Beyond random and forbidden interactions in plant-pollinator networks : how optimizing energy gain results in morphological matching among subalpine Asteraceae and their flower-visitors

Klumpers, S.G.T.

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Author: Klumpers, Saskia
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2

Interaction patterns among generalized plants species and their flower-visitors: are these interactions less structured?

Saskia G.T. Klumpers, Peter G.L. Klinkhamer & Martina Stang

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Abstract

Plant-flower-visitor networks show general structures, such as asymmetry, nestedness and size-matching between plants and their flower-visitors, which are determined by species abundance and morphological traits. However, interactions among generalized plants, such as the Asteraceae, are assumed to be less structured. Here, we examined the interactions among 16 co-occurring Asteraceae species and their nectar-foraging flower-visitors. We investigated structural features, including network generalization, connectance, asymmetry and nesteness, and analysed how local flower density, nectar tube depth and display area affect both the number of flower-visitor species and visits. In addition, we investigated how size-matching patterns between the nectar tube depth of flowers and the proboscis length of flower-visitors differed among bumblebees, solitary bees, butterflies and flies. Surprisingly, although the Asteraceae were visited by a wide range of flower-visitor taxa, some plant species were extremely specialized. Plant-flower-visitor interactions were highly connected, even and generalized but also asymmetric and nested. Further, the number of flower-visitor species increased with flower head density and display area and decreased with nectar tube depth. In contrast, the number of visits did not increase with flower head density nor nectar tube depth, but only with increasing display area. Interestingly, nectar tube depth and flower head density were negatively correlated and on average, flower-visitor species visited more dense and shallow flowers less frequently. Finally, patterns of size-matching differed among flower-visitor taxa. Overall, our results indicate that even interactions among generalized plant species and their flower-visitors are structured and influenced by plant morphological traits and flower density. Moreover, flower density might not affect plant-flower-visitor interactions by itself but rather through correlation with morphological traits and the frequency distribution of these morphological traits. Consequently, our results highlight the importance of quantitative data analyses, in addition to qualitative data analyses, and trait-abundance correlations to understand plant-flower-visitor network structure.

Key-words: Asteraceae, asymmetry, display area, generalization, nectar tube depth, plant-pollinator community structure, proboscis length, specialization

Introduction

Plants, flower-visitors and their interactions form complex networks in which species differ widely in their generalization degree. While some plant species are visited by numerous flower-visitors, and vice versa, others only have one or a few interaction partners and thus are more specialized. In general, specialized flower-visitors and plants often interact with generalist plants and flower-visitors respectively, resulting in an asymmetric and nested (a core group of generalists interact with each other and a few specialists mostly interact with generalists) community structure (Bascompte *et al.* 2003; Vazquez & Aizen 2004; Bascompte, Jordano & Olesen 2006). Understanding how these structures emerge is important, since they influence species coexistence, community stability (Thebault & Fontaine 2010; Tylianakis *et al.* 2010; Kaiser-Bunbury *et al.* 2017) and ecosystem functions (Blüthgen & Klein 2011; Schleuning, Fründ & Garcia 2015).

Key to understanding plant-pollination network structure is to have insight in the mechanisms that determine why some species are generalized while others are more specialized. It has been suggested that species abundances per se determine their generalization degree and plant-flower-visitor interaction patterns (Dupont, Hansen & Olesen 2003; Vazquez & Aizen 2004; Vazquez *et al.* 2007). This implies that interactions occur based on random encounters among individuals of different species. Consequently, abundant plant- and flower-visitor species will be more generalized as they will interact more frequently and with more species than rare plant- and flower-visitor species. However, other aspects, such as species phenology and traits, may also shape plant-flower-visitor interactions. Species phenology and traits constrain the type, number and strength of interactions between plants and flower-visitors and consequently determine species generalization degree (Stang, Klinkhamer & van der Meijden 2006; Junker *et al.* 2013; Kaiser-Bunbury *et al.* 2014; Renoult *et al.* 2015) and the structure of pollination networks (Rezende, Jordano & Bascompte 2007; Santamaria & Rodriguez-Girones 2007; Stang, Klinkhamer & van der Meijden 2007; Ibanez 2012).

Often phenotypic traits of plants and their flower-visitors correspond. For example, certain type of flower-visitor preference flowers with a particular colour (Renoult *et al.* 2015) or nectar sugar type or -concentration (Baker & Baker 1983). Moreover, the morphology of plants and their flower-visitors often match, which is referred to as size-matching. For example, plants species with flowers with long corollas are often visited by animals with a longer proboscis or bill. This because animals with short proboscis are not able to access the nectar of flowers with long corollas. Consequently, the length of the corolla of flowers and the proboscis (or bill) of flower-visitors are also expected to determine species generalization degree. Plant species with flowers that have short corollas can be visited by a wider variety of flower-visitor species, compared to plant species with flowers that have long corollas. In turn, animals with a long proboscis (or bill) can visit a wide variety of flowers, compared to animals with a short proboscis (or bill) (Agosta & Janzen 2005; Stang, Klinkhamer & van der Meijden 2006; Stang *et al.* 2009; Maglianesi *et al.* 2014). Consequently, morphological constraints also results in an asymmetric and nested community structure (Rezende, Jordano & Bascompte 2007; Santamaria & Rodriguez-Girones 2007; Stang, Klinkhamer & van der

Meijden 2007; Ibanez 2012). Surprisingly, only few studies have indeed shown that corolla tube length and/or proboscis (or bill) length are associated with species generalization degree and mostly among moths (Agosta & Janzen 2005; Johnson *et al.* 2017) and hummingbirds (Maglianesi *et al.* 2014; but see Stang, Klinkhamer & van der Meijden 2006 for a community level study). Moreover, the influence of other morphological traits, such as display area, and their co-variation with flower abundance, on plant-flower-visitor network structure have been rarely considered (but see Junker *et al.* 2013). Display area might constrain the number of flower-visitors, as a smaller display can make it more difficult for large bodied flower-visitors which do not hover, to land on a flower and unfold a long proboscis and therefore to access and extract nectar. Studies that did investigate the effect of display area on the number of flower-visitor species found contrasting results (Hegland & Totland 2005; Stang, Klinkhamer & van der Meijden 2007; Junker *et al.* 2013).

In this study, we used the Asteraceae to investigate how local flower density, nectar tube depth and display area affect the interaction patterns among generalized plants and their flower-visitors. To this end we collected quantitative data on both the number of flower-visitor species and number of flower-visitor visits. The Asteraceae are visited by a wide variety of insects, including bumblebees, solitary bees, butterflies and flies (Graenicher 1909; Lane 1996; Proctor, Yeo & Lack 1996; Torres & Galetto 2002). They have simple, tubular flowers which are aggregated in flower heads (capitula) and easy accessible. We expect that interactions among these plant species and their flower-visitors are more generalized and connected, as flower morphology might not pose strong constraints. Moreover, we expect that these interactions are less structured, specifically less asymmetric and nested, because these structures depend on the presence of specialists plant species, which are mostly plants with complex flowers (Kaiser-Bunbury *et al.* 2014). Furthermore, we investigated how patterns of size-matching differed among flower-visitor taxa, including bumblebees, solitary bees, butterflies and flies, e.g. because these flower-visitor taxa differ in their proboscis length. We asked the following questions: (1) Are interactions among generalized plants and their flower-visitors more generalized and connected and less asymmetric and nested? (2) Are plant generalization degree (both the number of flower-visitor species and Shannon diversity index) and the number of flower-visitor visits related to local flower head density, nectar tube depth and display area? And (3) are plant species with flowers with deeper nectar tubes visited by flower-visitors species which have a longer proboscis and how does this degree of size-matching differ among flower-visitor taxa, including bumblebees, solitary bees, butterflies and flies?

Materials and methods

Plant species and flower-visitor observations

Data were collected in the Colorado Rocky Mountains, USA, in the vicinity of the Rocky Mountain Biological Laboratory, during six weeks in June-August 2011. We included the sixteen most abundant, co-flowering Asteraceae species (Table 1). Plant-flower-visitor interactions were observed in five different meadows, which were approximately 100-200 meters apart. Flower-visitors could move freely between these meadows. The field sites were at approximate 3,000 m elevation, with slopes between 0° and 34°, exposed mostly to the south. Within these meadows, plant-flower-visitor interactions were observed at locations (patches) where the plant species were flowering abundantly. All plant species were observed separately for four hours, by walking through the patch in the meadow were the focal plant species was flowering abundantly. Sampling time was divided into eight sampling intervals of about ½ hour. These sampling intervals were spread over several days, during different times of day (morning and afternoon). All observations took place between 09:00-16:00h at days where there was no strong wind or rain. Flower-visitor species were not caught in order to avoid changing their densities, distributions and behaviour. Instead, the observed flower-visitors were filmed for later identification. These videos were used to determine whether flower-visitors visit flowers for nectar and/or pollen. We only included those flower-visitor species that were foraging for nectar. Flower-visitor species were identified up to species- or morphospecies level. We noted both the flower-visitor species and how many times they visited the plant species (number of visits).

Proboscis length

Proboscis length measurements of solitary bees, butterflies and flies were based on specimens from a reference collection. These specimens were collected in 2009 and 2010, at the same study site as where our study was conducted. Proboscis length of these specimens was measured as described in Stang, Klinkhamer and van der Meijden (2006). For bumblebees, data on the average proboscis lengths were obtained from Macior (1974) (for queens and workers) and Inouye (1976) (for males).

Plant traits

The Asteraceae have relatively small flowers which are aggregated in flower heads (capitula). For most species these heads have two basic types of flowers: tubular, bell-shaped disk flowers in the centre of the head, and strap-shaped ray flowers, in the peripheral, although the heads of some species have only disk or only ray flowers. The studied species belong to the subfamilies Carduoideae (which have only disk flowers) and Asteroideae (which have disk and ray flowers) (Table 1).

We measured the depth of disk flowers since these flowers produce nectar (Mani & Saravanan 1999). The corolla tube of disk flowers consists of two parts: a (very) narrow lower part and an upper wider bell-shaped part. The wider part roughly begins where the stamens insert and ends at the beginning of the corolla lobes, at the base of the deepest cleft in the corolla. It is unlikely that flower-visitors can access the lower part of the corolla tube, since this part is almost filled by the style (Graenicher 1909). Therefore, for each plant species, we measured the depth of the upper wider part of the corolla tube, hereafter referred to as nectar tube (Fig. 1). For each species, we measured the nectar tube depth for at least twenty freshly picked flowers, under a dissecting microscope. Floral display area we defined as the surface area of the flower heads, including the rays for the species that have ray flowers.

In patches where the plants were flowering abundantly and flower-visitors were observed, we counted the number of flower heads per square meter (local flower head density). For most plant species, we calculated the average number of flower heads per square meter, based on ten one square meter plots that were randomly placed within the patch. For *Cirsium sp.2* and *Arnica parryi*, we measured flower head density based on one plot of ten square meter, since these species were flowering at only one location in the meadow.

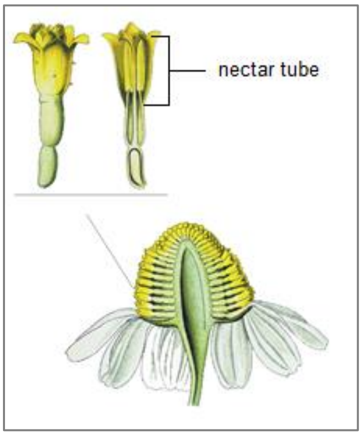

Fig. 1. Morphology of the Asteraceae

Species generalization degree

Species generalization degree was estimated both as the number of interaction partners, e.g. the number of flower-visitor species with which a given plant species interacts or the number of plants species with which a given flower-visitor species interacts, and the Shannon diversity index (Shannon 1948). In contrast to the number of species, the Shannon diversity index takes the number of visits of each flower-visitor species into account. For each plant species, we also calculated the potential number of flower-visitor species, e.g. the number of flower-visitor species that can visit a plant given the nectar tube depth of flowers and proboscis length of the flower-visitors. We assumed that flower-visitors could potentially visit a plant species if their average proboscis length +0.5 mm (natural variation) was equal to or exceeded the nectar tube depth.

Network parameters

We calculated qualitative and quantitative parameters which are indicative of species- and interaction diversity and network generalization and stability. The qualitative parameters included the number of plant species (P), number of flower-visitor species (A), network size (i.e. total number of potential interactions, $S = P\ x\ A$), total number of interactions recorded (I), network and connectance, $C = I/(P\ x\ A)$, which is the realized proportion of all possible interactions (Jordano 1987). Following Bersier, Banasek-Richter and Cattin (2002) we calculated quantitative parameters including weighted plant (lw_p) and flower-visitor (lw_a) linkage, which is the mean diversity of interaction partners across all species within a guild (plants or animals) (hence, the average plant and flower-visitor species partner diversity), quantitative connectance (C_q), which is the connectance weighted by the quantitative visitation rate of each species and interaction evenness (*IE*), which is a measure of the homogeneity of interaction frequencies across all links in the network (Tylianakis, Tscharntke & Lewis 2007). High values reflect more uniform spread of interactions among the species in the communities. In contrast, IE is low if some species and their links dominate the community while most others are rare. Moreover, we calculated nestedness weighted by sample size (*WNODF*; Weighted Nestedness based on Overlap and Decreasing Fill) (Almeida-Neto & Ulrich 2011) and network specialization (H_2') which is a measure of how selective species are in the network by quantifying how the observed interaction frequencies departs from a theoretical random distribution of interactions driven by species abundances (Blüthgen, Menzel & Blüthgen 2006). It characterizes the distribution of interactions relative to each other, namely their mutual exclusiveness. This metric increases in value when species are highly specialized on specific partners (high partitioning). High specialization indicates high dependency of each species on a few exclusive partners. Low specialization indicates higher functional redundancy. All network parameters were computed in R 3.3.2 (R Development Core Team 2014), using the “networklevel” command in the package bipartite (Dormann, Gruber & Fründ 2008).

Statistical analyses

Statistical analyses were performed, using R 3.3.2 (R Development Core Team 2014). Prior to all analyses, we tested whether the variables were normally distributed, using a Shapiro-Wilk test. To achieve normality, flower head density, floral display area and both plant and flower-visitor generalization degree were $\log_{10}$ transformed prior to all analyses. To analyse whether plant generalization degree (both the number of flower-visitor species and Shannon diversity index) and the number of flower-visitor visits were related to local flower head density, nectar tube depth and display area, we used both single- and multiple ordinary least square (OLS) regression analyses, using backward selection of variables. Moreover, we analysed whether plant species with deeper flowers were visited by flower-visitors with a longer proboscis, using OLS regression analyses. For these analyses, for each plants species, we calculated the average proboscis length of their flower-visitors, divided by the four taxonomic groups: bumblebees, solitary bees, butterflies and flies.

Results

Network structure: generalization, nestedness and asymmetry

Table 1 summarizes the floral traits. Nectar tube depth varied between 1.76 (± 0.08) and 6.83 (± 0.07) mm, display area varied between 10.97 (± 0.46) and 64.09 (± 2.48) mm and local flower head density varied between 4.15 (± 0.59) and 83.55 (± 7.95) heads/m^2. Nectar tube depth decreased with increasing flower head density (r=-0.69, P=0.003). Display area was not correlated with nectar tube depth (r=0.36, P=0.17) nor flower head density (r=-0.15, P=0.57).

In total we observed 64 flower-visitor species, mostly bumblebees, solitary bees, butterflies and flies. Some plant species were also visited by ants, beetles, bugs, hummingbirds and wasps (Fig. 2). The sixteen plant species differed greatly in the number of flower-visitor species visiting them, ranging from 2-29 species. The Asteraceae were most frequently visited by bumblebees, as they made up almost 54% of all visits. The proboscis length of the flower-visitor species varied between 1.40 mm and 19.00 mm.

Interaction network parameters are presented in Table 2. There were almost four times more flower-visitor species (64) than plant species in the community (16). In total, we observed 181 interactions which were 18% of all possible interactions (16*64=1024). The connectance weighted by the number of visits (Cq) was 0.09. Plants shared more flower-visitors than flower-visitors shared plants, as was indicated by the higher linkage of plants (lw_p=9.24 and lw_{fv}=5.83). The interactions in the network were nested (WNODF=14.49; Fig. 2) and uniform distributed across species, as was indicated by the relatively high IE (0.67). Overall, the network can be characterized as generalized, because of the relatively low H_2’ (0.35). Plant species that were more generalized were visited by flower-visitor species that were more specialized. Plant species which were visited by more flower-visitor species were visited by flower-visitor species which visited on average less plant species (r=0.6, P=0.003, n=16; Fig. 3a). In addition, plant species which had a higher Shannon index, and thus a higher diversity of interactions, were visited by flower-visitor species which had a lower Shannon diversity index (r=0.72, P=0.001; Fig. 3b). This indicates that interactions between plants and flower-visitors were asymmetric.

Table 1. Nectar tube depth, flower head density and display area of the Asteraceae species studied. The table gives mean ± s.e.m. and sample size (n). Plant species are named according to the accepted names in The Plant List.

Subfamily	Tribe	Plant species	Nectar tube dept (mm)	Flower head density (number heads/m^2)	Display area (mm^2)
Carduoideae	Cardueae	*Cirsium sp.1*	6.31 ± 0.16 (36)	7.40 ± 2.12 (10)	25.76 ± 0.59 (8)
		Cirsium sp.2	4.33 ± 0.07 (39)	9.65 *	20.89 ± 2.55 (8)
Asteroideae	Senecioneae	*Senecio bigelovii*	4.76 ± 0.06 (24)	18.30 ± 2.90 (10)	13.30 ± 0.41 (8)
		Packera werneriifolia	3.29 ± 0.06 (36)	36.30 ± 5.12 (10)	14.94 ± 0.57 (30)
		Senecio integerrimus	4.19 ± 0.08 (36)	20.70 ± 1.89 (10)	22.38 ± 0.65 (21)
	Astereae	*Erigeron flagellaris*	2.17 ± 0.04 (22)	32.90 ± 3.49 (10)	19.58 ± 0.33 (28)
		Erigeron speciosus	2.60 ± 0.04 (20)	83.55 ± 7.95 (10)	30.44 ± 0.54 (32)
		Heterotheca villosa	3.56 ± 0.03 (22)	55.69 ± 14.64 (10)	27.01 ± 1.18 (12)
		Pyrrocoma crocea	5.23 ± 0.05 (37)	10.91 ± 1.07 (10)	61.07 ± 2.77 (11)
		Solidago multiradiata	1.76 ± 0.08 (20)	47.60 ± 19.81 (10)	10.97 ± 0.46 (10)
	Heliantheae	*Arnica parryi*	4.64 ± 0.05 (40)	6.78 *	11.54 ± 0.43 (16)
		Helianthella quinquenervis	4.13 ± 0.05 (32)	9.10 ± 1.58 (10)	64.09 ± 2.48 (24)
		Heliomeris multiflora	2.33 ± 0.03 (22)	28.10 ± 2.77 (10)	35.60 ± 1.50 (19)
		Rudbeckia occidentalis	4.22 ± 0.06 (43)	4.15 ± 0.59 (10)	40.85 ± 1.95 (13)
		Wyethia amplexicaulis	6.83 ± 0.07 (40)	13.6 ± 1.19 (10)	99.00 ± 3.93 (13)
	Helenieae	*Hymenoxys hoopesii*	3.20 ± 0.04 (38)	21.50 ± 3.48 (10)	74.32 ± 4.11 (11)

* Flower head density was measured based on one plot of ten square meters instead of ten plots of one square meter.

Table 2. Qualitative and quantitative network parameters.

Statistics	**Value**
Qualitative parameters	
Number of plant species (P)	16
Number of flower-visitor species (A)	64
Ratio A/P	4
Number of interactions (I)	181
Network size (S)	1024
Connectance (C)	0.18
Quantitative parameters	
Weighted connectance (C_q)	0.09
Interaction evenness (IE)	0.67
Weighted plant linkage (lw_p)	9.24
Weighted flower-visitor linkage (lw_{fv})	5.83
Network specialization (H_2’)	0.35
Weighted nestedness (WNODF)	14.49

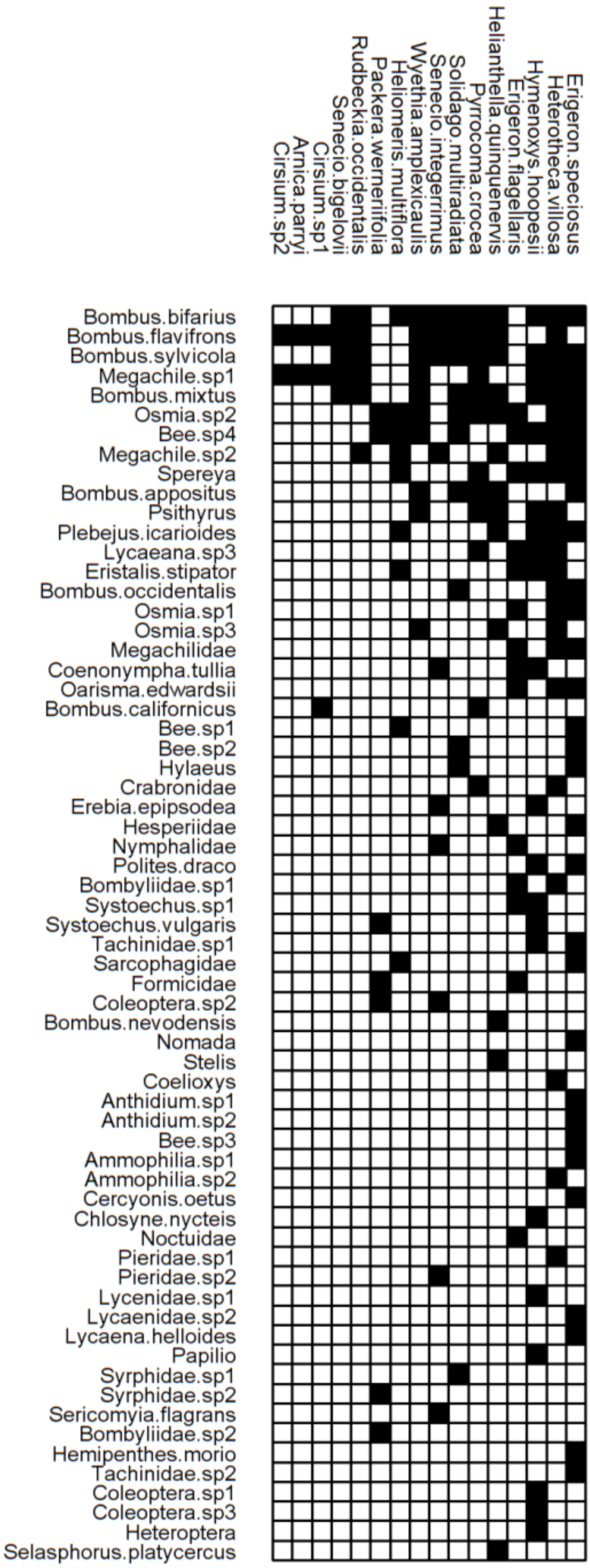

Fig. 2. Qualitative interaction matrix, with the rows representing the plant species, and the columns representing the flower-visitors, identified up to species or morphospecies level.

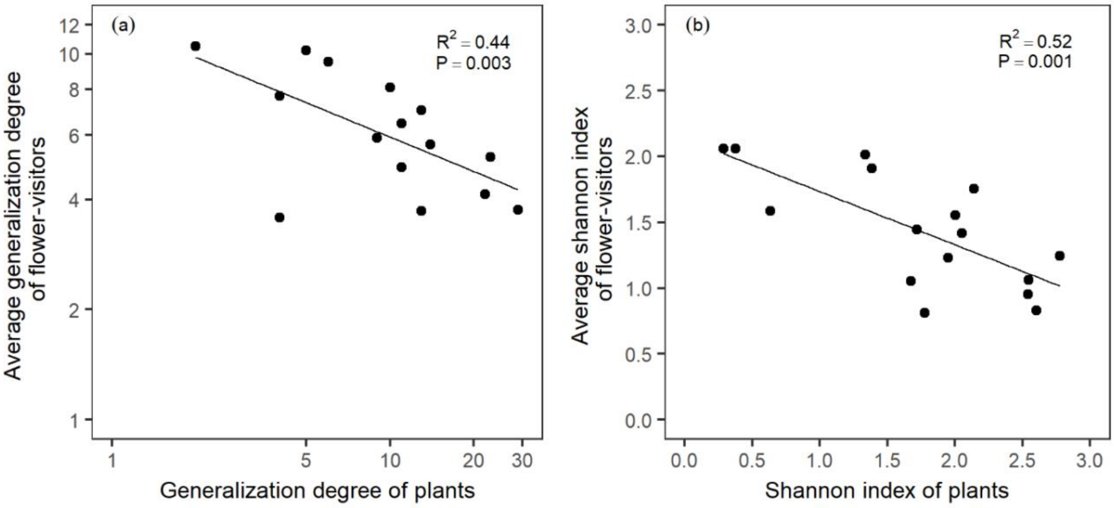

Fig. 3. Generalization degree of the plants in relation to the generalization degree of their flower-visitors, based on (a) the number of interactions and (b) the Shannon diversity index. Each data point corresponds to a single plant species (n=16) and is shown as the mean generalization degree of the flower-visitors observed at that plant.

Floral traits and number of flower-visitors species and -visits

Plant species with a higher flower head density (r=0.61, P=0.006; Fig. 4a), were visited by more flower-visitor species, but did not receive more visits (r=0.20, P=0.23; Fig. 4d). In contrast, plant species with a flowers with larger display were visited by more flower-visitor species (r=0.47 P=0.03; Fig. 4c) and received more visits (r=0.62 P=0.005; Fig. 4f). Plant species with flowers with deeper nectar tubes were visited by fewer species (r=-0.45, P=0.04; Fig. 4b), but did not receive fewer visits (r=-0.25, P=0.17; Fig. 4e). Moreover, the average number of visits per flower-visitor species increased with increasing flower head density (r=0.70, P=0.001) and decreased with increasing nectar tube depth (r=-0.47, P=0.03). The average number of visits per flower-visitor species was not related to the display area of their flower heads (r=-0.06, P=0.83; Fig. 4i). Flower head density, display area and nectar tube depth together explained 82% of the variation in number of flower-visitor species (Table 3).

Nectar tube depth posed a size constraint on the proboscis length of flower-visitors, as interactions occurred mainly between plant- and flower-visitor species that had a proboscis longer than the nectar tube depth of the flowers they visited (Fig. 5). Deeper flowers were visited by a higher proportion of the potential flower-visitor species than shallow flowers, indicated by the steeper slope of the potential number of species compared to observed number of species (b= -8.09, CI= -8.65 - 7.53vs b= -2.27, CI= -5.16 - 0.63; Fig. 6). Plant species were at the most visited by 49% of the potential number of flower-visitor species.

Table 3. Multiple regression with $\log_{10}$ number of observed flower-visitor species as the dependent variable and $\log_{10}$ flower head density (number of flower heads/m^2), nectar tube depth (mm) and $\log_{10}$ display area as independent variables. The explained variance of the presented model is 82% ($R^2=0.82$, $F=18.18$, $DF = 12$, $P<0.001$).

Independent variables	**Standardized coefficient**	**T-value**	**P-value**
Intercept	-0.66	-1.75	0.11
Nectar tube depth	-0.08	-1.96	0.04
Log_{10} flower head density	0.53	3.39	0.003
Log_{10} floral display area	0.85	5.44	<0.001

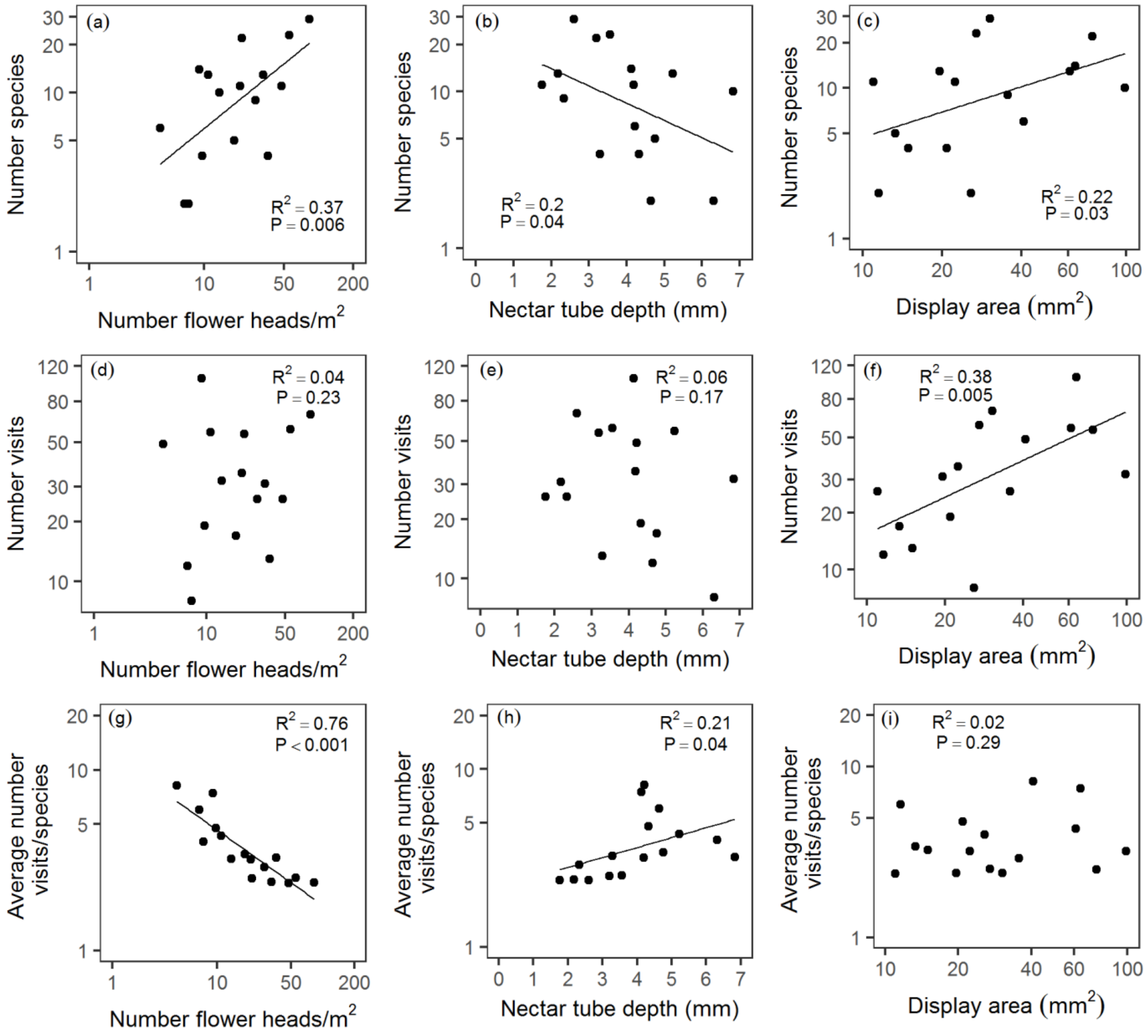

Fig. 4. The observed number of flower-visitor species, number of visits and the average number of visits per flower-visitor species in relation to flower head density (number of flower heads/m^2), nectar tube depth and display area. Each data point corresponds to a single plant species (n=16) and is shown as the total number of flower-visitor species, total number of visits or the mean number of visits of all flower-visitor species observed at that plant species. Number of flower-visitor species, number of visits, flower head density and display area are $\log_{10}$ transformed before statistical analysis to achieve normality. The axes of these variables are therefore on a logarithmical scale.

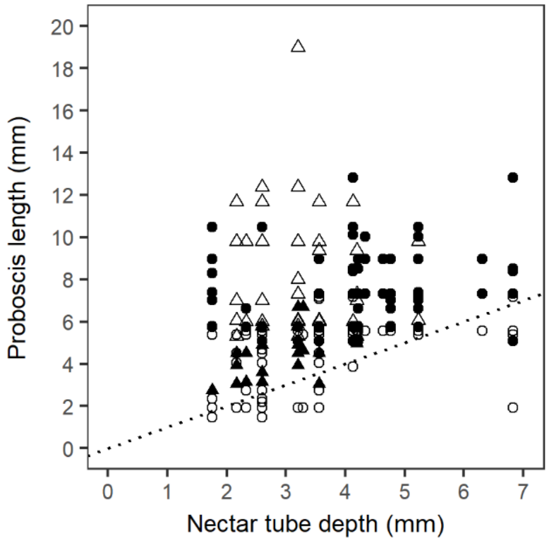

Fig. 5. Observed proboscis lengths in relation to nectar depths of flowers. Each data point represents an interaction between a single plant species and flower-visitor species. Closed circles are bumblebee species, open circles are solitary bee and wasp species, closed triangles are fly species and open triangles are butterfly species. The dotted line (x=y) represents the size threshold, which is the line at which proboscis length equals nectar tube depth. The interactions occur mainly within a triangle above the threshold.

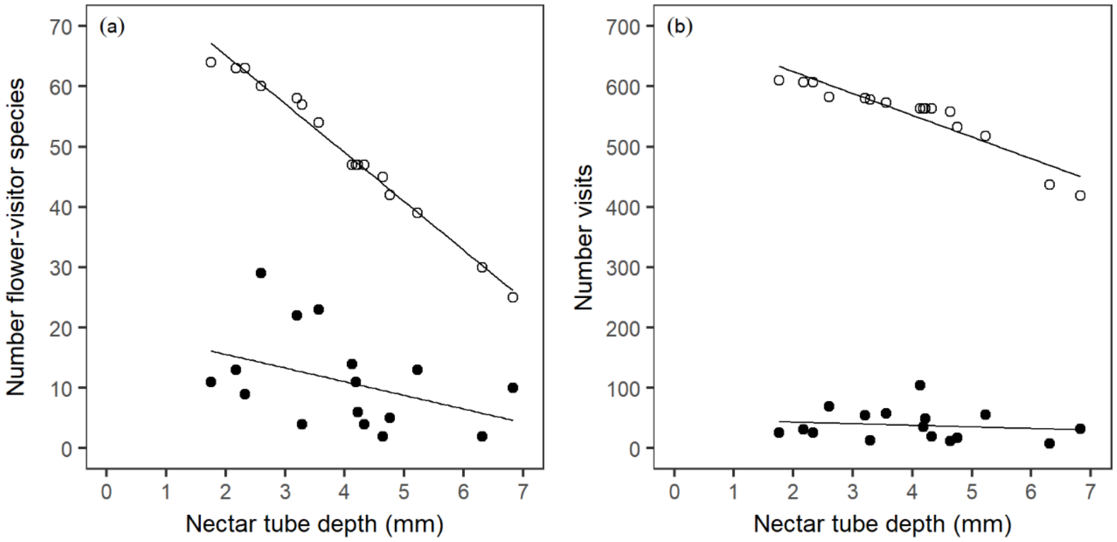

Fig. 6. Observed (filled circles) and potential (open circles) number of (a) flower-visitor species and (b) visits in relation to nectar tube depth of flowers. Each data point corresponds to a single plant species.

Patterns of size-matching

Approximately 60% of the flower-visitor species, which accounted for approximately 80% of the visits, had a relatively long proboscis (>5 mm) (Fig. 7). Plant species with deeper flowers were visited by bumblebees-, solitary bee- and fly species with a longer proboscis, both when accounting for the number of species and the number of visits (Fig. 8a-d,g-h). The degree of size-matching between the nectar tube depth and flower-visitor proboscis length differed among these flower-visitor taxa. In contrast, among butterflies, there was no relationship between nectar tube depth and their average proboscis length (Figs 8e-f). Bumblebees and butterflies had a proboscis long enough to visit all plant species. Therefore, apparently, bumblebee species with a short proboscis avoided flowers with a deeper nectar tube.

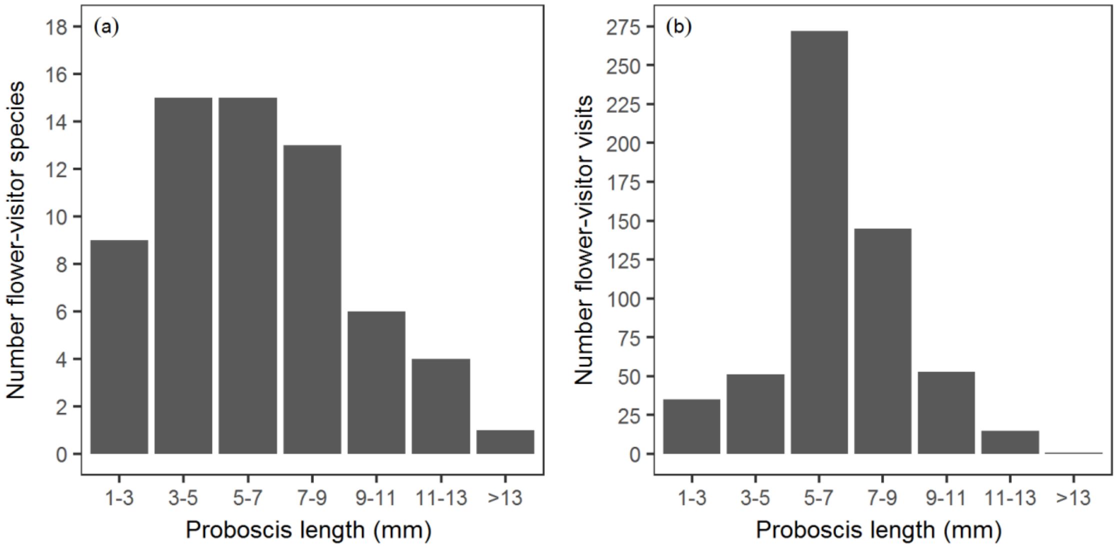

Fig. 7. Distribution of (a) the number of flower-visitor species and (b) the number of visits, based on their proboscis length.

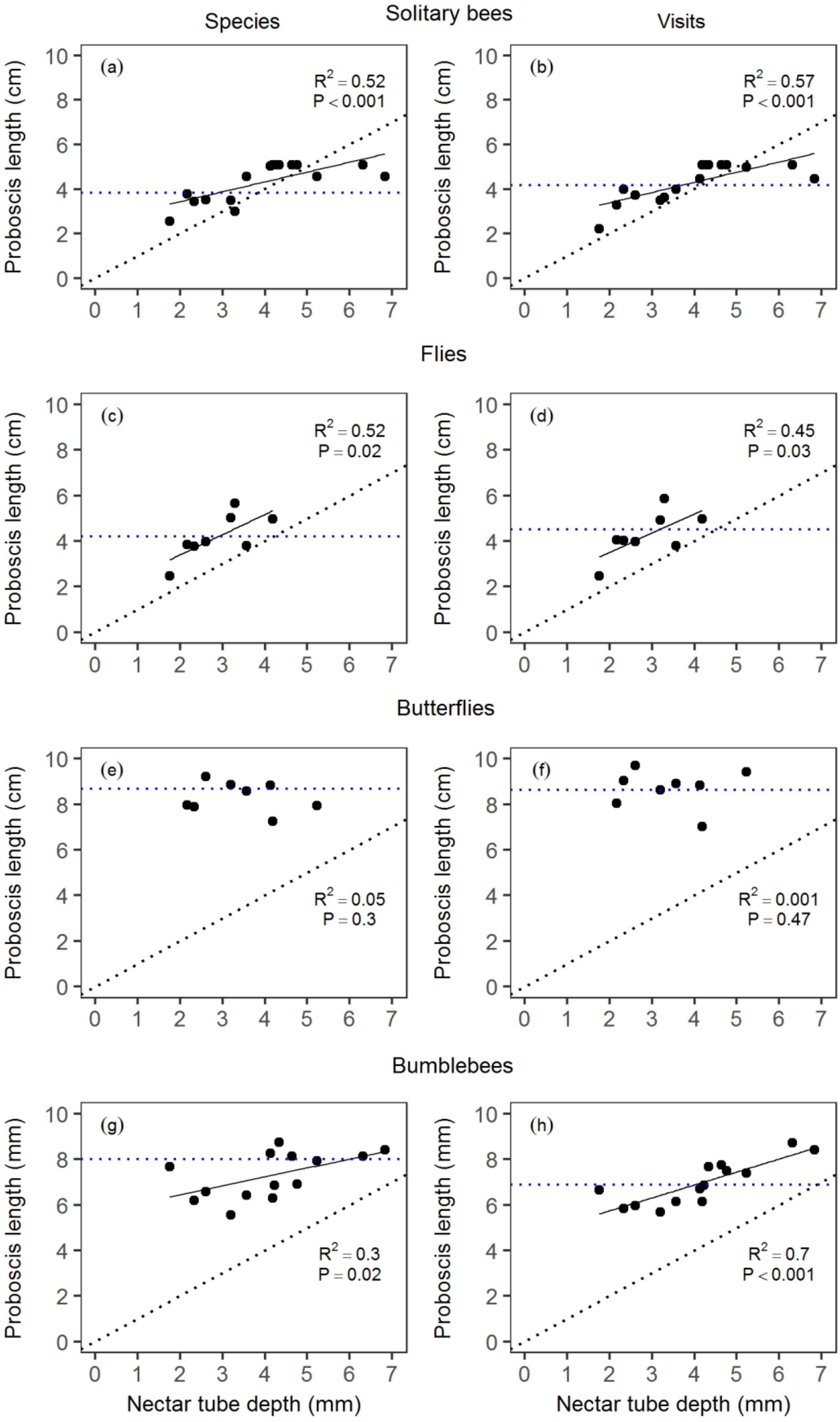

Fig. 8. Observed proboscis lengths in relation to nectar depths of flowers based on the number of (a) species of solitary bees, (b) visits of solitary bees, (c) species of flies, (d) visits of flies, (e) species of butterflies, (f) visits of butterflies, (g) species of bumblebees, and (h) visits of bumblebees. Each data point corresponds to a single plant species (n=16) and is shown as the mean proboscis length of all species observed at that plant. The dotted black line (x=y) represents the expected size threshold and the dotted blue line presents the expected average proboscis length, based on random encounters.

Discussion

Network structure: generalization, nestedness and asymmetry

Although plant species in the Asteraceae family are considered to be generalized, as they are visited by a high diversity of flower-visitor species and taxa (Graenicher 1909; Lane 1996; Proctor, Yeo & Lack 1996), we found that some plant species were highly specialized. We expected the interactions among the Asteraceae and their flower-visitors to be more generalized and connected and less asymmetric and nested, compared to other pollination networks, because the Asteraceae have shallow flowers which are easily accessible. Surprisingly, on average, species of the Asteraceae family were not visited by a higher number of flower-visitor species, compared to other pollination networks (Traveset *et al.* 2016). However, plant-flower-visitor interactions were more even, more connected and more generalized, compared to other pollination networks (Traveset *et al.* 2016). Apparently, these plants share a greater proportion of their flower-visitor species, which indicates plant phylogeny affects flower-visitor visitation patterns (e.g. Rezende, Jordano & Bascompte 2007). Furthermore, in contrast to our expectations, we found comparable levels of asymmetry and nestedness as in complete pollination networks (Traveset *et al.* 2016). This might be explained by the (unexpected) presence of morphological specialized flowers.

Determinants of plant generalization degree

Both nectar tube depth and flower head density determined the number of flower-visitor species by which plants were visited but not the number of visit that plants received. Plant species with a deeper nectar tube had a lower Shannon diversity index and were visited by fewer flower-visitor species, but did not receive fewer visits. On average, flower-visitor species visited plant species with a higher flower head density more frequently. Interestingly, plant species with deeper flowers were also visited by a higher proportion of the potential number of flower-visitor species, based on the floral nectar tube depth and flower-visitor proboscis length. Moreover, plant species with a higher flower head density had a higher Shannon diversity index and were visited by more flower-visitor species, but did not receive more visits per patch. On average, flower-visitor species visited plant species with a higher flower head density less frequently. From this positive relationship between flower density and number of flower-visitor species, which has often been found in plant-flower-visitor networks, it is generally concluded that plant-flower-visitor interactions occur randomly. Also, this relationship has often been explained by the fact that less abundant flower-visitor species have a higher chance of being detected on plant species with a high flower density (Dupont, Hansen & Olesen 2003; Vazquez & Aizen 2004; Vazquez *et al.* 2007) and thus that these results are due to sampling bias. However, our results indicate that plant-flower-visitor interactions do not occur randomly. If plant-flower-visitor interactions would occur at random, then plant species with a higher flower density should also have been visited more frequently. Rather, the flowers of plant species with a higher density of flower heads, were more shallow, and therefore could potentially be visited by more flower-visitor species. This negative correlation between flower head density and nectar tube depth may be an underlying cause for the positive relationship between flower head density and plant generalization degree. Moreover, a relatively high number of flower-visitor species with a short proboscis visited the Asteraceae less frequently and therefore are expected to be less abundant. Due to their short proboscis, they are restricted to plant species with shallow and thus more dense flower heads. This frequency distribution of flower-visitor proboscis length, may also be an underlying cause for the positive relationship between flower head density and plant generalization degree. We rather argue that there is a more ecological explanation for the positive relationship between flower head density and number of flower-visitor species, namely that less abundant flower-visitors may visit abundant plant species more frequently because they provide a more reliable food source (Memmott, Waser & Price 2004).

Although flower depth and animal proboscis- or bill length are generally assumed to influence species generalization (Herrera 1996; Agosta & Janzen 2005; Stang, Klinkhamer & van der Meijden 2006; Stang *et al.* 2009; Maglianesi *et al.* 2014; Johnson *et al.* 2017), this is mostly found among hummingbird and hawkmoth pollinated plants. Torres and Galetto (2002), did not find a significant correlation between nectar tube depth and the number of flower-visitor species among South American Asteraceae species. While Torres and Galetto (2002) measured the whole corolla tube length, we measured the functional length of the nectar tube, which is the part of the corolla tube in which flower-visitors can insert their proboscis. This highlights the importance of measuring the functional size of traits when relating morphological traits to flower-visitor visitation patterns.

In contrast to flower head density and nectar tube depth, both the number of flower-visitor species and visit increased with increasing flower display area. Previous studies have shown that among the Asteraceae, ray flowers play an important role in attracting flower-visitors (Stuessy, Spooner & Evans 1986; Andersson 2008). However, Pohl, Van Wyk and Campbell (2011) have shown that manipulating the size of ray flowers did not affect flower visitation patterns. Therefore, other aspects, e.g. flower-visitor foraging efficiency, may also affect flower-visitor flower selection and consequently plant generalization degree. For example, a larger display might make it easier for insects to land and to unfold a long proboscis and in turn to access and extract nectar. Moreover, flowers with larger displays might offer higher rewards, either because they produce more nectar per flower or have more open flowers per flower head. Especially for flower-visitors with a long proboscis, which have higher energetic requirements (Heinrich 1983), this would make flowers with larger displays more attractive. Consequently, the importance of display area for flower-visitor flower choice may depend on the type of flower-visitors and plants in the community. This may explain why previous studies, which examined different plant-flower-visitor communities, found contrasting results (Hegland & Totland 2005; Stang, Klinkhamer & van der Meijden 2006).

Patterns of size-matching among different flower-visitor taxa

Size-matching between the depth of flowers and the proboscis length of flower-visitors was inconsistent among flower-visitor taxa. Flowers with deeper tubes were, on average, visited by bumblebees, solitary bees and flies with a longer proboscis. We did not find this relationship among butterflies. It is surprising that even among bumblebees we found this pattern, since bumblebees had a proboscis long enough to visit all plant species. Consequently, this pattern suggests that shallow flowers were selectively avoided by species with a long proboscis, while flowers with deeper nectar tubes were selectively avoided by species with a short proboscis. This indicates that not only size constraints play a role in flower-visitor foraging choice. Although flower density might make flowers more attractive, flower head density can neither explain this preference for deep flowers because flowers with deeper tubes were less dense. Rather, patterns of size-matching may results because flower-visitors avoid resource competition (Rodriguez-Girones & Santamaria 2006). Moreover, the preference for deep flowers might also result from increased foraging efficiency of flower-visitors when they forage on flower which match the length of their proboscis, as (1) they can handle these flowers more quickly (Plowright & Plowright 1997) and (2) in some plant-flower-visitor communities, deeper flowers are more rewarding (Galetto & Bernardello 2004; Kaczorowski, Gardener & Holtsford 2005; Fenster *et al.* 2006; Ornelas *et al.* 2007; Gomez *et al.* 2008; Martins & Johnson 2013; Carvalheiro *et al.* 2014). These differences in foraging efficiency might result in competitive niche differentiation between species with longer and shorter proboscises. Like bumblebees, butterflies had a proboscis long enough to visit all plant species studied. Butterflies may select different flowers, based on sugar concentration (Watt, Hoch & Mills 1974; Pyke & Waser 1981) or display area, compared to bumblebees and solitary bees. Overall, incorporating traits which are related to flower-visitor foraging efficiency, such as nectar production, in community studies will be an important next step to better understand plant-flower-visitor network structure.

Further, patterns of size-matching differed among flower-visitor taxa. In accordance with a Spanish community, among flies, the degree of size-matching was independent of the nectar tube depth (Stang *et al.* 2009). However, among bumblebees, there was a closer matching between the nectar tube depth and proboscis length on flowers with deeper nectar tubes, while among solitary bees, there was actually the closest matching among medium-sized flowers. This pattern can be explained by the frequency distributions of flower-visitor proboscis lengths. Bumblebee species had a relatively long proboscis, compared to the depth of the flowers, while solitary bees did not. Moreover, among solitary bees, species with a medium-size proboscis length most frequently visited the plants.

Overall, we have shown that even among generalized plants, plant-flower-visitor interactions are structured and predictable. Flower-visitors did not forage randomly, as only the number of flower-visitor species, but not the number of visits was related to flower head density. Rather, plant generalization degree was determined by nectar tube depth and display area, the negative correlation between nectar tube depth and flower head density and flower-visitor proboscis length frequency distributions. Our results highlight the importance of quantitative data analyses, rather than qualitative data analyses and trait-abundance correlations to understand pollination community structure.

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