pollination) and insularity of the network as fixed factors. Here, we also included the plant family as a fixed factor to, at least partly, account for taxonomic relatedness. Alien plant species identity was included as a random effect to account for non-independence of the observations of the same species in different networks (Bolker *et al.*, 2009; Zuur *et al.*, 2009). We ran models separately for each of the five distinct species-level network indices. The full models included all predictors and were compared to reduced models using the function dredge in R package MuMIn (Barton, 2014), according to their Akaike information criteria (AIC) values, corrected for small sample sizes (AICc – Bolker *et al.*, 2009; Zuur *et al.*, 2009). Models with  $\Delta\text{AICc} \leq 2$  were considered to be equivalent. We also estimated the proportion of variance explained by the fixed factors in the selected best model as marginal  $R^2$ , and the proportion of variance explained by fixed and random factors as conditional  $R^2$  (Nakagawa & Schielzeth, 2013; Barton, 2014). For 12 of the networks (57.1% of the dataset), floral abundance data were available, and thus, we conducted additional analyses evaluating its role on species-level network indices. Following the same procedure to what was done for the entire dataset, we fitted LMMs to evaluate simultaneously the effect of alien plant traits, floral abundance and insularity on the species-level network indices. Here, as for indices, the floral abundance was standardized within each network.

### Question 3: Do hummingbird traits relate to facilitation of alien plant integration?

Finally, we asked whether hummingbird bill length, a functional bird trait associated with flower choice (Dalsgaard *et al.*, 2009; Maglianesi *et al.*, 2014; Maruyama *et al.*, 2014), was related to the probability of hummingbirds including alien plants in their array of interactions. Longer-billed hummingbirds are considered functionally more specialized (Dalsgaard *et al.*, 2009; Maglianesi *et al.*, 2014; Maruyama *et al.*, 2014). For this, we compiled information on hummingbird bill lengths (Table S3) and assessed whether a given hummingbird species interacted with an alien plant across the networks (a list of the data sources is found in Data S1). Then, we fitted a generalized linear model with binomial error distribution containing hummingbird bill length as predictor of the probability that a hummingbird species interacted with alien plant species (Zuur *et al.*, 2009). This analysis was conducted at species level, contrasting each species' bill length to the presence of interaction with alien plants across all the networks in which a given hummingbird species occurred. We also conducted a similar analysis excluding hummingbird species occurring on Caribbean islands where networks are small (Dalsgaard *et al.*, 2009), as well as using the body mass instead of the bill length. As bill length and body mass in hummingbirds show strong phylogenetic signal (Graham *et al.*, 2012), we also included the

hummingbird clades (McGuire *et al.*, 2014) as another fixed factor in these analysis. The models with and without clade identity were compared by an analysis of deviance test and their AIC values (Zuur *et al.*, 2009).

## RESULTS

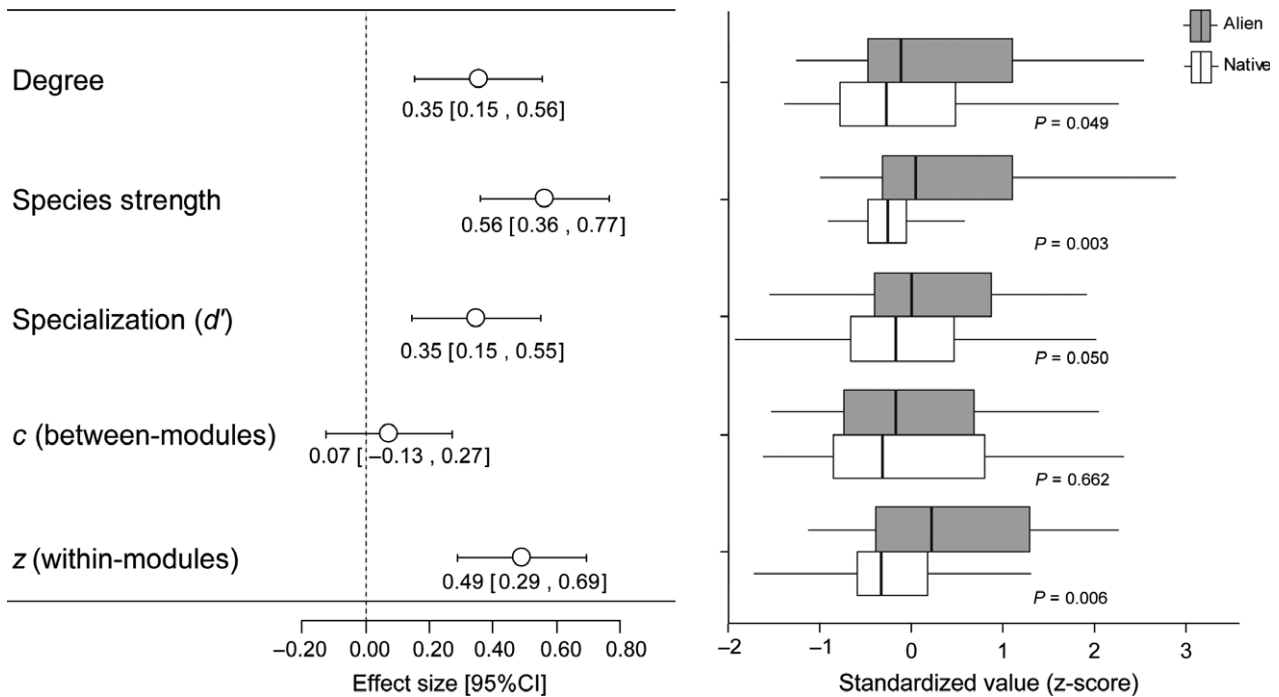
The 21 plant–hummingbird networks included a total of 74 hummingbird and 384 plant species, of which 32 plants were classified as being alien to the networks in which they occurred. Individual networks contained between seven and 65 plant species, with a mean of  $10.8 \pm 8.2\%$  ( $\pm\text{SD}$ ) and up to 28.6% alien plant species (Fig. 1, Table S7). Alien plants belonged to 16 plant families, with Musaceae and Myrtaceae constituting the most frequent families (Tables S5 & S6). Most alien plant species (~63%) had tubular flowers, and about half of them (~47%) had previous association with bird pollinators (Tables S5 & S6). Around 50% of alien species originated from Asia, about 19% originated from Africa and 19% from other regions of the Americas (Table S5).

### Question 1: Are alien plants topologically more important than native plants in the networks?

Overall, alien plant species had higher values of species strength than native species (effect size,  $s$ : Cohen's  $d = 0.56$ ; 95% confidence interval = 0.36–0.77; null model  $P = 0.003$ ; Fig. 2). Likewise, alien plants also had higher values of within-module connectivity ( $z$ : Cohen's  $d = 0.49$ ; 95% CI = 0.29–0.69;  $P = 0.006$ ; Fig. 2). For degree ( $k$ ) and complementary specialization ( $d'$ ), 95% CI of effect sizes did also not overlap zero and null models were significant ( $k$ : Cohen's  $d = 0.35$ ; 95% CI = 0.15–0.56;  $P = 0.049$ ;  $d'$ : Cohen's  $d = 0.35$ , 95% CI = 0.15–0.55;  $P = 0.050$ ; Fig. 2). However, alien plants did not differ from native species in connecting distinct modules ( $c$ : Cohen's  $d = 0.07$ ; 95% CI = –0.12 to 0.27;  $P = 0.662$ ). Hence, an average alien plant is more important for hummingbirds than an average native plant in terms of relative interaction frequency. There is also a tendency for alien plant species to have more partners and for some hummingbird species to interact more exclusively with alien plants than natives.

### Question 2: Do plant traits and insularity affect the network roles of alien plants?

Alien plant traits did not relate to species-level network indices, except for between-module connectivity ( $c$ ), since the model containing only the intercept was always included within the best models (Table S8). For  $c$ , the best two models included insularity and size of the alien plants; the model containing both terms had  $R^2$  marginal = 0.22 and  $R^2$  conditional = 0.33. Specifically, aliens on islands (estimate = 0.35, SE = 0.30) and larger alien plants (estimate = 0.75, SE = 0.27) had higher values for connectivity, that is, were more important for interconnecting modules. Plant family



**Figure 2** Species-level network indices for 352 native and 32 alien plant species across 21 plant–hummingbird networks. On the left, we show the effect sizes (Cohen’s *d*) comparing alien and native plant species for various indices; an effect size is considered significant if the 95% CI of the mean differences does not overlap zero (Nakagawa & Cuthill, 2007). On the right, box plots illustrate the distribution of standardized index values along with their significance, as obtained from null model analysis. With the exception of *c*, both approaches found that an average alien plant has higher network index values than an average native plant.

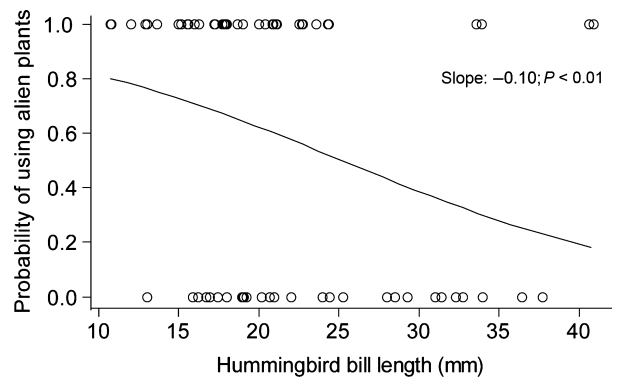
was not included in any of the best models. Considering the subset of networks for which we had floral abundance data, this did not relate to species topological roles in any of the LMMs, as in all cases the intercept-only model was as good as models including floral abundance (Table S9). Importantly, the results of LMMs for this reduced dataset were fairly consistent and we again have that insularity (estimate = 0.68, SE = 0.18) and plant size (estimate = 1.18, SE = 0.36) relate to *c* ( $R^2$  marginal = 0.42 and  $R^2$  conditional = 0.97).

**Question 3: Do hummingbird traits relate to facilitation of alien plant integration?**

We found that short-billed hummingbirds were more likely to interact with alien plants than were long-billed hummingbirds (slope:  $-0.10$ ;  $P < 0.01$ ; Fig. 3). The model including the hummingbird clades did not differ from the one without (deviance = 6.68,  $P > 0.46$ ) and had higher value of AIC ( $\Delta$ AIC = 9.32). Excluding the hummingbird species occurring in the Caribbean islands did not change our results (slope:  $-0.08$ ;  $P = 0.036$ ; Fig. S1), and body mass was found unrelated to the probability of visiting alien plants ( $P = 0.091$ ).

**DISCUSSION**

We have shown that alien plants are strongly integrated into plant–hummingbird networks, playing key roles in the



**Figure 3** Probability of hummingbird species incorporating alien plant species into their interactions in relation to their bill length. Each circle illustrates whether a given hummingbird species incorporates alien plants (1) or not (0). The fitted line reflects the modelled probability of hummingbird species feeding on alien plants, showing that short-billed hummingbirds have a higher probability of feeding on alien plants than do long-billed hummingbird species. We used generalized linear models with a binomial error distribution to assess the significance of the relationships. A Mann–Whitney test likewise shows a significant difference between the bill length of those hummingbirds incorporating and those not incorporating alien plants in their interactions ( $P = 0.004$ ).

networks where they occur. Alien plants have more partners (higher degree) and hummingbirds show higher dependency on them than on an average native plant, both across the

entire network and within their modules. Although we note that the networks contained many more native than alien plant species (352 vs. 32 species, range 2.0–28.6% of alien species), these results suggest that alien plants are important and act as core generalists in these networks (Aizen *et al.*, 2008; Bartomeus *et al.*, 2008; Vilà *et al.*, 2009; Stouffer *et al.*, 2014; Traveset & Richardson, 2014). Moreover, some alien plants may function as private or somewhat exclusive floral resources for some hummingbird species, as revealed by their high degree of complementary specialization (Blüthgen *et al.*, 2006; Stouffer *et al.*, 2014).

The traits we hypothesized *a priori* to determine how alien plants would integrate into the networks showed little importance. For instance, convergent evolution to bird pollination has been suggested as an example of previous adaptation to specific pollinator types aiding the incorporation of aliens to novel plant–pollinator networks (Richardson *et al.*, 2000; Ollerton *et al.*, 2012). However, this pre-adaptation did not apply to network roles of alien plants in plant–hummingbird networks. Hummingbirds may favour specific floral traits (Cronk & Ojeda, 2008; Ferreira *et al.*, 2016), but they may also show opportunism in flower use by legitimately visiting plants that do not obviously conform to the bird pollination syndrome of ornithophily (e.g. Dalsgaard *et al.*, 2009; Maruyama *et al.*, 2013). Due to this opportunism, specialized floral traits may not relate to plant species roles in plant–hummingbird networks (Maruyama *et al.*, 2013). Nevertheless, one possible limitation is the fact that we only considered plant species recorded as visited by hummingbirds. It is possible that other alien plants were present in the studied communities and that these were not visited by hummingbirds. If such non-participating alien species had been considered, plant traits, including the previous adaptation to bird pollination, could have emerged as important for alien integration into the plant–hummingbird web. Likewise, we did not include non-hummingbird pollinators, such as insects, which may overlap with hummingbirds on the phenotypically more generalized plant species (e.g. Dalsgaard *et al.*, 2009; Maruyama *et al.*, 2013); thus, other pollinators may also influence alien plant integration.

It has been suggested that invasive plants, i.e. widespread and abundant alien plants, may become core components of plant–insect pollinator networks due to their high abundance in invaded communities (Lopezaraiza-Mikel *et al.*, 2007; Aizen *et al.*, 2008; Albrecht *et al.*, 2014). However, recent studies have shown that abundance has minor importance in structuring interactions among plants and hummingbirds, in contrast to more generalized insect pollination systems (Maruyama *et al.*, 2014; Vizentin-Bugoni *et al.*, 2014, 2016). In accordance, analyses conducted with the subset of the networks for which we have floral abundance data show that there is no association between floral abundance and their species-level indices. Thus, for plant–hummingbird networks, floral abundance seems a poor predictor of alien topological importance. Instead, we suggest that other plant traits that we lack in our dataset, such as the temporal availability of

alien flowers in relation to native plants (i.e. phenology), or higher nectar secretion rates, could be important for explaining the integration of alien species in these networks (see Chittka & Schürkens, 2001; Godoy *et al.*, 2009).

Although most plant traits evaluated here did not relate to the role of alien plants in the networks, we found that larger alien plants had higher values of between-module connectivity than smaller alien plants. Thus, presumably those alien plants that have bigger floral display distribute their interactions more widely among modules in networks, acting as connectors in these networks. This is important because connectors are suggested to blur the boundaries between modules and affect network dynamics (Albrecht *et al.*, 2014). Alien plants occurring in depauperate island networks were also better connectors than alien plants on the mainland, which indicates that they may have greater potential to affect insular than mainland communities (e.g. Traveset *et al.*, 2013; but see Kaiser-Bunbury *et al.*, 2011).

From the hummingbird perspective, we show that shorter-billed hummingbirds have higher probabilities of incorporating alien plant species in their web of interactions. Although there is variation in this trend, because some longer-billed hummingbirds used alien plants (Fig. 3), this result is consistent to the setting in which longer-billed hummingbirds avoid interacting with more generalized flowers due to competition with shorter-billed hummingbirds (Maglianesi *et al.*, 2015). Studies have suggested that generalist insect pollinators facilitate alien plant establishment, because these often include alien plants in their interactions (Richardson *et al.*, 2000; Memmott & Waser, 2002; Olesen *et al.*, 2002; Lopezaraiza-Mikel *et al.*, 2007; Aizen *et al.*, 2008; Bartomeus *et al.*, 2008; Traveset *et al.*, 2013; Stouffer *et al.*, 2014). In previous studies, however, ‘generalists’ were defined based in their roles in networks, for example the number of partners. Here, we show a link between integration of alien plants and a functional trait of the pollinators, i.e. hummingbird bill length.

## CONCLUSION

Invasive plants are regarded as one of the major current threats to biodiversity. One of the key factors for alien plants to establish in novel ecosystems is their successful integration into mutualistic networks (Richardson *et al.*, 2000; Traveset & Richardson, 2014). Although examples of successful integration of alien species in temperate and insular insect–plant systems are common (e.g. Olesen *et al.*, 2002; Aizen *et al.*, 2008; Bartomeus *et al.*, 2008; Vilà *et al.*, 2009; Stouffer *et al.*, 2014), here we show that alien plants are strongly integrated into the web of interactions even for more specialized tropical pollination systems, such as hummingbird pollination. Further research incorporating complementary data, such as interspecific pollen deposition or the contribution of hummingbirds to alien plant reproduction, are essential next steps to fully assess the impact and integration of alien plants in this system (Richardson

*et al.*, 2000; Lopezaraiza-Mikel *et al.*, 2007; Bufford & Daehler, 2014; Traveset & Richardson, 2014). By acting as core generalist species in the networks, these plants may impact the entire plant–pollinator network (Traveset *et al.*, 2013) and even modify their eco-evolutionary dynamics (Guimarães *et al.*, 2011). In sum, our results here show that plant–hummingbird networks are dynamic and open for invasion, emulating what happens in other plant–pollinator systems.

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## REFERENCES

- Aizen, M.A., Morales, C.L. & Morales, J.M. (2008) Invasive mutualists erode native pollination webs. *PLoS Biology*, **6**, e31.
- Albrecht, M., Padrón, B., Bartomeus, I. & Traveset, A. (2014) Consequences of plant invasions on compartmentalization and species' roles in plant–pollinator networks. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 2014077320140773.
- Bartomeus, I., Vilà, M. & Santamaría, L. (2008) Contrasting effects of invasive plants in plant–pollinator networks. *Oecologia*, **155**, 761–770.
- Barton, K. (2014). *MuMIn: Multi-model inference*. R package version 1.10.5. Available at: <http://CRAN.R-project.org/package=MUMIn> (accessed 30 September 2014).
- Bascompte, J., Jordano, P. & Olesen, J.M. (2006) Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science*, **312**, 431–433.
- Bates, D., Maechler, M., Bolker, B. & Walker, S. (2014) *lme4: Linear mixed-effects model using Eigen and S4*. R package version 1.1–6. Available at: <http://CRAN.R-project.org/package=lme4> (accessed 21 June 2014).
- Bawa, K.S. (1990) Plant–pollinator interactions in tropical rain forests. *Annual Review of Ecology, Evolution, and Systematics*, **21**, 399–422.
- Bjerknes, A.L., Totland, Ø., Hegland, S.J. & Nielsen, A. (2007) Do alien plant invasions really affect pollination success in native species? *Biological Conservation*, **138**, 1–12.
- Blüthgen, N., Menzel, F. & Blüthgen, N. (2006) Measuring specialization in species interaction networks. *BMC Ecology*, **6**, 9.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.S. (2009) Generalize linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution*, **24**, 127–135.
- Bufford, J.L. & Daehler, C.C. (2014) Sterility and lack of pollinator services explain reproductive failure in non-invasive ornamental plants. *Diversity and Distributions*, **20**, 975–985.
- Chittka, L. & Schürkens, S. (2001) Successful invasion of a floral market. *Nature*, **411**, 653.
- Colautti, R.I. & MacIsaac, H.J. (2004) A neutral terminology to define “invasive” species. *Diversity and Distributions*, **10**, 135–141.
- Cronk, Q. & Ojeda, I. (2008) Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, **59**, 715–727.
- Dalsgaard, B., Martín González, A.M., Olesen, J.M., Ollerton, J., Timmermann, A., Andersen, L.H. & Tossas, A.G. (2009) Plant–hummingbird interactions in the West Indies: floral specialisation gradients associated with environment and hummingbird size. *Oecologia*, **159**, 757–766.
- Dalsgaard, B., Magård, E., Fjeldså, J., Martín González, A.M., Rahbek, C., Olesen, J.M., Ollerton, J., Alarcón, R., Araujo, A.C., Cotton, P.A., Lara, C., Machado, C.G., Sazima, I., Sazima, M., Timmermann, A., Watts, S., Sandel, B., Sutherland, W.J. & Svenning, J.C. (2011) Specialization in plant–hummingbird networks is associated with species richness, contemporary precipitation and Quaternary climate-change velocity. *PLoS ONE*, **6**, e25891.
- Davis, M.A., Chew, M.K., Hobbs, R.J., Lugo, A.E., Ewel, J.J., Vermeij, G.J., Brown, J.H., Rosenzweig, M.L., Gardener, M.R., Carroll, S.P., Thompson, K., Pickett, S.T.A., Stromberg, J.C., Del Tredici, P., Suding, K.N., Ehrenfeld, J.G., Grime, J.P., Mascaro, J. & Briggs, J.C. (2011) Don't judge species on their origins. *Nature*, **474**, 153–154.
- Dormann, C.F. & Strauss, R. (2014) A method for detecting modules in quantitative bipartite networks. *Methods in Ecology and Evolution*, **5**, 90–98.
- Dormann, C.F., Gruber, B. & Fründ, J. (2008) Introducing the bipartite package: analysing ecological networks. *R News*, **8**, 8–11.
- Ferreira, C., Maruyama, P.K. & Oliveira, P.E. (2016) Convergence beyond flower morphology? Reproductive biology of hummingbird-pollinated plants in the Brazilian Cerrado. *Plant Biology*, **18**, 316–324.
- Fleming, T.H. & Muchhala, N. (2008) Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of



- vertebrate pollination systems. *Journal of Biogeography*, **35**, 764–780.
- Gibson, M.R., Richardson, D.M. & Pauw, A. (2012) Can floral traits predict an invasive plant's impact on native plant–pollinator communities? *Journal of Ecology*, **100**, 1216–1223.
- Godoy, O., Castro-Díez, P., Valladares, F. & Costa-Tenorio, M. (2009) Different flowering phenology of alien invasive species in Spain: evidence for the use of an empty temporal niche? *Plant Biology*, **11**, 803–811.
- Graham, C.H., Parra, J.L., Tinoco, B.A., Stiles, F.G. & McGuire, J. (2012) Untangling the influence of ecological and evolutionary factors on trait variation across hummingbird assemblages. *Ecology*, **93**, S99–S111.
- Guimarães, P.R. Jr, Jordano, P. & Thompson, J.N. (2011) Evolution and coevolution in mutualistic networks. *Ecology Letters*, **14**, 877–885.
- Gurevitch, J. & Padilla, D.K. (2004) Are invasive species a major cause of extinctions? *Trends in Ecology and Evolution*, **19**, 470–474.
- Janeček, Š., Bartoš, M. & Njabo, K.Y. (2015) Convergent evolution of sunbird pollination systems of *Impatiens* species in tropical Africa and hummingbird systems of the New World. *Biological Journal of the Linnean Society*, **115**, 127–133.
- Johnson, S.D. & Raguso, R.A. (2016) The long-tongued hawkmoth pollinator niche for native and invasive plants in Africa. *Annals of Botany*, **117**, 25–36.
- Kaiser-Bunbury, C.N., Valentin, T., Mougil, J., Matatiken, D. & Ghazoul, J. (2011) The tolerance of island plant–pollinator networks to alien plants. *Journal of Ecology*, **99**, 202–213.
- Lopezaraiza-Mikel, M.E., Hayes, R.B., Whalley, M.R. & Memmott, J. (2007) The impact of an alien plant on a native plant–pollinator network: an experimental approach. *Ecology Letters*, **10**, 539–550.
- Maglianesi, M.A., Blüthgen, N., Böhning-Gaese, K. & Schlenning, M. (2014) Morphological traits determine specialization and resource use in plant–hummingbird networks in the Neotropics. *Ecology*, **95**, 3325–3334.
- Maglianesi, M.A., Böhning-Gaese, K. & Schlenning, M. (2015) Different foraging preferences of hummingbirds on artificial and natural flowers reveal mechanisms structuring plant–pollinator interactions. *Journal of Animal Ecology*, **84**, 655–664.
- Manly, B.F.J. (1997) *Randomization, bootstrap and Monte Carlo methods in biology*, 2nd edn. Chapman & Hall/CRC, London.
- Martín González, A.M., Dalsgaard, B., Nogués-Bravo, D. *et al.* (2015) The macroecology of phylogenetically structured hummingbird–plant networks. *Global Ecology and Biogeography*, **24**, 1212–1224.
- Maruyama, P.K., Oliveira, G.M., Ferreira, C., Dalsgaard, B. & Oliveira, P.E. (2013) Pollination syndromes ignored: importance of non-ornithophilous flowers to Neotropical savanna hummingbirds. *Naturwissenschaften*, **100**, 1061–1068.
- Maruyama, P.K., Vizentin-Bugoni, J., Oliveira, G.M., Oliveira, P.E. & Dalsgaard, B. (2014) Morphological and spatio-temporal mismatches shape a Neotropical savanna plant–hummingbird network. *Biotropica*, **46**, 740–747.
- Maruyama, P.K., Vizentin-Bugoni, J., Dalsgaard, B., Sazima, I. & Sazima, M. (2015) Nectar robbery by a hermit hummingbird: association to floral phenotype and its influence on flowers and network structure. *Oecologia*, **178**, 783–793.
- McGuire, J.A., Witt, C.C., Remsen, J.V. Jr, Corl, A., Rabosky, D.L., Altshuler, D.L. & Dudley, R. (2014) Molecular phylogenetics and the diversification of hummingbirds. *Current Biology*, **24**, 910–916.
- Memmott, J. & Waser, N.M. (2002) Integration of alien plants into a native flower–pollinator visitation web. *Proceedings of the Royal Society B: Biological Sciences*, **269**, 2395–2399.
- Morales, C.L. & Traveset, A. (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecology Letters*, **12**, 716–728.
- Nakagawa, S. & Cuthill, I.C. (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews*, **82**, 591–605.
- Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining  $R^2$  from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, **4**, 133–142.
- Olesen, J.M., Eskildsen, L.I. & Venkatasamy, S. (2002) Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distributions*, **8**, 181–192.
- Ollerton, J., Watts, S., Connerty, S., Lock, J., Parker, L., Wilson, I., Schueller, S., Nattero, J., Cocucci, A.A., Izhaki, I., Geerts, S., Pauw, A. & Stout, J.C. (2012) Pollination ecology of the invasive tree tobacco *Nicotiana glauca*: comparisons across native and non-native ranges. *Journal of Pollination Ecology*, **9**, 85–95.
- Pyšek, P., Richardson, D.M., Rejmánek, M., Webster, G.L., Williamson, M. & Kirschner, J. (2004) Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. *Taxon*, **53**, 131–143.
- R Development Core Team (2016) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.R-project.org> (accessed 04 February 2016).
- Richardson, D.M. & Ricciardi, A. (2013) Misleading criticisms of invasion science: a field guide. *Diversity and Distributions*, **19**, 1461–1467.
- Richardson, D.M., Allsopp, N., D'Antonio, C.M., Milton, S.J. & Rejmánek, M. (2000) Plant invasions—the role of mutualisms. *Biological Reviews*, **75**, 65–93.
- Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E. & Vilà, M. (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology and Evolution*, **28**, 58–66.
- Snow, D.W. & Snow, B.K. (1980) Relationships between hummingbirds and flowers in the Andes of Colombia. *Bulletin of the British Museum of Natural History (Zoology)*, **38**, 105–139.

- Stiles, F.G. (1981) Geographical aspects of bird–flower coevolution, with particular reference to Central America. *Annals of the Missouri Botanical Garden*, **68**, 323–351.
- Stouffer, D.B., Cirtwill, A.R. & Bascompte, J. (2014) How exotic plants integrate into pollination networks. *Journal of Ecology*, **102**, 1442–1450.
- Sullivan, G.M. & Feinn, R. (2012) Using effect size—or why the p value is not enough. *Journal of Graduate Medical Education*, **4**, 279–282.
- Traveset, A. & Richardson, D.M. (2014) Mutualistic interactions and biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, **45**, 89–113.
- Traveset, A., Heleno, R., Chamorro, S., Vargas, P., McMullen, C.K., Castro-Urgal, R., Nogales, M., Herrera, H.W. & Olesen, J.M. (2013) Invaders of pollination networks in the Galapagos Islands: emergence of novel communities. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 20123040.
- Vidal, M.M., Hasui, E., Pizo, M.A., Tamashiro, J.Y., Silva, W.R. & Guimarães, P. Jr (2014) Frugivores at higher risk of extinction are the key elements of a mutualistic network. *Ecology*, **95**, 3440–3447.
- Vilà, M., Bartomeus, I., Dietzsch, A.C., Petanidou, T., Stefan-Dewenter, I., Stout, J.C. & Tscheulin, T. (2009) Invasive plant integration into native plant–pollinator networks across Europe. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 3887–3893.
- Vizentin-Bugoni, J., Maruyama, P.K. & Sazima, M. (2014) Processes entangling interactions in communities: forbidden links are more important than abundance in a hummingbird–plant network. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20132397.
- Vizentin-Bugoni, J., Maruyama, P.K., Debastiani, V.J., Duarte, L.S., Dalsgaard, B. & Sazima, M. (2016) Influences of sampling effort on detected patterns and structuring processes of a Neotropical plant–hummingbird network. *Journal of Animal Ecology*, **85**, 262–272.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009) *Mixed effects models and extensions in ecology with R*. Springer, New York.

## SUPPORTING INFORMATION

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

**Figure S1** Probability of hummingbirds incorporating alien plants into their interactions in relation to their bill length, excluding island networks.

**Table S1** Coordinates, description, location and data references for each studied plant–hummingbird network.

**Table S2** List of plant species found across plant–hummingbird networks.

**Table S3** List of hummingbird species found across plant–hummingbird networks.

**Table S4** Pearson's correlation  $r$  among distinct species-level network indices.

**Table S5** List of the alien plant species found across plant–hummingbird networks.

**Table S6** Details on the assessment of alien plants' pollination system.

**Table S7** Proportion of alien plant species and their interactions across networks.

**Table S8** Model selection results for linear mixed effect models explaining species-level network indices of the alien plant species.

**Table S9** Model selection results for the subset of 12 networks with floral abundance data.

**Data S1.** References: data sources.

## BIOSKETCH

**Pietro K. Maruyama** is an ecologist, especially interested in natural history and plant–animal mutualistic interactions in megadiverse tropical ecosystems, such as the Cerrado and Atlantic Rainforest. This study is part of an ongoing research collaboration on plant–hummingbird networks across the Americas, involving numerous researchers.

Author contributions: P.K.M., J.V.B., J.S., A.M.M.G., M.Sc. and B.D. contributed to the design for the study. P.K.M., J.V.B., J.S., A.M.M.G., A.C.A., A.C.B., J.C., P.C., P.A.C., G.K., C.L., T.M., O.H.M., A.M.R., A.T., I.G.V., T.B.Z., M.Sa. and B.D. provided and/or assembled the data used. P.K.M. analysed the data with advice from M.Sc., J.O. and B.D. and led the writing. All authors critically read the subsequent versions of manuscript.

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