The structure of flower visitation webs: how morphology and abundance affect interaction patterns between flowers and flower visitors

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chapter 4

Morphological matching of flowers and flower visitors: the role of size thresholds and size distributions

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Abstract

Plants attract animals to pollinate their flowers by providing rewards such as nectar and pollen. These rewards differ greatly in their accessibility, which constrains who visits whom. In earlier studies we showed that the size threshold that the depth of nectar concealment places on the proboscis length of nectar-searching flower visitors is an important factor determining the degree of ecological generalization and interaction asymmetry in flower-visitation webs. Here we analyze the influence of this rule on the degree of size matching between flowers and flower visitors. The threshold rule should lead on average to a closer match to nectar depth for flower visitors with a short proboscis than for visitors with a long proboscis. Accordingly, plant species with hidden nectar should match their visitors more closely than plant species with openly-presented nectar. However, distributions of proboscis length and flower depth across species or individuals will strongly influence the average degree of matching. By using a simple modeling approach we can show that particular size distributions will lead to equal degrees of matching for all species, whereas other distributions will produce stronger differences. The analysis of a Mediterranean plant–flower visitor web revealed that both proboscis length and nectar holder depth resemble right-skewed lognormal size distributions. We can demonstrate, consistent with the model predictions based on observed size distributions, that flower visitors with a short proboscis matched the nectar depth of flowers more closely on average than those with a long proboscis, while plant species with hidden nectar and openly-presented nectar matched their interaction partners equally closely. The observed patterns differed only slightly between a species- and an individual-based analysis. Deviations from expectations will serve as a starting point to search for additional factors that influence interaction patterns. Overall we can say that both size thresholds and size distributions are essential to explain the degree of matching. The degree of morphological matching can serve, along with the degree of ecological generalization and interaction asymmetry, as an essential ecological property of flower visitation webs, with important implications for coevolution and biodiversity conservation.

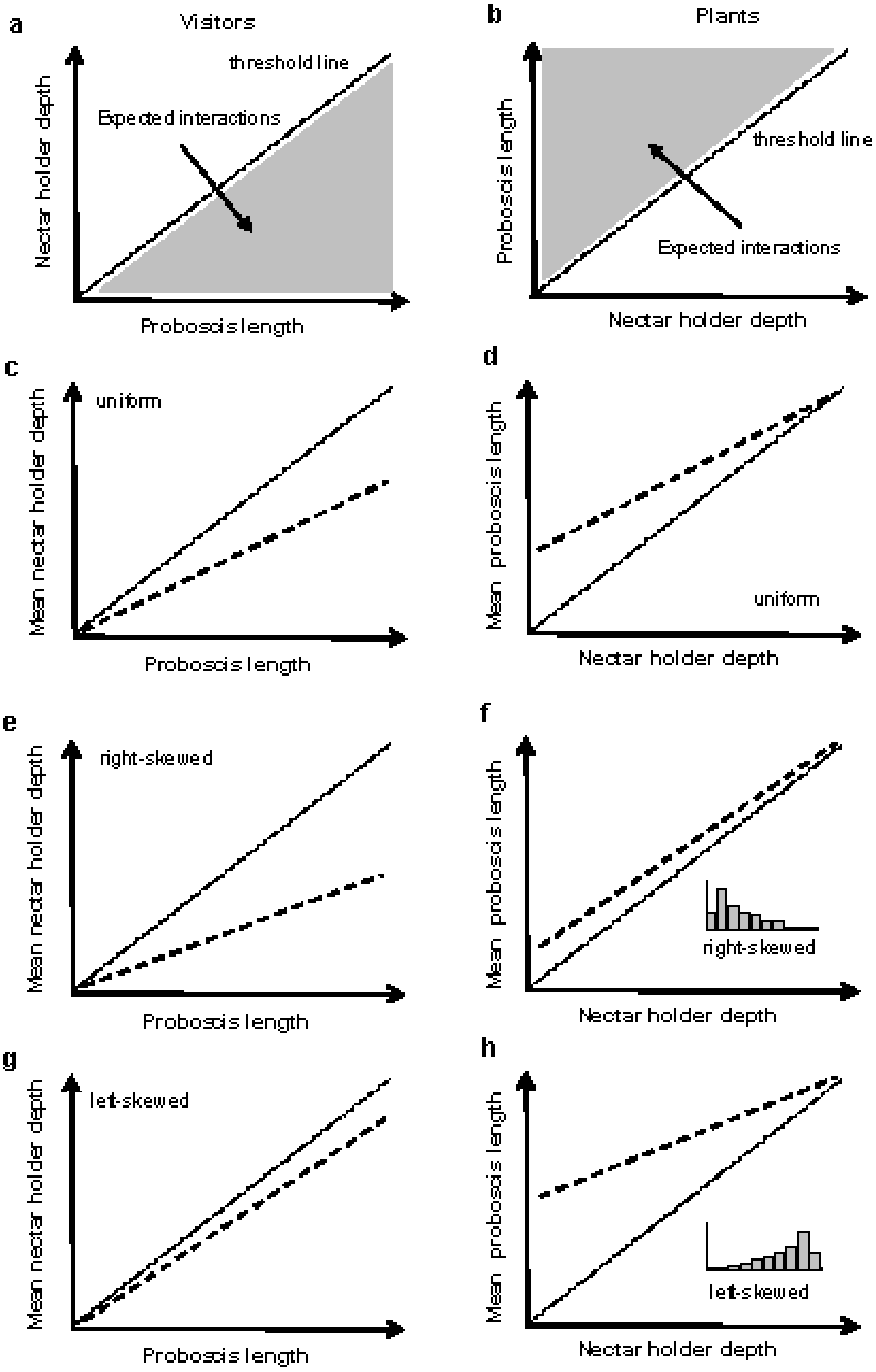
Introduction

Most species of angiosperms attract animals to pollinate their flowers (*e.g.* Nabhan & Buchmann, 1997; Renner, 1988). Attraction usually is achieved by providing rewards of nectar, pollen, oils or other substances to pollinators. In some flowers these rewards are easily accessible, but in others they require particular behaviours and/or morphologies of pollinators to obtain. Perhaps the clearest example is the concealment of nectar within deep tubes or other floral structures. Putting aside those animals that pierce deep tubes to 'rob' the nectar (Irwin *et al.*, 2001), it seems logical that concealed nectar will be accessible only to animals with mouthparts longer than the depth of the structure (tube, spur, etc.) that holds the nectar (hereafter termed the 'nectar holder depth'). In earlier studies this size threshold was successful in predicting several general properties of an actual web of interactions between flowers and their visitors, including the numbers of insect species visiting each plant species and the proboscis lengths of these visitors (Stang *et al.*, 2006), and the asymmetry of interactions between plants and insects (*i.e.*, the fact that specialists mostly interact with generalists) along with the correlation between the number of interaction partners of a species and the level of generalization of its partners (Stang *et al.*, 2007).

In this paper we ask whether the threshold rule can explain another important characteristic of plant–flower visitor interactions, the degree of size matching between proboscis length and nectar holder depth. A close morphological match between flowers and their flower visitors can be an important component of high visitation rates (Inouye, 1980; Peat *et al.*, 2005; Ranta & Lundberg, 1980) or high per-visit pollination efficiencies of flower visitors (Campbell *et al.*, 1996; Johnson & Steiner, 1997; Nilsson, 1988; although see Wilson, 1995). An analysis of published records of flower visits across north-western Europe (Knuth, 1906) indeed points in the direction of size matching: plants of certain nectar depths are visited mainly by insect groups with corresponding proboscis lengths (Corbet, 2006; Ellis & Ellis-Adam, 1993). However, this size matching seems at odds with the fact that pollinators with long proboscises will have access to shallow as well as deep flowers (Stang *et al.*, 2006, 2007). But this conclusion misses the fact that the degree of matching will also be influenced by the frequencies of species and individuals with shallow and deep flowers or with short and long proboscises (even leaving aside any behavioural preferences which lead individual animals to visit flowers that match their proboscis; *e.g.* Harder, 1985, Ranta & Lundberg 1980). For example, visitor groups (*e.g.* species or individuals with the same proboscis length) that are more abundant can visit more of their potential plant species and can visit these species more often than rare visitor groups, and so will have a higher impact than rare visitor groups on the average proboscis length that we observe at flowers of a plant species.

Our intent is to use the depth threshold and the assumption of interactions proportional to the frequency of traits to estimate the effect of trait distributions on the degree of size matching. The depth threshold by itself should force individual interactions between nectar producing plants and nectar searching flower visitors to occur below (FIGURE 4.1a, nectar holder depth vs. proboscis length) or above the threshold line (FIGURE 4.1b, proboscis length vs. nectar holder depth), leading to a triangular distribution of possible interactions. If traits are uniformly distributed across plants and visitors, visitors with a short proboscis (morphological specialists) will match on average the plants they visit more closely than visitors with a long proboscis (morphological generalists, FIGURE 4.1c); and plant species with deeply-hidden nectar (morphological spe-

FIGURE 4.1 – Conceptual model depicting the relationship between trait distributions and degree of size matching under the threshold rule and interactions proportional to abundance. The graphs on the left are from the visitors' and those on the right from the plants' point of view. The interactions are expected to fall within a triangular below (a) or above (b) the threshold line. The threshold line is the $x = y$ line where proboscis length equals nectar holder depth. The expected range of nectar holder depths increases with increasing proboscis length and that of proboscis lengths decreases with increasing nectar holder depth. The expected degree of matching is expressed as the regression of mean nectar holder depth on proboscis length (c, e and g) and mean proboscis length on nectar holder depth (d, f and h). To illustrate the influence of trait distributions we used three combinations of proboscis length and nectar holder depth distributions: both uniform (c and d), both right-skewed (e and f), and both left-skewed (g and h). The trait distributions had equal minimum and maximum values. The relationship is not by definition linear and depends on the shape of the trait distribution. The model incorporates a weighting factor (see methods) that accounts for differences in probability of observing species-species interactions in relation to the number of potential interaction partners.


a
Visitors
threshold line
Expected interactions
Nectar holder depth
Proboscis length
b
Plants
threshold line
Expected interactions
Proboscis length
Nectar holder depth
c
uniform
Mean nectar holder depth
Proboscis length
d
uniform
Mean proboscis length
Nectar holder depth
e
right-skewed
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right-skewed
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Nectar holder depth

cialists) will be visited by insects that match the nectar depth more closely than plants with openly-presented nectar (morphological generalists, FIGURE 4.1d). However, the picture changes if trait distributions are not uniform. Thus a right-skewed, lognormal distribution of nectar holder depths and proboscis lengths should decrease matching for generalized visitors (FIGURE 4.1e) but increase it for generalized plants (FIGURE 4.1f), whereas conversely a left-skewed distribution for both traits should increase matching for generalized visitors (FIGURE 4.1g) but decrease it for generalized plants (FIGURE 4.1h). Extrapolating from these patterns suggests that the best matching across all morphologies would be achieved by a combination of left-skewed nectar holder depths and right-skewed proboscis lengths, whereas the worst matching would follow from right-skewed nectar holder depths and left-skewed proboscis lengths.

Little effort has been made to date to explore actual patterns of size distribution across species and individuals in local communities, and their role for the organization of flower visitation webs (Agosta & Janzen, 2005; Woodward *et al.*, 2005). There also are few comparative, community based studies analyzing the degree of morphological matching for morphologically generalized vs. specialized species. The few existing studies have taken the visitors' point of view and restricted their analysis to groups of closely-related species such as hoverflies (Gilbert, 1981), long-proboscid flies (Goldblatt & Manning, 2000), euglossine bees (Borrell, 2005), bumblebees (Brian, 1957; Harder, 1985; Ranta & Lundberg, 1980), butterflies (Corbet, 2000), or hawkmoths (Haber & Frankie, 1989). Overall, these studies revealed that animal species with long proboscises visit on average a wider range of flowers than species with short proboscises, supporting the threshold hypothesis. All studies also reported a positive relationship between proboscis lengths of visitors and average nectar holder depth of the plants visited. However, none of the studies just cited tested whether the observed degree of matching could result from proboscis length or nectar depth distributions in the local community. Furthermore, these animal-centred studies do not allow an extrapolation to how plant species match the morphology of their visitors (the plants' perspective), given that many of the plants studied were probably visited by more than the visitor group under investigation (Herrera, 1996; Olesen, 2000; Waser *et al.*, 1996)

We used a Mediterranean flower-visitation web to explore whether size thresholds in combination with frequency distributions of proboscis lengths and nectar holder depths can explain observed size matching in a local community. First we calculated the degree of matching from species-based and individual-based means of proboscis lengths and nectar holder depths. We compared the observed patterns with theoretical expectations based on the threshold rule and the observed size distributions under the assumption that interactions are proportional to the frequency of traits values in the species pool. We wanted to know firstly, whether the degree of matching of morphologically generalized and specialized plant and visitor species differ in this visitation web, and secondly, whether the threshold rule in combination with the size distribution reproduces the observed degree of morphological matching between flowers and their visitors. As an additional factor potentially influencing matching we tested nectar holder width, a trait that was found to be constraining the number of visitor species (Stang *et al.*, 2006). Specifically we asked:

- How are proboscis lengths and nectar holder depths distributed among species and individuals in the actual Mediterranean web?
- What is the expected degree of matching based on the threshold rule and observed trait distributions among species and individuals?
- What is the observed degree of matching and does this differ from the theoretically predicted matching?

Methods

Study system, sampling method, and trait distributions

The empirical data used in this paper come from a Mediterranean flower visitation web in the southeast of Spain consisting of 25 nectar-producing plant species spread over 11 plant families, and 111 nectar-collecting flower-visitor species spread over five insect orders (Stang *et al.*, 2006). We determined the number of visitor species and visitor individuals searching for nectar on these plant species during 6 weeks in March and April 2003. Each plant species was observed for a total of 60 min (comprising totals of 15 min observation during each of the four two-hour periods between 10 AM and 6 PM). Observations (on average about 12 per plant species) were randomly distributed over 15 sampling days when the species in question was in full bloom, and over 10 sampling plots (3.6 ± 1.6 plots per species [mean ± standard deviation]). We concentrated the sampling within a plot in patches were the observed plant species was relatively frequent and sampled only under optimal conditions for flower visitors. The latter means that we tried to sample a plant species only when we noticed visitation activity. With these methods we optimized sampling effort across species (number of observed plant and visitor individuals per observation period and plant species). During peak flowering time of a plant species we also counted the number of flowering individuals, the number of open inflorescences per individual and the number of open flowers per inflorescence.

We used the total number of nectar-searching animal species and individuals on the 25 plant species as an estimate of the total number of visitor species (111) and individuals (887) in our study area during the observation period. We caught the majority of observed nectar searching visitor individuals but kept only one specimen from each insect species per plant species and sampling interval (in total 278 individuals) to minimize disturbance. *Apis mellifera* was the most abundant species in the area; one third of the observed individuals belong to this species. Here we caught only a very small fraction of the observed individuals so that we are not sure how many individuals were actually in the sampling area. Per plant species we observed on average 36 ± 25 visitor individuals (or 24 ± 18 excluding honey bees). Per visitor species we observed on average 8.0 ± 28.5 individuals (or 5.4 ± 7.5 excluding honey bees). 34% of the insect species were represented by only a single individual during the whole observation period. We did not determine visitation rate of individual visitors (*e.g.* number of flowers visited per minute), so that per observation period and plant species the number of observed visitor individuals is approximately the actual number of individuals of that animal species in the sampling plot. The inclusion of visitation rates of individual flower visitors to flowers would be desirable but was not feasible given that we had to catch visitors for identification and size measurements almost immediately after we observed them at flowers.

We measured nectar holder depth and width for 5 to 10 flowers of each plant species, and proboscis length, proboscis diameter, and body length of all insects captured at flowers. Body mass of visitors was estimated from length as $M = 0.0305 L^{2.62}$ with M = body dry mass in mg and L = body length in mm (Rogers *et al.*, 1976). In all analyses we used the minimum value measured for each species for nectar holder depth, and the maximum value measured for nectar holder width, to allow the most liberal interpretation of the threshold that would exclude visitors (Stang *et al.*, 2006). Nectar standing crop was generally small, so that the nectar holder depth we measured will come close to actual nectar level depths. We used a Kolmogorov-Smirnov test to determine if the traits were normally or log-normally distributed across species and individuals and determined the kurtosis and skewness of distributions. Additionally we tested flower visitors for a positive correlation between body mass and proboscis length.

Observed degree of matching

As explained in the Introduction, the threshold rule by itself predicts a triangular distribution of interactions in a graph with values of the traits as its axes (see FIGURE 4.1). To get a first impression of the 'degree of triangularity', *i.e.* how evenly interactions were distributed within this triangle, we used linear regression as a heuristic tool. The more evenly the data points are distributed in the area where the highest variance occurs, the closer the regression coefficient will be to 0.5 (high degree of triangularity). The more data points occur near the threshold, the closer the slope will be to 1.0 (low degree of triangularity).

To estimate observed size matching for each species separately we calculated mean and standard deviation of trait values for its mutualistic partners – for plants this means proboscis length of visitors to their flowers, and for insects it means nectar holder depths of the flowers they visit. Observed mean trait values per species were calculated by weighting all species of insects or plants equally (hereafter 'species-based means') or by weighting all individuals equally ('individual-based means'). The species-based approach gives an impression of the potential influence of trait distributions across species and can be easily applied to existing qualitative (species-based) datasets of interaction webs. Moreover, published body size distributions for flower visitors are mostly species-based. The individual-based approach determines the influence of the frequency of individuals and is a first step toward fully quantitative community-level studies incorporating visitation rates of individual visitors to flowers.

We applied linear regression as a method to assess whether generalized and specialized species differ in their degree of matching. To do so we tested if the slope of the regression lines of mean proboscis length vs. nectar holder depth, and vice versa, differed significantly from one. To assess if the observed degree of matching could be a result of the threshold rule and interactions proportional to the frequency of traits we compared the observed slope with the expected slope, as calculated below, based on these rules. To compare the matching of generalized and specialized species and to compare expected and observed slopes we used a partial F-test following Potthoff (1966). Statistical analyses were performed in SPSS 12.0 (SPSS Inc., Chicago, USA).

Expected degree of matching

To calculate expected mean proboscis lengths and nectar holder depths we assumed that visitors distribute themselves conform to the threshold rule over plants and plants over visitors. This means, for the species–based analysis that we assumed that the probability that a certain visitor species interacts with a certain plant species depends on the number of plant species available to an insect species and on the number of insect species that can visit this plant species. For the individual–based analysis the number of individuals instead of the number of species was used. We assumed further that the available resources per plant species do not differ and visitors perform equally well on all flowers that confirm to the threshold rule. The latter means that handling time on a flower and flight time between flowers for the animal species do not differ across plant species. Thus in our model the chance to observe a visitor species will not be influenced by assumptions others than the threshold and the distribution of proboscis lengths and nectar holder depths across species or individuals.

We assumed that differences in visitation rate play a minor role because we caught most of the visitor individuals immediately after visiting a few flowers on a plant. Nevertheless, the frequency of observed individuals of a given insect species to a plant can be seen as one of the quantity components of pollinator importance (Herrera, 1989; *sensu*

Waser, 1983). We also assumed that the total amount of resources provided per plant species do not differ because we sampled each plant species during peak flowering time and secondly, we supposed that the amount of nectar per flower and number of open flowers per inflorescence cancel each other out. In support of the latter assumption, the number of open flowers per inflorescence is negatively correlated with nectar holder depth (after log transformation $r = -0.51$, $p = 0.01$, $N = 25$), whereas amount of nectar is positively correlated (Petanidou & Smets, 1995).

For the calculation of the expected means the species were arranged in a matrix. Columns represent plant species and rows represent insect species. The expected mean proboscis length for a given plant species j is:

$$\bar{p}_j = \frac{\sum_i^{N_a} p_i f_i M_{ij}}{\sum_i^{N_a} f_i M_{ij}}, \tag{1}$$

where p_i is the proboscis length of insect species i, f_i is the frequency of this species in the visitor fauna, and M_{ij} is the weighting factor that reflects the threshold rule (see below).

Similarly, the expected mean nectar holder depth for a given animal species i is:

$$\bar{h}_i = \frac{\sum_j^{N_p} h_j F_j M_{ij}}{\sum_j^{N_p} F_j M_{ij}}, \tag{2}$$

where h_j is the nectar holder depth of plant species j, F_j is the frequency of this species in the flora, and M_{ij} is again the weighting factor. In both calculations the weighting factor is:

$$M_{ij} = m_{ij} \frac{f_i}{\sum_i^{N_a} m_{ij} f_i} \cdot \frac{F_j}{\sum_j^{N_p} m_{ij} F_j} \quad \text{and} \quad m_{ij} = \begin{cases} 1 & \text{for } p_i \geq h_j \\ 0 & \text{otherwise} \end{cases} \tag{3}$$

The plant or animal frequencies were 1 for the species-based means or equaled the number of individuals for the individual-based means. If nectar holder width was included as a size constraint we extended the depth threshold rule m_{ij} in eq. (3) with the additional rule that proboscis diameter is equal to or less than the width of the nectar holder.

The weighting factor M_{ij} reflects how the probability of an interaction between two species depends on the potential number of animal and plant species (or individuals). An example may clarify the weighting factor for the species-based model where we assumed that f and F are set to 1. An insect species with a proboscis of 3 mm can exploit all plant species with a nectar holder ≤ 3 mm; if 5 plant species meet this criterion, the probability to observe this insect on each of these plant species is 0.2. Similarly, an insect species with a proboscis of 1 mm can only visit flowers of ≤ 1 mm; if there is only 1 plant species that meets this criterion the probability to observe an interaction is 1. This distribution of insects over accessible plants is substantially represented in the right fraction of eq. (3). On the other hand, a plant species with a nectar holder depth of 3 mm can be visited by insects with a proboscis ≥ 3 mm; if 20 insect species meet this criterion the probability for each visitor species is 0.05. The distribution of plants over insects is substantially represented in the left fraction of eq. (3). For each potential pair of species we multiplied both parts as shown in this equation.

Because of the threshold rule and our modelling approach the expected mean proboscis length of the visitors for a plant with open nectar will be relatively more influenced by species with short proboscises (specialists which are restricted to such flowers) than with long proboscises (generalists with access to a wider range of flowers). A parallel argument holds for the mean nectar holder depth of the plants visited by an animal species: the expected mean nectar holder depth of the plants visited by a visitor with a long proboscis is relatively more influenced by flowers with deeply hidden nectar. Without taking the weighting factor into account, we would unrealistically increase the frequency of a species proportional to the number of potential interaction partners; for example, visitor species with a long proboscis would be more frequent than visitors with a short proboscis solely because they can potentially visit more plant species. This would overestimate the mean proboscis length for generalized plants and underestimate the mean nectar holder depth for generalized visitors.

One may regard our modelling approach as the appropriate ‘null model’ for our specific sampling method. Our sampling method is characterized, firstly, by a low but equal sampling effort per plant species; secondly, by a low chance to observe many visitations per visitor species because of catching away of individuals (with the exception of honey bees). We found that the ratio of observed to potential visitors on a plant species increased with decreasing potential number visitor species (Stang *et al.*, 2006). So indeed not only the observed mean proboscis length for a plant species with open nectar should be more influenced by visitors with a short proboscis but also the mean nectar holder depth of a visitor species with a long proboscis by plants with deeply hidden nectar.

Results

Observed trait distributions and covariation among species traits

The observed proboscis lengths of the 111 visitor species ranged from 0.1 to 14.0 mm with a mean of 3.5 mm and a median of 2.3 mm. The distribution was unimodal and right-skewed (FIGURE 4.2a, kurtosis = 1.36, skewness = 1.43). After log transformation the proboscis lengths were normally distributed (Kolmogorov-Smirnov test, $z = 1.01$, $p = 0.26$, $n = 111$). The frequency distribution based on the number of individuals (excluding *Apis mellifera*) resembles the distribution based on species number (again right-skewed, FIGURE 4.2b); in this case a log transformation did not normalize the distribution. Estimated dry body mass of the insect species ranged from 0.1 and 67.4 mg with a mean of 12.7 mg and a median of 7.8 mg. The distribution was right-skewed and was normalized by a log transformation ($z = 0.90$, $p = 0.39$, $n = 111$). Log proboscis length and log body mass were significantly positively correlated across visitor species ($y = 0.72\, x^{0.61}$, $r^2 = 0.67$, $p < 0.001$, $n = 111$), so that proboscis length had a positive allometric scaling relationship with body mass.

The depth of nectar holders ranged from zero to 9.5 mm with a mean of 3.5 mm and a median of 2.7. The maximum value was 4.5 mm smaller than the maximum for visitor species; but the minimum, mean and median differed only slightly between nectar holder depths of plants and proboscis lengths of animals. The frequency distribution of nectar holder depths was right-skewed (FIGURE 4.2c, kurtosis = –0.25, skewness = 0.73) but could not be distinguished statistically from a normal distribution

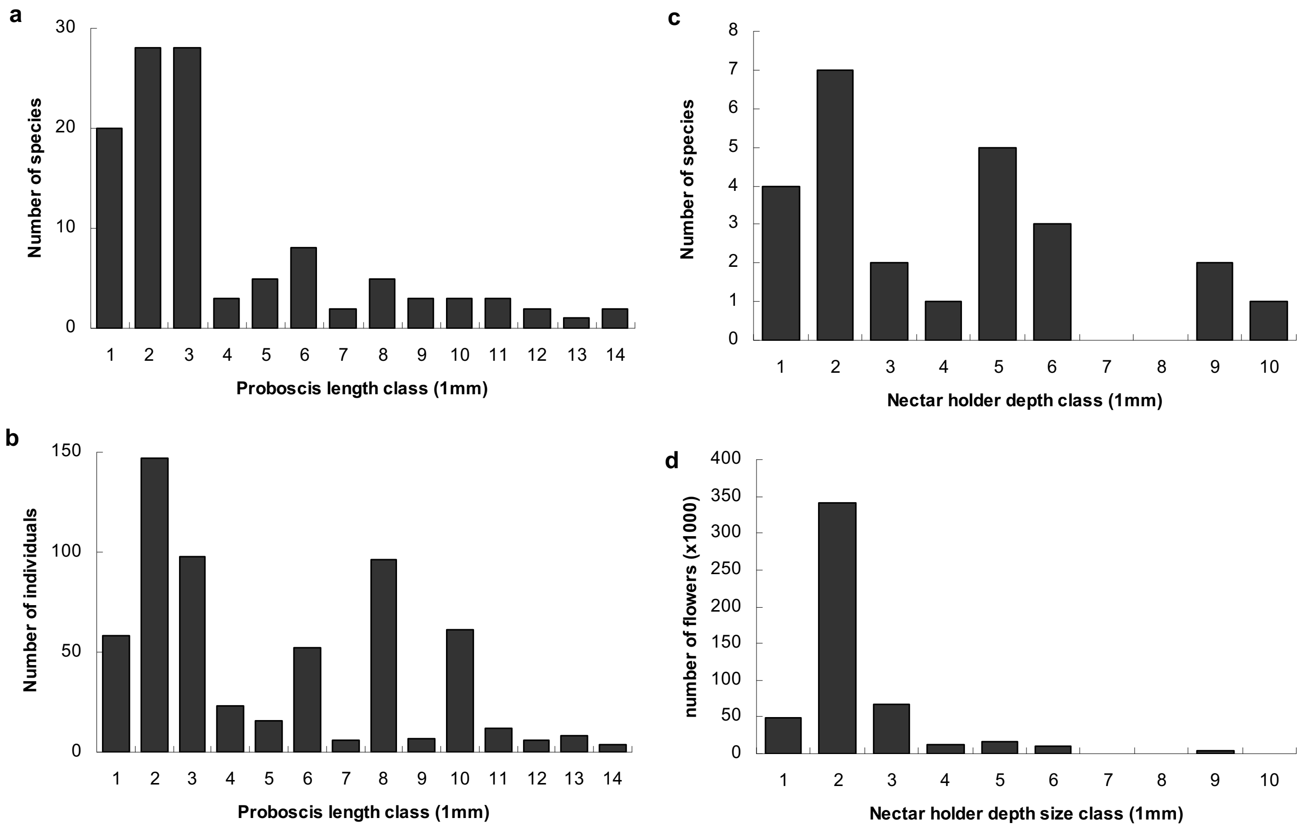

FIGURE 4.2 – Observed proboscis length and nectar holder depth distributions. The number of species (a, c) individuals (b) or flowers (d) per size class interval of 1 mm is given. The 300 individuals of honey bees (*Apis mellifera*, proboscis length = 5.95 mm) were excluded from (b).

($z = 0.84$, $p = 0.49$, $n = 25$). The distribution of the total number of open flowers across plant species was also right-skewed, with a maximum within the same size class as visitor individuals (FIGURE 4.2d). It was normally distributed after log-transformation ($z = 0.68$, $p = 0.75$, $n = 25$). Number of observed visitor individuals and total number of open flowers were positively correlated ($r_S = 0.58$, $p = 0.002$, $N = 25$).

Observed distribution of interactions

The observed use of flowers of different nectar holder depths by visitors of increasing proboscis length (which can be considered the visitors' point of view) falls into a triangle below the threshold line, *i.e.*, the line $x = y$ on which proboscis length exactly matches nectar holder depth (FIGURE 4.3a; compare to FIGURE 4.1a). Applying a linear regression to this triangular distribution gives a slope of 0.54 which is clearly smaller than 1.0 and indicates that the degree of triangularity is relatively large. Similarly, the observed use of visitors of different proboscis lengths by plants of increasing nectar holder depth (which can be considered the

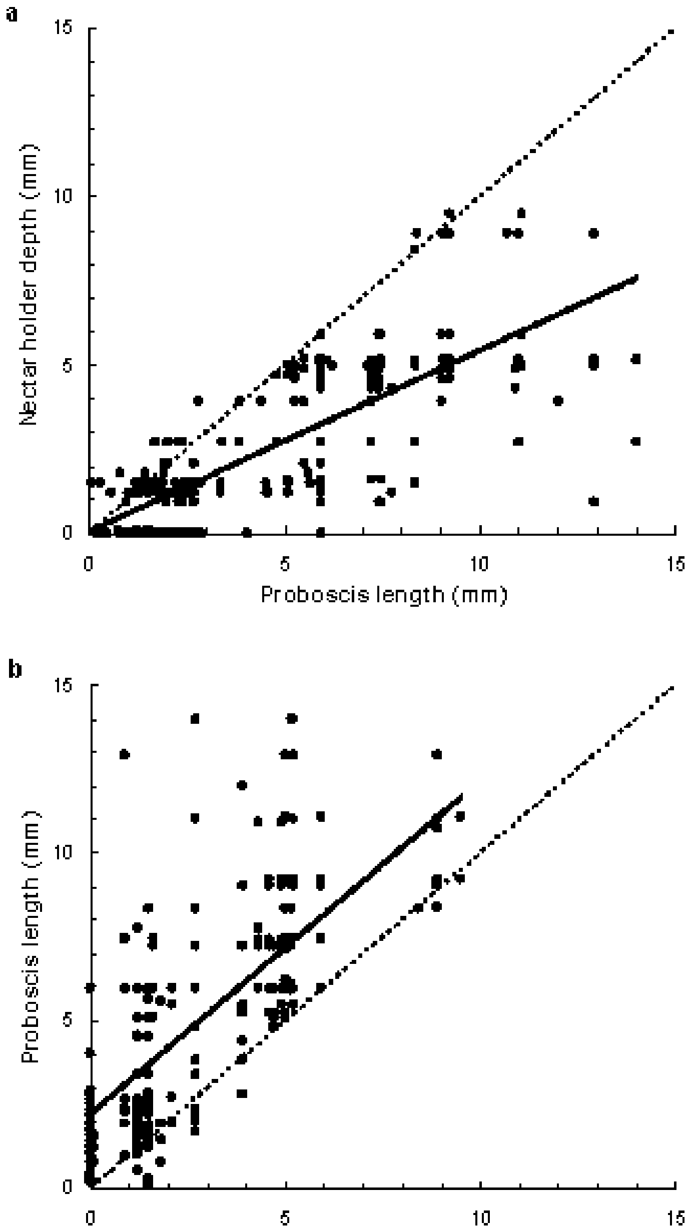

Figure 4.3 – Observed distribution of plant–visitor interactions. The observed interactions are distributed within a triangle. In 3a the interactions were found mainly below the threshold line (visitors' point of view). In 3b the interactions occur mainly above the threshold line (plants' point of view). Each data point represents one species–species interaction (*n* = 231). The regression lines are based on insect individual–plant species interactions (*n* = 887). The x = y threshold line is indicated with a dotted line.

plants' point of view) falls into a triangle above the threshold line (FIGURE 4.3b, compare to FIGURE 4.1b). In this case, however, linear regression gives a slope of 0.99; the degree of triangularity is low.

Matching of observed and expected in the mean of trait values

Regressing observed mean nectar holder depths on proboscis lengths (the visitors' point of view) yields a significant positive slope, both for

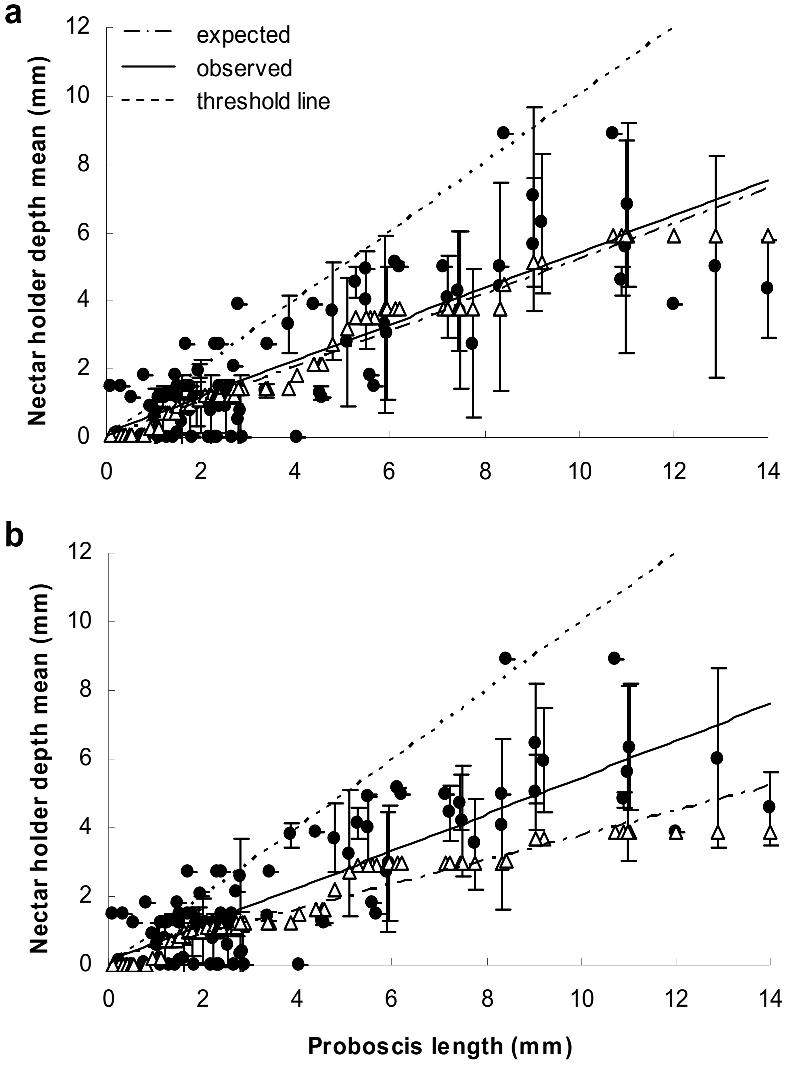

FIGURE 4.4 – Observed and expected mean nectar holder depths in relation to proboscis length of the visitor species based on species (a) or on individuals (b). Observed values are indicated with black circles and are given with their standard deviation in a and b. Expected values are indicated with open triangles. For the sake of simplicity we used linear regression as a first approximation of the relationship. The linear regression line of the observed values is indicated with a continuous line, for the threshold model with a dashed-dotted line, and for the $x = y$ line with a dotted line. Each data point represents one insect species ($n = 111$).

species-based (= 0.53) and individual-based means (= 0.54, Figures 4.4a and 4.4b, continuous lines, and Table 4.1, visitors). However, flower visitors with a short proboscis matched the flowers they visit more closely than flower visitors with a long proboscis, because both the species-based slope and the individual-based slope were significantly smaller than 1.0 (results of the partial F-test: delta = –0.47, $t = -14.17$, $p < 0.001$ for species, and delta = –0.46, $t = -14.57$, $p < 0.001$ for individuals).

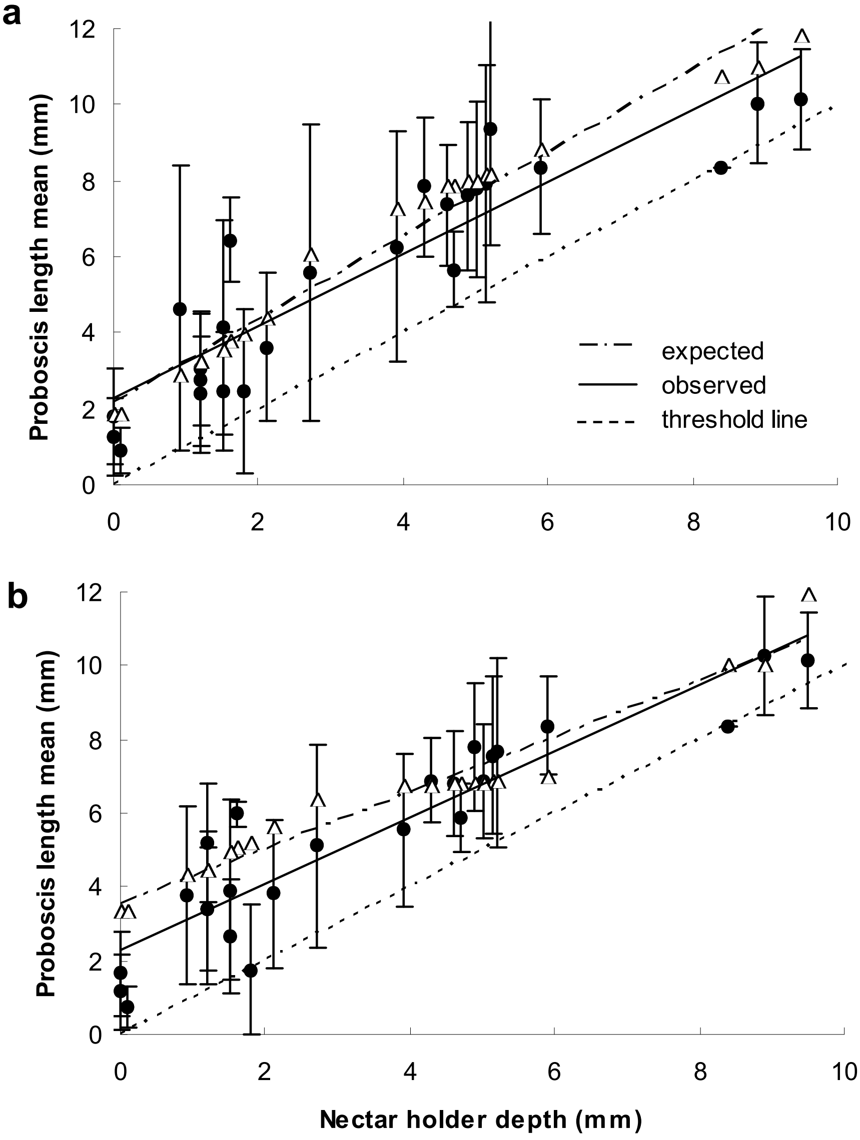

Figure 4.5 – Observed and expected mean proboscis length in relation to nectar holder depth of the plant species based on species (a) or on individuals (b). Observed values are indicated with black circles and are given with their standard deviation in a and b. The linear regression line of the observed values is indicated with a continuous line, for the threshold model with a dashed-dotted line, and for the x = y line with a dotted line. Each data point represents one plant species ($n = 25$). Further explanations see Figure 4.4.

TABLE 4.1 – Degree of observed and expected morphological matching estimated with the mean trait values of the interaction partners. Expected values of species-based means are based on the depth threshold and interaction proportional to potential number of species; individual-based means are based on the depth threshold and interaction proportional to number of potential individuals. The table gives the observed slope, intercept, r^2 and the significance of the regression between plant and visitor traits, and the expected slope and intercept based on the threshold models without or with nectar holder width threshold (D or D +W). The individual-based models are based on observed visitor individuals. Delta indicates the difference in slope between observations and expectations. The significance of the difference in slope is indicated with p (ns: non significant). For further explanations see text.

	rules	slope	intercept	r^2	Delta	p
species-based						
visitors	observed	0.53	+0.14	0.70	–	–
	D	0.52	–0.01	0.96	0.01	ns
	D+W	0.51	–0.01	0.97	0.02	ns
plants	observed	0.95	+2.26	0.82	–	–
	D	1.09	+2.18	0.97	–0.14	ns
	D+W	1.08	+2.2	0.97	–0.13	ns
individual-based						
visitors	observed	0.54	+0.11	0.72	–	–
	D	0.36	–0.18	0.95	0.18	<0.001
	D+W	0.31	+0.05	0.87	0.23	<0.001
plants	observed	0.90	+2.27	0.85	–	–
	D	0.75	+3.54	0.95	0.15	ns
	D+W	0.74	+3.62	0.94	0.16	ns

The observed slope based on species means was not significantly different from the expected slope under the depth threshold rule (0.53 compared to 0.52 in TABLE 4.1), whereas the slope based on individuals was significantly steeper than expected (compare 0.54 to 0.36 in TABLE 4.1). Nevertheless, the difference was small compared to the difference with a slope of 1.0. Thus, with increasing proboscis length visitor individuals matched the flowers they visit slightly but significantly more than expected but the difference in matching of species with short and long proboscises remains large. The inclusion of nectar holder width yielded no change in the expected slopes for species-based means and individual-based means compared to the depth threshold alone (TABLE 4.1, see rules D compared to D+W).

Regressing observed mean proboscis length on nectar holder depths (the plants' point of view) also yields a significant positive slope both for species-based (= 0.95) and individual-based means (= 0.90, Figures 4.5a and 4.5b and Table 4.1, plants). In contrast to the visitors' point of view, the slopes did not differ significantly from 1.0 (Figures 4.5a and 4.5b dotted lines, species based delta = –0.05, $t = -0.949$, $p = 0.348$, individual based delta = –0.10, $t = -1.638$, $p = 0.108$). The observed slopes did also not differ from the expectations of the threshold model (Figures 4.5a and 4.5b, dashed-dotted lines and Table 4.1, 0.95 compared to 1.09 and 0.90 compared to 0.75). As for the visitors, the inclusion of nectar holder width yielded no difference in the expected slopes for species-based means and individual-based means compared to the depth threshold alone (Table 4.1, see rules D compared to D+W).

Discussion

Trait distributions and morphological matching

In the Mediterranean flower visitation web we studied, visitor species with a short proboscis matched the flowers they visited on average more closely than species with a long proboscis. Flowers with open and hidden nectar, on the contrary, did not differ on average in their degree of morphological matching, *i.e.*, morphologically specialized and generalized visitors diverge more in their degree of matching than plants did. This was true for the species-based as well as the individual-based means. The threshold rule alone cannot explain the observed pattern. Only when the null model included the observed trait distribution in the local species pool was the threshold rule able to reproduce to a great extent the observed pattern. These results suggest that in addition to the threshold rule trait distributions play an important role in determining the degree of morphological matching between flowers and their visitors.

Proboscis lengths in the web showed a right-skewed, lognormal distribution across species; most species had a short proboscis. As a result the majority of visitors of morphologically generalized plants matched the nectar holder depth very closely so that the average difference in matching of generalized vs. specialized plants was small. A right-skewed, lognormal distribution is the prevailing distribution for body mass of animal species (*e.g.* Allen *et al.*, 2006; Kozlowski & Gawelczyk, 2002; Ulrich, 2006). Because proboscis length and body mass were positively correlated among visitor species, as has been reported for solitary bees, bumblebees, butterflies and sphingid moths (Agosta & Janzen, 2005; Corbet, 2000; Haber & Frankie, 1989; Harder, 1985; Shmida & Dukas, 1990), a right-skewed, lognormal proboscis length distribution should also be the rule.

Nectar holder depths in our web were right-skewed distributed and the mean and mode of nectar holder depths resemble those of proboscis lengths except that the longest proboscis exceeded the deepest nectar by 4.5 mm so that flowers and visitors do not fully match one another in size distributions. The larger range of proboscis lengths could be the reason that even deep flowers attracted a range of visitors, contributing to the equivalent degree of matching for morphologically generalized vs. specialized plants. Comparing the observed nectar holder depth distribution with published data, we found that, on a broad scale, plant species with shallow flowers seem to be more species rich than plant species with deep flowers (Ollerton & Watts, 2000). A right-skewed distribution was found for flowers in alpine communities in North America, Austria, and Australia, and for the visitors of these plants (Inouye & Pyke, 1988), as well as for plant species visited by Costa Rican dry forest moths and for the moths (Agosta & Janzen, 2005).

Deviations between expected and observed matching

Nevertheless, despite the good agreement between observed and expected patterns, there was some variation in how close the mean of single species agreed with theoretical expectation based on our simple rules. The reason for this variation at the level of single species could be variation due to chance, because of the short observation time. However, we also found systematic deviations from the expected slopes: species matched closer than expected, especially if the calculations were based on individual means. We suppose that a systematic deviation from the expected degree of matching would occur if traits that lead to matching are correlated with proboscis length or nectar holder depth. Nectar holder depth and width were not significantly correlated (Stang *et al.*, 2006). Accordingly, we did not find a systematic influence of nectar holder width on the degree of matching. However, plant species with accessible nectar may produce less nectar and thus be less attractive for insects with a long proboscis compared to flowers with deeply hidden nectar. We found that proboscis length and body mass were positively correlated. The bigger a visitor species, the more energy it needs and the higher its threshold of expected profit (or energetic cost) beyond which flowers are rewarding enough to visit (Corbet, 2006). If the amount of nectar cannot be counterbalanced by the number of flowers as assumed in our simple model, the cost threshold could restrict the observed maximum proboscis length on a flower (Corbet *et al.*, 1995) and leads to a tighter match than predicted by our simple model.

Influence of resource partitioning on the degree of matching

That the size threshold and interactions proportional to trait distributions were able to reproduce a great deal of the overall community pattern seems surprising given studies that show that competition and resource partitioning are plausible mechanisms to explain interaction patterns between plants and visitors. One reason could be that studies of plant–flower visitor interactions testing competition are normally based on visitation rates to flowers of a small set of interacting species within restricted time intervals and small distances. Community level studies normally do not take into account visitation rates to flowers and are based by definition on a large number of species and broader scales in space and time. Here the set of interacting partners and the conditions for visitation often will change dramatically, even at a relatively small scale, and thus modify the expectations based on short-term competition effects. Observing plant species only during peak flowering times, as we did, should further reduce the potential influence of competition. Moreover, studies analysing resource partitioning normally have not tested whether the pattern found can be a result of trait distributions. A positive relationship between mean nectar holder depth and proboscis length is not a proof for resource partitioning because a size threshold will always lead to a positive relationship between depth and length.

Implications for the adaptiveness of generalization

We were able to show that a size threshold is not at odds with relatively high degrees of morphological matching for generalized plants. Yet the reason for a high degree of matching differs between generalized and specialized plants, because generalized plants can only indirectly achieve high degrees of matching. Plant species with deeply hidden nectar, on one hand, restrict the potential visitors to species with a long proboscis and, as a result, the degree of morphological matching is high. Plant species with openly presented nectar, on the other hand, allow a high diversity of proboscis lengths. Because visitor species with a short proboscis are restricted to plant species with openly presented nectar and are more species-rich than visitors with a long proboscis, most of the visitors of a plant species with open nectar will match the nectar holder depth closely. As a consequence, most of the visitors of generalized plant species are very likely functionally equivalent (*sensu* Zamora 2000) with regard to their proboscis length. In this case a large number of visitor species and a high degree of matching are not a contradiction. Instead this high number could even be necessary for sufficient pollination.

Even so, the question arises whether plant species with accessible nectar will suffer from a higher degree of morphological mismatching than plant species with hidden nectar. It might not be necessary that there is a tight match between both interaction partners, because trade-offs between morphology and pollination efficiency may be weak in some cases (Aigner, 2004, 2006). Flowers that put no restrictions on pollinator morphology and behaviour might be adapted to a wide range of pollinators because of diffuse pollen presentation (Faegri & van der Pijl, 1979). For the visitor species additional morphological, physiological or behavioural constraints or preferences might lead to a higher degree of matching than expected solely on basis of size threshold and size distribution patterns. Nevertheless, apart from the fact that flowers with accessible nectar may not rely on a close fit and visitors might be more restricted in their choice, the frequency distribution alone, providing there is a size threshold, can tighten the degree of morphological matching.

Implications for biodiversity conservation

Overall, our results imply that it could be important, at least for plants, that a certain trait distribution exists in a community, because the probability that interacting species and individuals match each other's morphology will depend on the size distributions of the interaction partners. If there are only few species with short proboscises or deep nectar holders, the pollination of morphologically generalized plant species und the food resource of morphologically generalized visitor species will be less certain. Biesmeijer *et al.* (2006) reported a parallel decline of flower visitors and insect pollinated plant species in the Netherlands and the UK. Visitor species with long proboscises declined especially strongly. The analysis of trait distributions would help to understand and eventually avert biodiversity loss of flowering plants and their pollinators in local communities. Trait distributions that provide an optimal morphological matching for all species could serve as a testable reference point to estimate the potential stability and health of a flower visitation web.

Conclusion

This study shows that a simple threshold rule gives biologically predictable patterns, even if based on uncertain or changing species-specific relationships (*i.e.* which exact species are interacting). The size threshold in combination with a seemingly ubiquitous right-skewed frequency distribution of proboscis lengths will ensure that morphologically generalized plant species will be mainly visited by visitors that match the depth of the nectar holder with their proboscis length. Even if a tight match might not originally be essential for successful pollination, the high number of species and individuals with a predictable morphology (in our case a certain proboscis length and body size) would increase the probability that plant species can adapt to the most common visitor type, thus increasing their per-visit pollination efficiency. In this case a preference of visitors for flowers that match their proboscis can occur but will not be necessary for a tight match. Nevertheless, a high degree of size matching for all plant and visitor species can hypothetically occur simultaneously if certain trait distribution patterns are found at the community level (right-skewed for proboscis lengths and left-skewed for nectar holder depths). The presented results, in combination with results of previous studies of this interaction web (Stang *et al.*, 2006, 2007) show that both size thresholds and frequency distributions are necessary to explain simultaneously numerical (*e.g.* generalization, asymmetry) and biological characteristics (morphological matching) of an interaction web. Given these results, we emphasize the importance of measuring trait distributions across species and individuals in community-level studies of plants and flower visitors, and of including these distributions in simulation models, so that observations can be compared with theoretical expectations to yield a better understanding of plant–pollinator interactions. Deviations from expectations may serve as a starting point for the search for additional factors that influence interaction patterns, such as energy requirements of flower visitors.

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