# The structure of flower visitation webs

## How morphology and abundance affect interaction patterns between flowers and flower visitors

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

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# The structure of flower visitation webs

## How morphology and abundance affect interaction patterns between flowers and flower visitors

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## Promotiecommissie

*Promotor*
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*Co-promotor*
Dr. P.G.L. Klinkhamer

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# chapter 1

## General introduction

Pollination, the transfer of pollen grains to the stigma of the plant gynoecium is a crucial step in the sexual reproduction of flowering plants. The majority of flowering plants rely on animals for the transfer of pollen (Nabhan & Buchmann, 1997; Renner, 1988). Because flower visitors gain no direct benefit by pollinating flowers, rewards must lure them. The most common way plants attract animals to visit their flowers is by providing food such as nectar, pollen or oils. While searching for these rewards in the flower, pollen from the flower's anthers may stick to the body of the animal. When the animal visits subsequent flowers in search of more rewards, pollen from its body may adhere to the stigma of these flowers and again, new pollen may stick to the body of the animal.

Flowers differ tremendously in colour, scent, size and shape; and they are visited by an equally diverse morphological and taxonomic array of animals. The most common flower visitors are insects belonging to the orders Hymenoptera, Lepidoptera, Diptera and Coleoptera. But several species of birds, bats, and other mammals also regularly visit and pollinate flowers. A common and longstanding view in pollination biology is that plants should specialize on a small subset of these visitors in order to ensure effective pollination. And indeed, despite the huge morphological and taxonomical diversity of potential interaction partners, flowers show trait combinations that seem to reflect the morphology, behaviour and physiology of certain pollinator types (*e.g.* Faegri & van der Pijl, 1979). For example, red coloured, odourless flowers with deeply hidden and dilute nectar seem to be adapted to hummingbirds or perching birds; blue coloured bilaterally symmetric flowers with moderately hidden and relatively concentrated nectar combined with a pleasant odour are thought to be adapted to bees. These typical trait combinations (termed ‘pollination syndromes’ in the literature) are found across diverse taxonomic groups of plants and seem to be a result of specialization and convergent evolution.

The prevalence of plants specializing on one taxonomical group of animals has been questioned, however, because community-level studies reveal that most plant species are visited by species belonging to different animal orders or even classes (Herrera, 1996; Waser *et al.*, 1996) and pollination syndromes are not as distinct as they seem to appear (Ollerton & Watts, 2000). Moreover, the concept of pollination syndromes depicts only the taxonomic variation among pollinators. Within a taxonomic group there might be a much greater variation in size and behaviour than among taxonomic groups. For example, flowers that show the typical hawkmoth syndrome (pale coloured flowers with a strong, heavy-sweet perfume which open at night in combination with narrow nectar tubes with ample nectar) differ in the depth at which the nectar is hidden in the flower from a few millimetres up to several centimetres, and hawkmoths differ to the same extent in the length of their mouthparts (Agosta & Janzen, 2005; Haber & Frankie, 1989). But not only field studies question the prevalence of specialization, there are also theoretical doubts that specialization should always be promoted in nature. Because relying on one species or type of pollinator causes variable reproductive success across years, plants might do better to generalize, so long as pollinator population sizes vary independently (Waser *et al.*, 1996). In such cases, a plant may be at an advantage if it attracts several species or types of pollinators, ensuring sufficient pollen transfer every year.

Doubts about the significance of specialization in plant–pollinator interactions and about the existence of discrete pollination syndromes have resulted in a renewed interest in how important and common specialization actually is, and what kind of traits really determine who visits whom (Waser & Ollerton, 2006). The essential first steps for this re-evaluation are an objective quantification of the degree of generalization and specialization and the search for trait combinations that can explain the whole set of interactions in flower visitation webs, rather than explaining only restricted portions of such webs. As an indicator of the degree of generalization a large number of studies follow a pragmatic approach and count the number of species that interact with each other, *i.e.* the number of visitor species observed on a plant species and the number of plant species visited by a flower visitor species (*e.g.* Dupont *et al.*, 2003; Jordano, 1987; Moldenke, 1975; Olesen & Jordano, 2002; Ollerton & Cranmer, 2002; Vázquez & Aizen, 2003). I follow this approach even though it has some drawbacks. Because of the large number of species normally encountered in community-level studies it is often not possible to distinguish whether flower visitors are pollinators or visit flowers without pollen transfer (flower larceny; *e.g.* Irwin *et al.*, 2001), or whether flower visitors are effective or non-effective in their pollen transfer. Yet community studies are a first essential step in the analysis of generalization and specialization.

Since the publication of the two influential papers that questioning the importance of specialization (Herrera, 1996; Waser *et al.*, 1996) a growing number of studies during the last 10 years has studied interaction patterns between flowers and flower visitors or reanalyzed existing community-level studies, with new mathematical and statistical approaches with exciting results (Waser & Ollerton, 2006). For example, not so long ago it was considered common sense (at least implicitly) that plant–pollinator interactions are symmetric (Vázquez & Aizen, 2004, and references therein), *i.e.* generalists interact mainly with generalists and specialists with specialists (FIGURE 1.1, top). However, community-level studies revealed that the interactions between plants and flower visitors are mainly asymmetric (Bascompte *et al.*, 2003; Lewinsohn *et al.*, 2006; Memmott *et al.*, 2004a; Vázquez & Aizen, 2004), thus specialists interact primarily with generalists, whereas generalists interact with specialists and generalists (FIGURE 1.1, bottom).

Fairly little is still known about the factors leading to patterns of specialization and generalization at the community level (Jordano *et al.*, 2006; Vázquez & Aizen, 2004, 2006) or the potential consequences of these patterns for species extinctions and the stability of whole plant–flower visitor interaction webs (Ashworth *et al.*, 2004; Memmott *et al.*, 2004a). There is also a lack of knowledge how the degree of generalization affects the degree of morphological matching which should influence the per-visit pollination efficiency of the visitors (Campbell *et al.*, 1996; Johnson &

Steiner, 1997; Nilsson, 1988; although see Wilson, 1995). In particular, the impact of plant and visitor traits that may constrain the kind and number of potential interaction partners, and the frequency of these traits across species and individuals in a local community, have rarely been investigated (Jordano *et al.*, 2006; Vázquez, 2005).

This thesis is an effort to evaluate the reasons for, and the importance and consequences of community-wide patterns of specialization and generalization. My intent is to assess the potential influence of morphology and abundance on the degree of ecological specialization and generalization (*i.e.* the number of plant species visited or the number of visitor species on a plant species), the asymmetry of interactions, the extinction risk of species, and the degree of morphological matching between plants and visitors. To do this I will compare the observed patterns with expected patterns based on the result of simulation models incorporating different combinations of the potential factors. The study system is a species-rich Mediterranean plant–flower visitor community in the southeast of Spain.

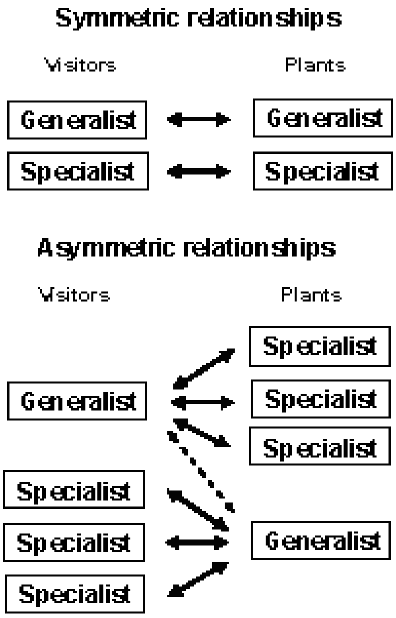

**Figure 1.1** – Reciprocity of relationships between generalized and specialized plants and visitors. An earlier view assuming 'symmetric' relationships (top) has been shown by recent community-level studies to be incorrect; instead interactions are 'asymmetric' (bottom), with specialist plants and animals tending usually to associate with generalist partners, although generalist plants and animals do also interact frequently.

I concentrate on the role of morphological traits that potentially constrain the interactions between nectar-producing flowers and nectar-foraging visitors: the depth at which the nectar is concealed inside the flower (which I refer to as 'nectar holder depth'), the width of the nectar hiding tube (which I refer to as 'nectar holder width'), and the size of the place where insects might alight on the flower as they feed (the 'alighting place'). The stronger the morphological restrictions a flower puts on the morphology of its potential visitors, the smaller the range of flower visitor traits that should be observed on a plant species and the more morphologically specialized this species is. This is shown in FIGURE 1.2 for nectar depth and proboscis length. I hypothesize that the smaller the expected morphological range of visitor traits, the fewer visitor species will be observed on a plant species and the closer the morphological fit. The same should be true for the visitor's point of view, thus the smaller the expected morphological range of plant traits, the fewer plant species a flower visitor should visit, and the closer the morphological fit with these plants. As estimates of abundance I chose the number of individuals (visitors) and the number of open flowers during peak flowering (plants). I hypothesize that the higher the abundance of species or

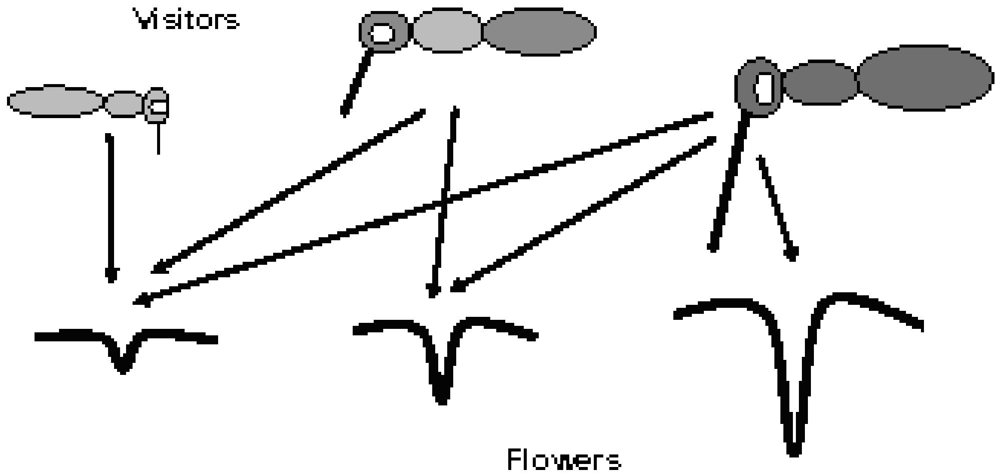

**FIGURE 1.2** – A cartoon depicting size constraints (nectar holder depth and proboscis length), which limit interactions between nectar-producing flowers and nectar-searching flower visitors. The insects possess short to long proboscises (top), and the flowers possess shallow to deep tubes (bottom). In principle, short proboscises can reach shallow but not deep nectar; longer proboscises can reach all nectar unless it is more deeply concealed than the proboscis is long.

resources, the larger the number of interaction partners and the higher the impact of an interaction partner on the degree of matching.

The thesis consists of five chapters of which this Introduction is CHAPTER 1 and the Summary is CHAPTER 5. In CHAPTER 2 I show that flower parameters set a size threshold on the morphology of flower visitors. I demonstrate that the number of observed visitor species decreases with increasing nectar holder depth and increases with increasing nectar holder width. Based on nectar holder depth and width the number of flower visitors that can potentially visit a plant species is determined. I demonstrate that the observed number of interaction partners is positively correlated with this potential number and that the observed interaction partners are a random draw out of the whole potential morphological range of visitor species. Within the constraints set by flower morphology, the number of flowers influences the number of interaction partners. The more flowers a plant species produces, the more animal species visit this plant species.

In CHAPTER 3 I ask whether there is a relationship between the degree of generalization of a species and the degree of generalization of its interaction partners and what the potential causes and consequences of this relationship are. In the first part of CHAPTER 3 I demonstrate that the Mediterranean flower visitation web I studied is asymmetrically organized, and that a size threshold in combination with random interactions proportional to species abundance among the potential interactions could be responsible for this asymmetric specialization. In the second part of CHAPTER 3 I study the influence of these factors on the extinction risk of species. The degree of asymmetry may have a profound impact on the extinction risk of a species. The more specialized the interactions, the more prone are the species to extinction by chance processes. If a flower visitation web is asymmetrically organized, this extinction risk might be equalized (Ashworth *et al.*, 2004) and the whole web might be more stable compared to a symmetrically organized one (Memmott *et al.*, 2004a). I show that, even if the web is asymmetrically organized, morphologically specialized species have higher extinction risks than morphologically generalized species. Because specialized species are less abundant in the studied web, the inclusion of species frequencies in the simulations increases the difference between specialists and generalists in extinction risk even more.

Chapter 4 takes up the influence of size thresholds on the degree of morphological matching between proboscis length and nectar holder depth. A close morphological match between flowers and 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 (Campbell *et al.*, 1996; Johnson & Steiner, 1997; Nilsson, 1988). 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). This size matching seems at odds with the fact that pollinators with long proboscises will in principle have access to shallow as well as deep flowers. However, the frequency of species and individuals with shallow and deep flowers or with short and long proboscises will influence the average degree of matching. My analysis of the Mediterranean flower visitation web reveals that flower visitors with a short proboscis indeed match on average the nectar depth of flowers more closely than those with a long proboscis. Conversely, plant species with hidden nectar and openly-presented nectar match their interaction partners on average equally closely. I show, under the assumption of random interactions proportional to abundance, that this overall relationship can be the result of the depth threshold and the observed proboscis length and nectar holder depth distributions. Both distributions are right-skewed and resemble seemingly ubiquitous log-normal body size distributions.

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chapter 2

# Size constraints and flower abundance determine the number of interactions in a plant–flower visitor web

*Martina Stang, Peter G.L. Klinkhamer & Eddy van der Meijden*

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## Abstract

The number of interactions with flower visitor species differs considerably among insect pollinated plants. Knowing the causes for this variation is central to the conservation of single species as well as whole plant–flower visitor communities. Species specific constraints on flower visitor numbers are seldom investigated at the community level. In this study we tested whether flower size parameters set constraints on the morphology of the potential nectar feeding visitors and thus determine the number of visitor species. We studied three possible constraints: the depth and width of tubular structures hiding the nectar (nectar holder depth and width) and the size of flower parts that visitors can land on (size of the alighting place). In addition we assess the role of flower abundance on this relationship. We hypothesized that the stronger size constraints and the smaller flower abundance, the smaller the number of visitor species will be. Our study of a Mediterranean plant–flower visitor community revealed that nectar holder depth, nectar holder width and number of flowers explained 71% of the variation in the number of visitor species. The size of the alighting place did not restrict the body length of the visitors and was not related to visitor species number. In a second step of the analyses we calculated for each plant species the potential number of visitors by determining for each insect species of the local visitor pool whether it passed the morphological limits set by the plant. These potential numbers were highly correlated with the observed numbers ($r^2 = 0.5$, $p < 0.001$). For each plant species we tested whether the observed visitors were a random selection out of these potential visitors by comparing the mean of the observed and expected proboscis length distributions. For most plant species the observed mean was not significantly different from the random means. Our findings shed light on the way plant–flower visitor networks are structured. Knowing the constraints on interaction patterns will be an important prerequisite to formulate realistic null models and understand patterns of resource partitioning as well as coevolutionary processes.

## Introduction

Plants pollinated by animals differ greatly in the number of interactions with visitor species, varying from one to more than hundred animal species (*e.g.* Ellis & Ellis-Adam, 1993; Jordano, 1987; Moldenke, 1975; Waser *et al.*, 1996). The mechanisms leading to this variation are still poorly understood (Johnson & Steiner, 2000). Especially the importance of species-specific constraints on this variation has seldom been investigated at the community level (Vàzquez, 2005; Waser *et al.*, 1996). In order to illustrate the role of constraints, we will use traits that are thought to have an important impact on flower visitors even if they are rarely tested as a factor determining the number of visitor species (*i.e.* the number of interactions with flower visitor species) in a community context. We will start from the most basic expectation that visitors will not be able to reach the nectar if their proboscis length is shorter than the depth of the nectar holder, or if their proboscis diameter is larger than the nectar holder width. Furthermore they may have difficulties landing on a flower if their body size exceeds the size of the alighting place; for example, butterflies prefer large blossoms (Corbet, 2000a). We hypothesize that the stronger the size constraints, the smaller the number of visitor species will be. Within the constraints set by flower morphology, the abundance of floral rewards may also influence the number of visitor species. Optimal foraging theory predicts that if a plant species offers a greater reward it will be visited by more individuals (*e.g.* Dreisig, 1995; Fretwell & Lucas, 1970; Pleasants, 1981) and, as a consequence, also by a higher number of visitor species (Possingham, 1992).

Only a few studies directly examined the relationship between size parameters and the number of visitor species. They do not show a clear picture. Herrera (1996) found that within the plant species he studied plants with a flower tube depth shorter than 10 mm were visited by a significantly larger number of visitor species than plants with a flower tube deeper than 20 mm. Agosta and Janzen (2005) analyzed data provided by Haber and Frankie (1989) and showed that there is a significant association between flower tube depth of hawkmoth flowers and visitor richness of hawkmoths. Yet, there was no relationship found between flower tube depth of Asteraceae species and visitor numbers (Torres & Galetto, 2002) or flower depth of *Echium* species and the number of visiting bee species

(Dupont & Skov, 2004). Likewise, an analysis of data provided by Harder (1985) and Corbet (2000a) revealed no significant relationship between flower tube depth and number of bumblebee or butterfly species, respectively. Conversely, it seems well established that there is a positive relationship between the total number of visitor species found in a community at a given time and the floral abundance of all plant species (Heithaus, 1974; Moldenke, 1975; Potts *et al.*, 2003; Steffan-Dewenter & Tscharntke, 1997) while nectar volume, nectar sugar composition or energy content of pollen were unrelated with the number of visitor species (Petanidou & Ellis, 1996; Petanidou & Vokou, 1990; Potts *et al.*, 2003; Torres & Galetto, 2002). Nevertheless, plant species based comparisons between resource parameters and visitor species numbers at the community level are rare or even missing.

Plant–flower visitor communities can be studied in the manner of food webs or networks (*e.g.* Dicks *et al.*, 2002; Memmott, 1999; Olesen & Jordano, 2002). An important parameter that might influence the stability of a food web is the connectance, *i.e.* the percentage of all possible interactions within a community that are actually observed. The number of all possible interactions (the size of the plant–flower visitor network) is calculated by multiplying the number of plant species with the number of flower visitor species. Yet, the number of interactions that is actually expected might be strongly reduced by morphological constraints and thus depends on the species composition (Jordano, 1987; Jordano *et al.*, 2003; Warren, 1994). Morphological traits of the plants act as filters allowing only certain visitors the access to nectar and or pollen. Constraints are usually ignored in flower visitation web analyses because of missing morphological information about whole plant–flower visitor communities (Olesen & Jordano, 2002; Vazquez, 2005). Yet, with this information we will better understand the frequency distribution of specialization levels within flower visitation webs and thus community wide patterns of linkage levels (Ollerton & Cranmer, 2002) and connectance (Olesen & Jordano, 2002). We also think that constraints on interaction patterns will be an important prerequisite to formulate realistic null models to understand interaction patterns.

In this study we examine whether the number of nectar feeding visitor species is related to flower size parameters and flower abundance in a local plant–flower visitor community. The restriction to nectar-feeding visitors is essential given the traits we want to investigate. What is more, nectar-producing flowers are normally better adapted to direct nectar-feeding visitors into an optimal position for pollination than visitors searching for pollen (Westerkamp, 1987). We chose a Mediterranean plant–flower visitor community because of the potentially high species diversity of flower visitors (Petanidou & Ellis, 1993). We based our analysis on a complete flower visitation web, *i.e.* we included all insect orders observed on the plant species. The total number of open flowers in the observation plots was used as a measurement of flower abundance. We decided to use equal observation periods for all plant species because differences in observation effort can alter the number of observed visitors independent of size constraints and flower abundance (Ollerton & Cranmer, 2002).

In order to test if a possible association between morphology and visitor numbers is based on a causal relationship we analyzed whether flower morphology constrains the morphology of nectar foraging visitors. We realize that visitors sometimes may overcome morphological limitations such as nectar robbers piercing corollas, small insects able to enter the nectar holder tube with parts of their body and hovering hawkmoths or beeflies that do not need to alight on a flower to feed nectar. However, if the traits chosen act as important constraints for the majority of the visitor species, we expect that the potential number of visitor species on a plant species is positively correlated with the actually observed number of visitor species on a plant species. We define the potential number of visitors of a plant species as those visitors that pass the morphological thresholds within the total sample of flower visitors observed in the flower visitation web. To test if our assumption holds that the observed visitors of a plant species came from the whole morphological spectrum of the potential visitors of that plant species, we performed Monte Carlo simulations tests and examined whether the observed visitors are a random draw out of the potential visitors. Specifically we want to test the following hypotheses based on null models using the frequency distribution of visitor traits in the local visitor community:

- The number of flower visitor species decreases with increasing size constraints and decreasing flower abundance.

- Flower morphology sets thresholds on the morphology of nectar foraging visitors.
- If so, the potential number of visitor species based on these thresholds is positively correlated with the observed number of visitor species.
- The observed visitors on a plant species are a random draw out of the whole potential morphological range of visitors of that plant species.

## Materials and Methods

### *Study site and selection of plants*

The study was conducted in a Mediterranean vegetation mosaic in the southeast of Spain (15 km to the west of Alicante, 38°22' N, 0°38' W). The vegetation was a combination of garigue, almond tree groves and roadside vegetation. We selected 10 observation plots of 200 $m^2$ within a 25 m wide strip of a road segment of 3 km length. In each of the plots we selected all nectar producing plant species with more than 5 flowering individuals in that plot. Observations were made during 6 weeks in March and April 2003. The selection resulted in 25 plant species distributed over 11 plant families, representing all main structural blossom types (Faegri & van der Pijl, 1979).

### *Flower size parameters*

From 5 to 10 individuals of each plant species, we selected flowers which were in the male or hermaphrodite phase. We measured depth and width of the nectar holder tube and size of the alighting place to the nearest 0.10 mm with a digital calliper under a dissecting microscope. Because tubes were formed by hairs, the receptacle, the calyx, the corolla, filaments or a combination of organs, we use the term nectar holder tube instead of the more widespread but in our case incorrect term corolla tube. In some species a nectar holder tube was absent and nectar glands were openly accessible. In this case nectar holder depth was scored as 0 mm. The depth of nectar holder was measured from the base to the top of the nectar holder. The top is the entrance of the nectar holder at the point where only a proboscis can enter, and is normally smaller than 1.0 mm. Nectar standing crop of the investigated species was generally small and the observed height of nectar levels in the field was low. This seems typical for plant species of Mediterranean dry habitats (Petanidou & Smets, 1995). Only in *Matthiola fruticulosa* (Loefl. ex L.) Maire we observed nectar levels of 1 to 2 mm above the nectaries so that a visitor with a shorter proboscis than the measured nectar holder depth can reach the nectar. This species opens its flowers late in the afternoon and seems to be adapted to night-flying visitors. In the Asteraceae we measured the depth of the upper wider part of the corolla, which roughly begins where the stamens insert and ends where the corolla flares out (Corbet, 2000a). At the bottom of the wider part you can find sometimes traces of nectar. None of the observed visitor species was physically able to enter the narrow part of the tube. The width of the nectar holder was measured at the middle of the tube after a cross section. If nectar was openly presented the diameter of the nectar glands was used. Almost all observed visitors landed on the flowers to get access to nectar (the observed exceptions were some large beeflies). The alighting place was measured as the distance between the entrance of the nectar holder tube and the functional border of the pollination unit or blossom (Faegri & van der Pijl, 1979).

### *Flower abundance*

We estimated the total number of open flowers in the 10 observation plots by multiplying the mean number of open flowers per blossom with the mean number of blossoms per individual and the total number of individuals. The total number of flowering individuals was counted once in the 10 observation plots during the observation period of a species. The number of flowering blossoms per individual and the number of open flowers per blossom were estimated by counting these parameters on 10 to 20 individuals within 3 plots of 10 by 10 m.

### *Flower visitor censuses*

Each plant species was observed four times 15 minutes long. Within each observation period we changed about every minute the observed individuals of a plant species within a plot. The four observation periods were evenly distributed between 10 am to 6 pm, including only day flying visitor species. Species that present their food only during a part of the day were observed only during that period (*e.g. Sonchus tenerrimus* L., *Reichardia tingitana* (L.) Roth., *Linum suffruticosum* L., *Matthiola fruticulosa* (Loefl. ex L.) Maire). We randomly spread the four observation periods over different observation plots and sampling days within a 15 day period for each species. We recorded if a visitor collected nectar, pollen or both, and counted the number of visiting individuals per visitor species. Only those visitors were included that were visiting a minimum of 3 flowers in sequence or stayed more than 3 seconds in a flower to exclude accidental visitors. We observed 1206 individuals of which 887 fed on nectar or nectar and pollen. The majority of the nectar feeding individuals in our study picked up pollen and touched stigmas during their visits. Nevertheless, pollination efficiency of the different species and even individuals may differ considerably.

### *Visitor traits*

The insect species were, if possible, identified to species level or otherwise to family or genus level and then assigned to 'morphospecies' categories. We are confident that these morphospecies represent in most cases single taxonomic species. One to 11 specimens of each species were collected. All voucher specimens are kept by the first author. Size parameters were measured from in total 278 specimens immediately after killing to ensure the flexibility of the mouthparts (hereafter called proboscis). We used a digital calliper and measured the proboscis and body dimensions to the nearest 0.10 mm under a dissecting microscope. For the Hymenoptera the length of the proboscis was measured as the length of the fully extended prementum and glossa. For long tongued bees it reflects the maximum depth to which an individual can probe, the normal extension of the proboscis during nectar feeding (functional length) is about 70% of the maximum length (Harder, 1982). For short tongued bees the length of prementum and glossa represents both the functional and maximum length of the mouthparts (Harder, 1983). The proboscis of the Diptera (labium) was measured after slightly pulling it out of the head because it often has a contractile basal part (*e.g.* Gilbert, 1981). The proboscis of the Lepidoptera was unrolled before measuring. Within the Coleoptera we used the length of the mandibles. The proboscis diameter was defined as the broadest part within the first millimetre of the tip of the proboscis after preparation for length measurements. Before measuring the body length insects were straighten and the length of the body parts (head, thorax, abdomen) was measured according to common determination literature instructions. Body length was functionally defined as the length of head, thorax and femur because the abdomen plays no role in the landing ability of the flower visitors. Total body length (head, thorax and abdomen) and functional body length were highly correlated ($r = 0.95$, $p < 0.001$, $n = 111$).

### *Data analysis*

Statistical analysis was performed using SPSS 11.0. A Kolmogorov-Smirnov test was used to test if the variables were normally distributed. Nectar holder width, total number of flowers, observed number of visitor species, potential number of visitor species and the ratio of observed to potential visitor species were log transformed to achieve normality. Relationships between flower parameters were tested with Pearson correlations. Correlations between size parameters of insects were tested with Spearman rank correlations because transformations did not result in normally distributed variables. We tested the association between flower parameters and number of visitor species with multiple least square regression and backward selection of variables.

We analysed which of the three size parameters restricted the observed visitors and used those that did so to determine the potential number of visitor species. We tested the minimum nectar holder depth, the maximum nectar holder width and the maximum alighting place length (APPENDIX 2.1). Minimum and maximum values better reflect the actual limits to potential flower–visitor interactions than mean values. Those insect species of the local visitor pool were treated as potential visitors of a plant species that met with their morphology the morphology of the flowers: a proboscis as long as or longer than the nectar holder depth and a proboscis as small as or smaller than the nectar holder width (see result section). We tested the relationship between potential and observed number of visitors with linear regression. The difference in the explained variance of the two regression models (the potential visitors based on nectar holder depth alone and that based on a combination of nectar holder depth and width) was tested with a paired samples t-test.

In order to estimate if the observed visitors that met the size criteria are a random selection out of the potential visitors we performed Monte Carlo simulation tests (Hood, 2005). We chose the mean proboscis length as a test variable and compared the observed mean with the means of 1000 random draws (without replacement) from the potential visitors. The observed number of visitors on a plant species was used as the sample size. We considered the observed mean to be significantly different from the random means if it was smaller than the 25 smallest or larger than the 25 largest random means. This difference statistic is provided by the programme poptools (Hood, 2005).

One drawback of equal and relatively short observation periods is that the ratio of observed to potential visitors may not be constant, but could decrease with weaker size constraints and thus an increasing potential number of visitors, likely because of the increasing time needed to encounter all potential species. To asses if the ratio of observed to potential visitors declined with increasing potential number of visitors we adopted the approach of Klinkhamer *et al.* (1990). The ratio of observed to potential visitors has to be calculated by dividing the potential by the observed number of visitors so that the potential number of visitors would be included in the independent and the dependent variable, which may result in an artificial correlation. To avoid this problem we tested with an F-test if the regression coefficient of the log transformed numbers of observed versus potential visitors was significantly smaller than 1.

## Results

### *Flower traits*

Among the 25 plant species nectar holder depths varied between 0 to 10 mm and nectar holder widths between 0.1 to more than 2 mm (APPENDIX 2.1). Depth and width of the nectar holders were not correlated ($r = -0.06$, $p = 0.79$, $n = 25$). The size of the alighting place varied between 2.9 and 15.7 mm. It was neither correlated with nectar holder depth ($r = 0.07$, $p = 0.75$, $n = 25$) nor with nectar holder width ($r = -0.01$, $p = 0.96$, $n = 25$). The number of flowers per plant species varied almost 900 fold with a minimum of about 400 flowers (*Erodium macaloides* (L.) L'Her.) and a maximum of almost 330 000 flowers (*Helichrysum stoechas* (L.) Moench). The deeper the nectar holder the smaller the number of open flowers that were available for the visitors ($r = -0.51$, $p = 0.01$, $n = 25$). There was no significant relationship between nectar holder width and number of flowers ($r = 0.14$, $p = 0.493$, $n = 25$).

### *Visitor traits*

The 111 nectar feeding visitor species covered 5 orders. The Hymenopterans were the species richest group with 55 species (42 bees, 9 wasps and 5 ants), followed by the Dipterans with 35 species (17 'muscoid' flies, 7 hoverflies, 5 beeflies and 6 other), the Lepidoptera with 9 species (7 butterflies and 2 moths), the Coleopterans with 7 species and the Heteropterans with 5 species. With 662 observed individuals the Hymenoptera were the most common visitors, even if the 298 individuals of *Apis melifera* were excluded. The distributions of proboscis length (0.1-14.0 mm) and diameter (0.1-0.6 mm) were positively skewed with a mean of 3.45 mm and 0.23 mm, respectively. Both parameters were not significantly correlated ($r_s = -0.13$, $p = 0.162$, $n = 111$) but the distribution was clearly triangular with a linearly decreasing upper ceiling (FIGURE 2.1a). Species with a short proboscis show a large variation of proboscis diameters. With increasing proboscis length mean proboscis diameter as well as variation in diameters decrease. Long proboscises are mostly thin. The number of individuals per insect species increased with increasing proboscis length ($r_s = 0.29$, $p = 0.002$, $n = 111$). Functional body length (1.5-11.2 mm) was normally distributed and positively correlated with proboscis length ($r_s = 0.84$, $p < 0.001$, $n = 111$), again, with an obviously triangular distribution (FIGURE 2.1b). Species with a short proboscis had small or large bodies, long proboscises were only found in species with large bodies.

### *Flower traits and observed number of visitor species*

The number of visitor species on each plant species ranged from 1 to 29 insect species (mean of 9.24, median of 7.0, APPENDIX 2.1). The number of visitor species decreased with increasing nectar holder depth ($r^2 = 0.36$,

$p = 0.002$, $n = 25$, FIGURE 2.2a) and decreasing nectar holder width ($r^2 = 0.25$, $p = 0.011$, $n = 25$, FIGURE 2.2b), while there was no significant correlation with the size of the alighting place ($r^2 = 0.004$, $p = 0.984$, $n = 25$, FIGURE 2.2c). Species with a large total number of open flowers were visited by more visitor species ($r^2 = 0.45$, $p < 0.001$, $n = 25$, FIGURE 2.2d).

The simple regressions are still significant after a Bonferroni correction for multiple single comparisons has been applied. (*i.e.* critical *p*-value

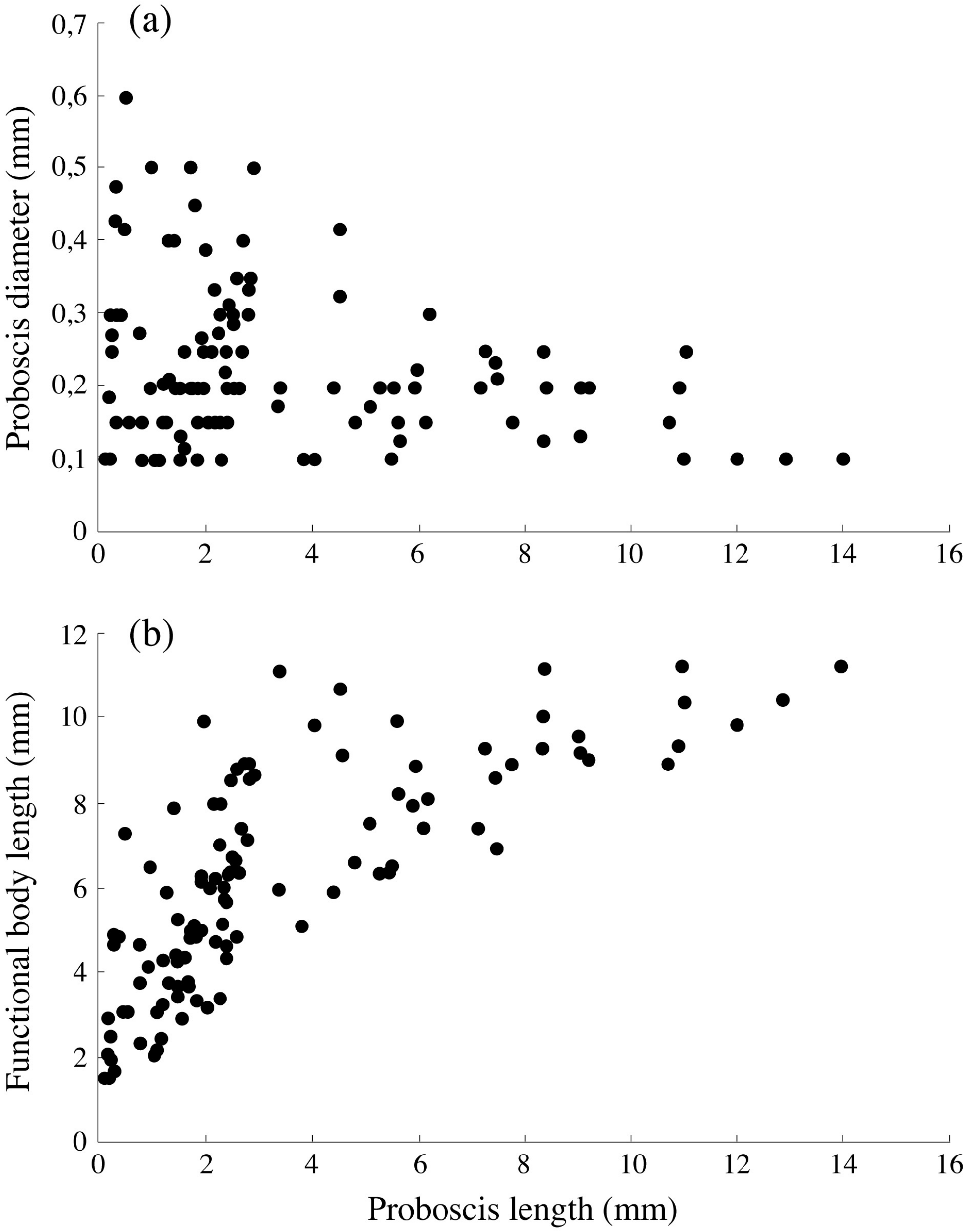

**FIGURE 2.1** – Relationship between proboscis length and (a) proboscis diameter and (b) functional body length (the length of head, thorax and femur). Each dot represents one insect species.

< 0.0125). A multiple regression analysis show that nectar holder depth, nectar holder width and flower abundance explained 71% of the variation in the observed number of visitor species ($r^2 = 0.71$, $p < 0.001$, $n = 25$, TABLE 2.1). The three variables contribute significantly and almost equally to the explained variation.

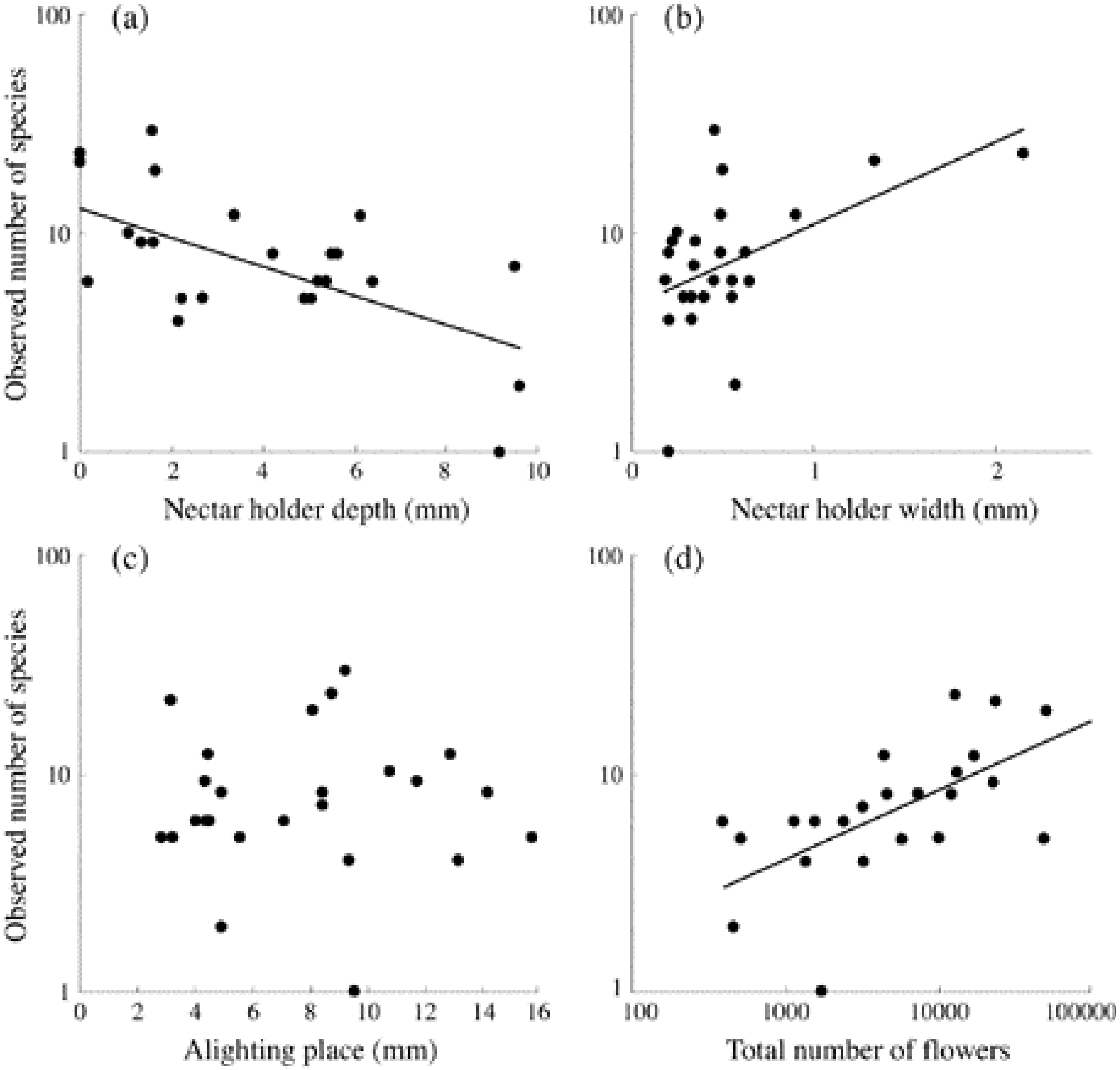

**FIGURE 2.2** – Relationship between flower traits and observed number of visitor species. (a) nectar holder depth, (b) nectar holder width, (c) length of the alighting place, (d) total number of flowers. Each dot represents one plant species. Y axes in all four graphs are on a logarithmical scale. Nectar holder width is log transformed before statistical analysis to achieve normality; values in the graph are given without transformation.

**Table 2.1** – Multiple regression (method backward selection of variables) with the number of observed visitor species as the dependent variable and nectar holder depth, nectar holder width, alighting place length and total number of flowers as independent variables. Alighting place length was excluded from the model: ($t$ = 1.53 and $p$ = 0.141). The explained variance of the presented model is 71% ($r^2$ = 0.71, $p$ < 0.001, $n$ = 25).

| Independent variables | Standardized coefficient (Beta) | $t$ | $p$ |
|---|---|---|---|
| (constant) | | 1.587 | 0.127 |
| nectar holder depth | -0.359 | -2.643 | 0.015 |
| log nectar holder width | 0.418 | 3.532 | 0.002 |
| log total number flowers | 0.429 | 3.134 | 0.005 |

### *Size constraints and potential number of visitor species*

Only 7.5% of the 887 observed insect individuals were insects with a proboscis shorter than the nectar holder depth and 3.5% were insects with a proboscis larger than the nectar holder width (together 8.7%). Nectar holder depth exceeded proboscis length by maximally 1.5 mm and proboscis diameter exceeded nectar holder width by maximally 0.1 mm. The observed minimum proboscis length of the insect species visiting a plant species was strongly correlated with nectar holder depth ($\text{length}_{\text{min}}$ = 0.95 * depth – 0.15, $r^2$ = 0.89, $p$ < 0.001, $n$ = 25). The alighting place did not restrict body length of the visitors. Almost 38% showed a longer functional body length than the length of the alighting place.

Based on the previous results we calculated the potential number of visitors, firstly by using the nectar holder depth alone and secondly by the combination of the nectar holder depth and width (see Appendix 2.1). In both cases a significant positive correlation with the observed number of visitors was found ($r^2$ = 0.39, $p$ = 0.001, $n$ = 25 and $r^2$ = 0.50, $p$ < 0.001, $n$ = 25, respectively, Figure 2.3). The explained variance was higher in the latter one, although the difference was not significant ($t$ = 1.787, $p$ = 0.087). In a multiple regression analysis with potential number of visitors (based on nectar holder depth and width) and flower abundance as independent variables, flower abundance ($p$ = 0.046) increased the explained variance in observed number of visitors even further ($r^2$ = 0.59, $p$ < 0.001, $n$ = 25).

The potential number of visitors (based on nectar holder depth and width) decreased exponentially with increasing nectar holder depth ($r^2$ = 0.88, $p$ < 0.001, $n$ = 25). Nectar holder width has the largest influence on the number of potential visitors for flowers with short nectar holder

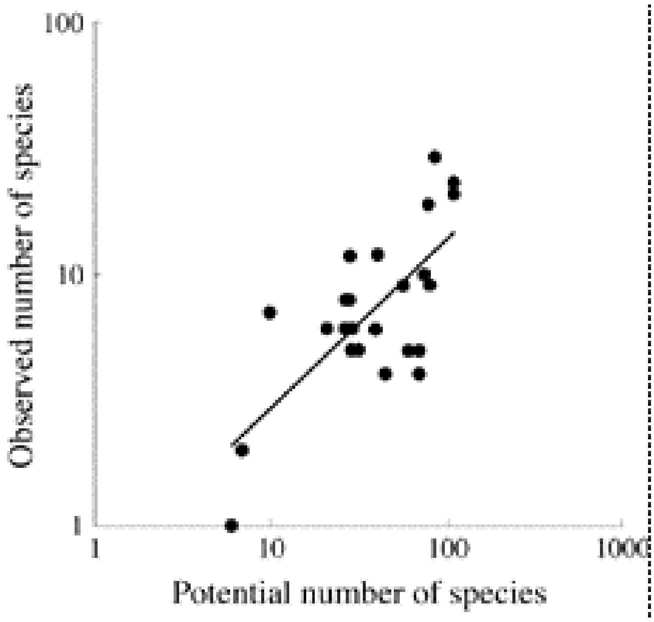

**FIGURE 2.3** – Relationship between potential and observed number of visitor species. Potential number of species was determined based on the nectar holder depth and width constraint. Each dot represents one plant species. Both axes are on a logarithmical scale to achieve normality.

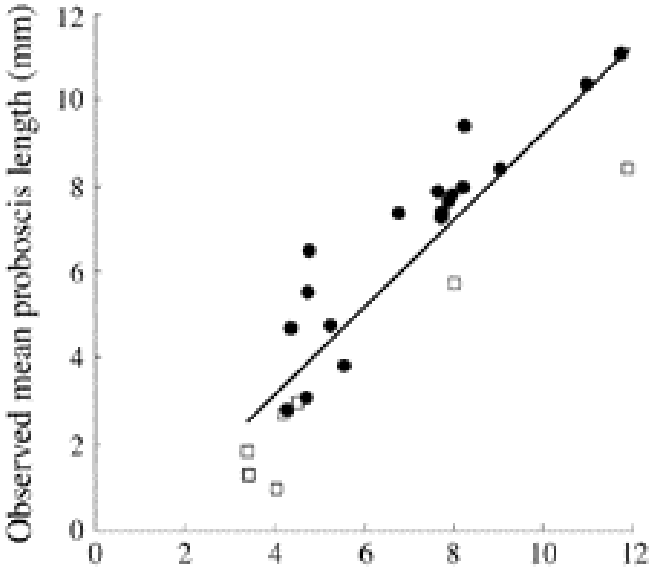

**FIGURE 2.4** – Observed versus random mean proboscis lengths. The observed means that differed significantly from the random means are indicated with an open square. Both variables are significantly correlated ($r^2 = 0.81$, $p < 0.001$, $n = 25$). Each dot represents one plant species. The plant species that differ significantly from a random draw are *Anacyclus valentinus*, *Erodium malacoides, Euphorbia serrata, Euphorbia terracina*, *Helichrysum stoechas, Matthiola fruticulosa* and *Sideritis leucantha.*

tubes (APPENDIX 2.1). For most of the plant species (72%) the proboscis lengths of the observed visitors are random selections out of the proboscis lengths of the potential visitors (FIGURE 2.4).

### *Ratio of observed to potential visitor species*

On average about a quarter of the potential visitors were observed on a plant species (0.23 ± 0.14). This ratio increased slightly but significantly with increasing nectar holder depth and decreasing nectar holder width ($r^2 = 0.384$, $p = 0.005$, $n = 25$), *i.e.* the stronger the size constraints, the higher the ratio of observed to potential visitors. The regression coefficient of the log transformed number of observed versus potential visitors was significantly smaller than 1 ($F = 5.846$, $p = 0.023$), indicating that the ratio of observed to potential visitors decreased significantly with increasing potential number of visitors. The ratio was not correlated with flower abundance ($r^2 = 0.00$, $p = 0.97$, $n = 25$), yet, a multiple regression of flower abundance and potential number of visitor species against this ratio revealed an almost significant contribution of flower abundance ($t = 2.08$, $p = 0.05$, $n = 25$).

The ratio of all 231 actually observed plant species–insect species interactions to all 2775 possible interactions was 0.08 (*i.e.* the connectance of this plant–flower visitor community). Due to the size constraints, the actually expected interactions were reduced by 57%, *i.e.* from 2775 to 1195. This means that more than half of the not observed interactions of the whole community can be explained by size constraints.

## Discussion

### *Flower parameters and number of visitor species*

Our results clearly show that most of the variation in the number of nectar foraging visitor species can be explained on basis of two simple morphological constraints and flower abundance. The number of visitor species significantly decreased with increasing nectar holder depth and decreasing nectar holder width. The size of the alighting place was unrelated to the number of visitor species. This is the first report of an association between nectar holder sizes and the number of visitor species in a community-based study. Most other studies analyzing size parameters have been based on broader geographical areas or have included only one plant family or one visitor group, and they have found only in part an association (Dupont & Skov, 2004; Haber & Frankie, 1989; Herrera, 1996; Torres & Galetto, 2002). Although the range of nectar holders in the community studied here was only one third to one half of the range analyzed in other studies (Herrera, 1996; Torres & Galetto, 2002), the relationship between nectar holder morphology and species number was still strong. The observed strength of the relationship could be partly caused by the fact that the analysis was, contrary to other studies (*e.g.* Dupont & Skov, 2004; Torres & Galetto, 2002), restricted to nectar foraging visitors. That the number of flowers per plant species was positively related to the number of nectar feeding insect species is in accordance with predictions of optimal foraging theory (Possingham, 1992) as well as with other empirical data (Heithaus, 1974; Moldenke, 1975; Potts *et al.*, 2003). Although each of the three flower parameters alone was significantly correlated with the number of visitor species, only the combination explained the high amount of variation in species numbers and stresses the importance to include all of them in a study which tries to explain the level of ecological specialization to flower visitors.

### *Size constraints as a determinant of the number of visitor species*

Although rarely, we sometimes observed insect species that seem to be able to overcome size constraints because they were visiting flowers that had longer and narrower nectar holders than their proboscis lengths and diameters would let expect. These observations may be explained by a number of reasons. Nectar can accumulate so that nectar levels can be considerably higher than the base of the nectar holder. Additionally, flowers with very short nectar holder tubes were sometimes visited by small insects with head diameters that are smaller than that of the nectar holder (personal observation). Species of the Brassicaceae and Fabaceae have often flexible nectar holder widths because the petals forming the nectar holder tube are not fused. Given these exceptions, the percentage of visitor species that fell outside the limits set by the nectar holder was with less than 9% remarkably low. Conversely, the percentage of visitors with a functional body length that exceeds the potential size limits of the alighting place was with almost 38% high. This is mainly a result of the flexibility of insect behavior. Sometimes visitors used the whole diameter of a flower or adjacent flowers to sit on, as well as parts of the calyx of sideward orientated flowers with a lower lip as alighting place. Some of the visitors (such as beeflies) hover in front of a flower.

As expected, potential and observed number of visitor species was positively related. The relationship was stronger if the potential visitors were determined with both nectar holder size constraints, although the increase in explained variance was marginally not significant. Visitors with short proboscises had a much higher variance of proboscis widths than visitors with a long proboscis (FIGURE 2.1a). Given this distribution, especially flowers with short nectar holder tubes can restrict the number of visitors by narrowing down nectar holder width. This explained why nectar holder width has the largest influence in restricting the number of potential visitors for flowers with short nectar holder tubes. For about three quarter of the plant species, the mean proboscis length of the observed visitors could not be distinguished from a random selection out of the potential visitors. For all cases that differ significantly from a random draw, the observed mean proboscis length was smaller than the random mean, indicating a better matching between nectar holder depth and proboscis length of the visitors than expected by chance. Five of the seven plant species that differ significantly from a random draw had dish-shaped blossoms with easily accessible nectar. It is very likely that these plant species have a low nectar production per flower. In Mediterranean shrublands nectar holder depth is positively correlated with nectar volume and negatively with nectar concentration (Petanidou & Smets, 1995). Flower visitors with a long proboscis often need more energy because of their larger body sizes (proboscis length and body size is positively correlated). For them it is not profitably to exploit flowers with a low nectar production if they are scarce. Visitors with a long proboscis may have also more difficulties to exploit highly concentrated nectar (Gilbert & Jervis, 1998).

### *Ratio of observed to potential visitors*

On average 23% of the potential visitors were observed on a plant species. This percentage was larger for plant species with stronger size constraints and thus a smaller potential number of visitor species. The decreasing ratio of observed to potential visitors might be an artefact caused by the sampling procedure. Observed species richness increases with increasing sampling effort (Ollerton & Cranmer, 2002), and with increasing potential number of species the time needed to observe all potential visitors will increase. This relationship was very likely intensified by flower abundance (even if the relationship with the ratio was only marginally significant) as well as by the increasing observed number of individuals per insect species with increasing proboscis length. As a result, the variation in the observed number of species was partly masked, and we expect that the difference in the number of flower visitor species (*i.e.* the level of ecological specialization to flower visitors) will be even larger when based on longer observation intervals.

### *Implications for the analysis of flower visitation webs*

Our findings have important implications for community based studies analysing the structure of whole plant-pollinator webs or interaction networks (Dicks *et al.*, 2002; Memmott, 1999; Memmott & Waser, 2002; Olesen & Jordano, 2002; Vàzquez & Aizen, 2003). In network analysis the number of possible interactions is defined as the product of the number of plant and animal species. Usually only a small number of these possible interactions are actually observed. The important question is whether the ones that are not observed are drawn by chance or for some reason cannot occur. In the latter case they are referred to as forbidden interactions or links (Jordano *et al.*, 2003; Vàzquez, 2005). Jordano (1987) suggested that an increasing corolla length would cause an exponential decrease in the fraction of potentially interacting mutualists in a plant-pollinator network. We were able to show this exponential decrease based on a local visitor species pool. As a result, size constraints explain in our system about half of the not observed interactions and only within the allowed insect species the visitors were a random draw. The restriction of an analysis to the frequency of visitors as the most parsimonious explanation for the number of insect species per plant species as proposed by Vàzquez (2005) will obscure the underlying mechanism of this relationship. We have shown that the number of insect individuals increased with increasing proboscis length, indicating that constraints are very likely the underlying cause of the association between the frequency of visitors and the number of plant species visited.

Connectance and the mean number of interactions per plant species within a community differs considerably (Olesen & Jordano, 2002; Ollerton & Cranmer, 2002). On basis of our results we suppose that this difference is caused by a shift in the morphological character distribution of the plant and visitor species. However, flowers are visited by nectar and pollen visitors. Following up studies should thus include pollen foraging visitors as well as the traits that will restrict them, *e.g.* whether pollen is free accessibility or hidden in flower structures. Phenological mismatching between flowers and visitors (Jordano *et al.*, 2003) were not likely for our dataset because of the restricted observation time of 6 weeks.

As far as we know, our study is the first that documented morphological constraints and their significance for the variation in the number of flower visitors in a local plant–insect visitor community including a broad range of plant families and insect orders. It is also the first that based the potential number of flower visitors on size constraints. Size constraints and floral abundance will provide an important basis to understand interaction patterns in flower visitation webs. Knowing the constraints on these patterns will be an important prerequisite to formulate realistic null models (Gotelli & Graves, 1996; Vàzquez, 2005; Vàzquez & Aizen, 2003) and understand resource partitioning and compartmentalization in studies that include the visitation frequency of the flower visitors (Dicks *et al.*, 2002). It may help to predict the susceptibility of flower visitation webs to disturbance and thus facilitate the conservation of species diversity (Corbet, 2000b; Memmott *et al.*, 2004a). Interactions patterns will on their part influence the co-evolution of flowers and their pollinators (Jordano, 1987; Jordano *et al.*, 2003).

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## APPENDIX 2.1

Flower size parameters, flower abundance and number of visitors. Total number of open flowers in the 10 observation plots; mean (range) of flower size parameters (in mm,

| Plant species (plant family) | Nu. of flowers | Nectar holder | |
|---|---|---|---|
| | | *depth* | *width* |
| *Anacyclus valentinus* (Asteraceae) | 51005 | 1.65 (1.5–1.8) | 0.5 (0.4–0.55) |
| *Anthyllis terniflora* (Fabaceae) | 12081 | 4.2 (3.9–4.4) | 0.2 (0.15–0.25) |
| *Asphodelus fistulosus* (Liliaceae) | 3191 | 2.16 (1.6–2.45) | 0.33 (0.25–0.4) |
| *Bituminaria bituminosa* (Fabaceae) | 1136 | 6.36 (5.9–6.8) | 0.45 (0.4–0.5) |
| *Centaurea aspera* (Asteraceae) | 4602 | 5.5 (4.9–5.9) | 0.63 (0.4–0.7) |
| *Convolvulus althaeoides* (Convolvulaceae) | 514 | 4.92 (4.3–5.3) | 0.55 (0.5–0.6) |
| *Coris monspeliensis* (Primulaceae) | 2404 | 5.38 (5.15–5.6) | 0.65 (0.6–0.7) |
| *Diplotaxis erucoides* (Brassicaceae) | 10103 | 2.66 (1.5–3.3) | 0.29 (0.25–0.35) |
| *Dorycnium pentaphyllum* (Fabaceae) | 50540 | 2.24 (2.1–2.5) | 0.4 (0.3–0.5) |
| *Echium creticum* (Boraginaceae) | 4454 | 6.1 (5–6.6) | 0.9 (0.85–0.95) |
| *Erodium malacoides* (Geraniaceae) | 385 | 0.19 (0.1–0.25) | 0.18 (0.15–0.2) |
| *Euphorbia serrata* (Euphorbiaceae) | 12781 | 0 | 2.16 (1.9–2.3) |
| *Euphorbia terracina* (Euphorbiaceae) | 23510 | 0 | 1.34 (1.3–1.4) |
| *Helichrysum stoechas* (Asteraceae) | 229840 | 1.58 (1.2–1.8) | 0.45 (0.35–0.5) |
| Linum *suffruticosum* (Linaceae) | 1366 | 2.12 (1.8–2.3) | 0.2 (0.15–0.25) |
| *Matthiola fruticulosa* (Brassicaceae) | 1720 | 9.14 (8.4–9.8) | 0.2 (0.15–0.25) |
| *Moricandia arvensis* (Brassicaceae) | 3163 | 9.54 (8.9–10.9) | 0.34 (0.3–0.4) |
| *Phagnalon saxatile* (Asteraceae) | 23026 | 1.62 (1.2–1.8) | 0.35 (0.3–0.4) |
| *Reichardia tingitana* (Asteraceae) | 22717 | 1.36 (1.2–1.7) | 0.22 (0.2–0.25) |
| *Rosmarinus officinalis* (Lamiaceae) | 1577 | 5.21 (4.6–6.2) | 0.55 (0.45–0.7) |
| *Sideritis leucantha* (Lamiaceae) | 5720 | 5.04 (4.7–5.5) | 0.33 (0.25–0.45) |
| *Sonchus tenerrimus* (Asteraceae) | 13027 | 1.08 (0.9–1.2) | 0.25 (0.2–0.3) |
| *Teucrium murcicum* (Lamiaceae) | 17101 | 3.36 (2.7–3.9) | 0.49 (0.4–0.55) |
| *Vella lucentina* (Brassicaceae) | 7324 | 5.62 (5.2–6.85) | 0.49 (0.4–0.65) |
| *Vicia pseudocracca* (Fabaceae) | 462 | 9.6 (9.5–9.8) | 0.57 (0.5–0.7) |

based on 5 to 10 flowers); number of individuals, observed number of visitor species and potential number of species (first column is based on nectar holder depth constraint, second column is based on nectar holder depth and width constraint).

| Alighting place | Observed nu. of | | Potential nu. of species | |
|---|---|---|---|---|
| *length* | *individuals* | *species* | *depth* | *depth + width* |
| 8.08 (7–9.2) | 41 | 19 | 78 | 78 |
| 4.9 (4.5–5.2) | 41 | 8 | 33 | 27 |
| 9.4 (8–10.25) | 56 | 4 | 76 | 71 |
| 4.42 (4.2–4.6) | 16 | 6 | 21 | 21 |
| 14.18 (13.1–15.5) | 32 | 8 | 28 | 28 |
| 15.74 (15.3–17) | 24 | 5 | 32 | 32 |
| 4.05 (3.5–4.85) | 23 | 6 | 27 | 27 |
| 5.56 (5.4–6) | 26 | 6 | 78 | 70 |
| 3.22 (2.6–3.6) | 41 | 5 | 62 | 61 |
| 12.9 (12.1–15.5) | 91 | 12 | 28 | 28 |
| 4.51 (3.4–5) | 13 | 6 | 110 | 39 |
| 8.74 (8.2–9.5) | 71 | 23 | 111 | 111 |
| 3.14 (2.5–3.5) | 47 | 21 | 111 | 111 |
| 9.18 (8.05–9.55) | 88 | 29 | 88 | 86 |
| 13.16 (10.8–16.3) | 7 | 4 | 71 | 45 |
| 9.54 (7.6–11) | 1 | 1 | 7 | 6 |
| 10.42 (9.5–12) | 14 | 7 | 10 | 10 |
| 4.32 (4.1–4.6) | 38 | 9 | 88 | 81 |
| 11.72 (10.5–12.2) | 23 | 9 | 88 | 57 |
| 7.1 (6–8.3) | 88 | 6 | 29 | 29 |
| 2.9 (2.5–3.5) | 11 | 5 | 29 | 29 |
| 10.8 (10.1–11) | 35 | 10 | 94 | 74 |
| 4.44 (3.7–5.4) | 34 | 12 | 40 | 40 |
| 8.45 (7.95–10.9) | 34 | 8 | 27 | 27 |
| 4.9 (4.1–5.5) | 2 | 2 | 7 | 7 |

# chapter 3

# Asymmetric specialization and extinction risk in plant–flower visitor webs: a matter of morphology or abundance?

*Martina Stang, Peter G.L. Klinkhamer & Eddy van der Meijden*

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## Abstract

A recently discovered feature of plant–flower visitor webs is the asymmetric specialization of the interaction partners: specialized plants interact mainly with generalized flower visitors and specialized flower visitors mainly with generalized plants. Little is known about the factors leading to asymmetry and their consequences for the extinction risk of species. Existing studies proposed random interactions proportional to species abundance as an explanation. However, the simulation models used in these studies did not include potential biological constraints. In the present study, we tested the potential role of both morphological constraints and species abundance in promoting asymmetric specialization. We compared actual field data of a Mediterranean plant–flower visitor web with predictions of Monte Carlo simulations including different combinations of the potential factors structuring the web. Our simulations showed that both nectar holder depth and abundance were able to produce asymmetry; but that the expected degree of asymmetry was stronger if based on both. Both can predict the number of interaction partners, but only nectar holder depth was able to predict the degree of asymmetry of a certain species. What is more, without the size threshold the influence of abundance would disappear over time. Thus, asymmetric specialization seems to be the result of a size threshold and only among the allowed interactions above this size threshold a result of random interactions proportional to abundance. The simulations also showed that asymmetric specialization could not be the reason that the extinction risk of specialists and generalists is equalized as suggested in the literature. In asymmetric webs specialists had clearly higher short-term extinction risks. In fact, primarily generalist visitors seem to profit from asymmetric specialization. In our web specialists were less abundant than generalists. Therefore, including abundance in the simulation models increased the difference between specialists and generalists even more.

## Introduction

The study of plant–flower visitor interaction webs can give important answers to fundamental ecological questions, such as the factors that determine the structure and stability of communities. The structure of an interaction web can be described in terms of the number of interaction partners. This number varies in plant–flower visitor webs from one up to more than several dozens (Waser *et al.*, 1996). A species with a low number of interaction partners in a local web is called an ecological specialist and a species with a high number of interactions partners an ecological generalist (Ferry-Graham *et al.*, 2002). A recently discovered structural feature of mutualistic interaction webs is the asymmetric specialization of the interacting partners (Bascompte *et al.*, 2003; Dupont *et al.*, 2003; Petanidou & Ellis, 1996; Vázquez & Aizen, 2004; Vázquez & Simberloff, 2002). Flower visitors that visit an ecologically specialized plant species tend to interact with a large number of plant species. Flower visitors that visit an ecologically generalized plant species tend to interact with a small number of plant species. Asymmetric specialization is an intriguing pattern that was found not only in plant–flower visitor webs but also in plant–fruit disperser (Bascompte *et al.*, 2003) and fish–parasite webs (Vázquez *et al.*, 2005). The asymmetric nature of interactions is intriguing because it contradicts the traditional view of symmetric interactions, *i.e.* generalist plants interact with generalist visitors and specialist plants with specialist visitors (Vázquez & Aizen, 2004 and references therein). Surprisingly little is known about the factors that promote asymmetry and the influence of these factors on the extinction risk of the interaction partners. In this paper we want to explore the impact of morphological constraints and species abundance on the degree of asymmetry in a Mediterranean plant–flower visitor interaction web and the influence of both factors on the short-term extinction risks of the species due to chance processes.

Asymmetric specialization in interaction webs seems to be based on a so-called nested structure of the interactions (Bascompte *et al.*, 2003; Dupont *et al.*, 2003; Ollerton *et al.*, 2003). In order to discover nestedness, the species in a plant–flower visitor matrix have to be arranged according to their number of interactions (the visitor species with the highest number of interactions will be found in the first row of the matrix and the plant species with the highest number of interactions in the first column). The interactions in a perfectly nested matrix will occur above a boundary threshold (Atmar & Patterson, 1993), *i.e.* a line from the bottom left corner to the top right corner (FIGURE 3.1a). As a result, generalists interact not just with specialists but also with generalists. A nested pattern of interactions necessarily means asymmetric specialization, the converse is not necessarily true (Vázquez & Aizen, 2004). The nested organization can be thought of as an alternative to a compartmentalized organization with only a small overlap of interacting partners between groups of species, *i.e.* the whole web is divided into a number of smaller webs with few interactions among these sub-webs (Dicks *et al.*, 2002). Compartmentalized webs can be asymmetrically organized, however, in this case without generalist–generalist interactions (FIGURE 3.1b). Both types of asymmetric organization are different from a purely random structure (FIGURE 3.1c).

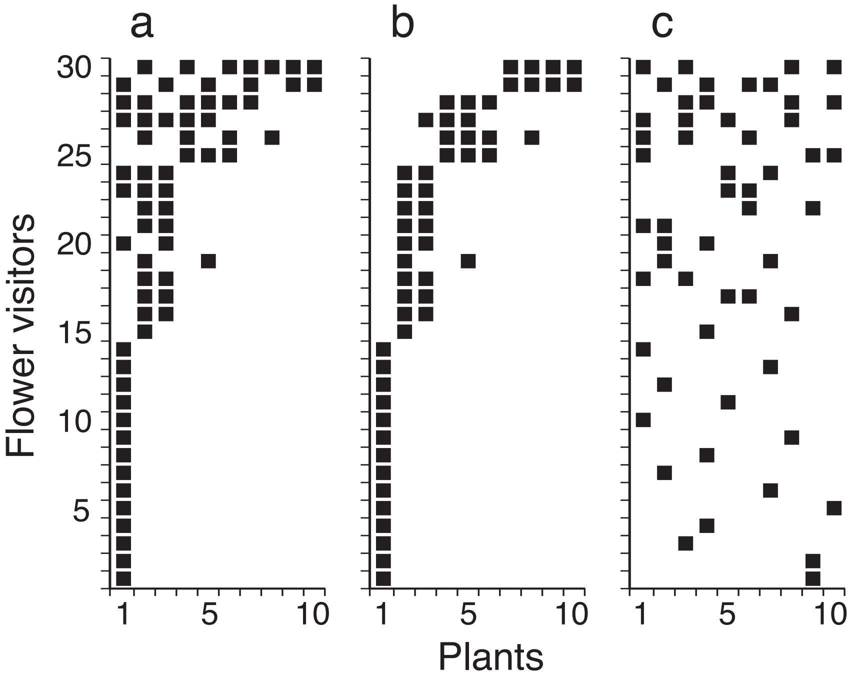

**FIGURE 3.1** – Examples of fictitious plant–flower visitor interaction matrices with different types of interaction patterns: (a) nested and asymmetric, (b) compartmentalized and asymmetric, (c) random. Each number labels either a plant or a visitor species. A species–species interaction is indicated with a black square. The species were ordered by the number of interaction partners. For example, in (a) visitor species number 30 visited seven plant species and plant species number 1 was visited by 20 visitor species.

Asymmetric specialization has been found to be related to the abundance of the species involved (Dupont *et al.*, 2003). Locally rare plants tend to interact with generalized, locally abundant visitors, and locally rare visitors appear to utilize generalized, locally abundant plants. Random interactions proportional to species abundance seemed to be sufficient to explain the observed asymmetric specialization in a null model analysis based on Monte Carlo simulations (Vázquez, 2005; Vázquez & Aizen, 2004). The idea behind this is that visitor individuals rather than visitor species distribute themselves randomly and proportional to the available resources over the plant species (following an ideal free distribution). Thus, abundant visitor species visit many plant individuals and, because they choose plant individuals randomly, many plant species. As a result, rare plant species are visited by few individuals and thus by few visitor species that are most likely ecologically generalized.

The biologically neutral mechanism of random interactions proportional to abundance is based on the assumption that no constraints exist that restrict this process. However, in a previous paper we have shown that nectar holder depth and width set limits to the morphology of nectar- gathering flower visitors (Stang *et al.*, 2006). Visitors rarely try to visit a flower for nectar if their proboscis is shorter and/or thicker than the flower structures hiding the nectar. The observed flower visitors in this study were a random selection out of the potential visitors, *i.e.* those species that have a proboscis as long as or longer than the depth of the nectar holder (Stang *et al.*, 2006). Other studies proposed that morphological mismatching could lead to forbidden interaction which would explain gaps in an otherwise perfectly nested matrix caused by abundance patterns (Dupont *et al.*, 2003; Jordano *et al.*, 2003). In the present study, we want to test the role of size constraints as a biological mechanism responsible for the overall pattern of asymmetric specialization and nestedness. We include both morphological constraints and species abundance in a null model approach to contrast the observed patterns with those obtained from simulation models including different combinations of the potential factors (Gotelli & Graves, 1996).

Asymmetric specialization might have important consequences for biodiversity conservation. The extinction risk of a plant or flower visitor species may not only depend on the number of interaction partners but also on the extinction risk of these interaction partners, which will be influenced by their level of specialization (Ashworth *et al.*, 2004; Memmott *et al.*, 2004; Vázquez & Simberloff, 2002). A specialized species that interacts with a generalist will be less prone to extinction then a specialized species that depends on a specialized interaction partner. The extinction risk of single species and the stability of the whole web in the long term will also be influenced by whether asymmetric specialization is based on a nested or a compartmentalized organization (Melian & Bascompte, 2002; Memmott *et al.*, 2004).

There are indications that generalist and specialist plant species do not differ in reproductive susceptibility due to disturbance (Aizen *et al.*, 2002). It is argued that generalist visitors will be less affected by habitat fragmentation than specialist visitors as they can change their food plants easily. If a flower visitation web is asymmetrically organized and only generalist pollinators will be left over after fragmentation, generalists and specialist plants might depend both on these generalists which would place them in similar conditions (Ashworth *et al.*, 2004). This idea depends on three prerequisites, which will be tested in this study. Firstly, species-specific traits determine the degree of ecological specialization. Secondly, asymmetry is based on nestedness which will provide the necessary redundancy to allow generalists to substitute for specialists (in addition to the possibility that specialists can substitute for other specialists). Thirdly, specialist and generalist plants will have the same chance of becoming extinct in the short term. The knowledge about species-specific short-term extinction risks will also provide the basis to model extinction cascades for plant–flower visitor webs (Memmott *et al.*, 2004).

Overall, we will answer the following questions:

- Is the flower visitation web asymmetrically organized and is this asymmetry a result of a nested structure of the interaction matrix?
- Are morphological thresholds (nectar holder depth and width), random interactions proportional to species abundance, or both responsible for this asymmetric specialization?
- Does asymmetry lead to similar short-term extinction risks due to chance processes for generalists and specialists and how do size thresholds and abundance influence the short-term extinction risk of ecological and morphological specialists and generalists?

## Methods

### *Study system, sampling procedure and size parameter estimation*

The data used in this paper come from a previously published study of a Mediterranean flower visitation web. A full description of field methods is given in Stang *et al.* (2006). This flower visitation web consisted of 25 nectar-producing plant species and 111 nectar-collecting flower visitor species spread over five insect orders. We measured size parameters of flowers (nectar holder depth and width) and insects (proboscis length and proboscis diameter), which were found to constrain the potential interactions between the mutualistic partners (Stang *et al.*, 2006). We estimated plant species abundance using two direct measurements: total number of individuals and total number of open flowers. We determined the number of insect individuals and species visiting a plant species during four 15 minutes intervals for each plant species. The intervals were randomly spread over a period of two weeks during the period when the plant was in full bloom. The total observation period of all plant species was 6 weeks during March and April 2003. We observed 1,206 visitor individuals of which 887 fed on nectar or nectar and pollen. The restriction to nectar-producing plant species and nectar-feeding visitors is essential given the morphological constraints we want to investigate.

### *Interaction asymmetry and nestedness*

Interaction asymmetry was estimated by the correlation coefficient between the number of interactions of a species $n$ and the mean number of interactions of its interaction partners $m$ (Vázquez & Aizen, 2004; Vázquez & Simberloff, 2002): a negative correlation between the two shows that interactions are asymmetric, a positive that interactions are symmetric.

We used the following definitions of $n$ and $m$:

$n_{vis}$ = number of visitor species of a plant species,
$n_{pla}$ = number of plant species visited by a flower visitor species,
$m_{vis}$ = mean number of interactions of the visitor species of a plant species

$$m_{vis} = \frac{\sum n_{pla}}{n_{vis}}$$

$m_{pla}$ = mean number of interactions of the plant species visited by a flower visitor species

$$m_{pla} = \frac{\sum n_{vis}}{n_{pla}}$$

The variables were tested for normality with a Kolmogorov-Smirnov test. Because of the triangular-like distribution of the data, which lead to non-normality, we used a Spearman rank-order correlation coefficient for both plants and visitors. Statistical analysis was performed using SPSS 11.0 (SPSS Inc., Chicago, USA) and Winstat for Microsoft Excel version 2005.1.

In order to test if asymmetry was associated with the nestedness of interactions, the species in the plant–flower visitor matrix were arranged according to the number of interactions with their mutualistic partners in descending order. We calculated one commonly used estimate of nestedness: system temperature T (Atmar & Patterson, 1993) by using the Nestedness Calculator software, which was developed by Atmar and Patterson in 1995 (AICS Research, University Park, NM). System temperature T is a measure of the number of deviations of unexpected presences and absences in the observed matrix above and below a calculated boundary threshold of a perfectly nested matrix. For each of these unexpected presences or absences, a normalized measure of global distance to the boundary is calculated, and these values are averaged. T has values ranging from 0° to 100° with T = 0° representing a perfectly nested matrix (no disorder). In a perfectly nested matrix with less than 50% fill the observed interactions will form a concave meniscus in the upper-left corner of the matrix. A matrix is considered significantly nested if the observed T value was smaller than a benchmark value (5%) of 1,000 randomly gathered T values using matrices of similar size and fill.

### *Species traits and interaction asymmetry*

As a first descriptive step of the analysis, we examined the relation between size parameters, abundance estimates and the number of observed interactions of a species (*n*) and the mean number of interactions of its interaction partners (*m*). The smaller *n* or *m*, the more ecologically specialized a species or species group is. For the statistical analysis we used again the non-parametric Spearman rank-order correlation coefficient, because visitor traits could not be transformed to achieve normality.

As a second step of the analysis, we estimated the contribution of size constraints and abundance to asymmetry (measured as the Spearman rank-order correlation coefficient between $n$ and $m$) by Monte-Carlos simulation tests based on four different null models. In the first fully random model (*a*) all interactions were possible with the same probability for each species. In the second model (*b*) the possible interactions were constrained by the morphology of the interaction partners, *i.e.* the proboscis of a visitor had to be as long or longer than the depth of the nectar holder of a plant. Within these limits the probability of an interaction was equal for all species. In the third model (*c*) the probability was proportional to the abundance of the species. Each interaction was allowed. The fourth model (*d*) combined morphological constraints with the probability of the interactions being proportional to the abundances of the species.

For the species based simulation models (*a* and *b*) we held the total number of species–species interactions (231) constant. For the individual based simulation models (*c* and *d*) we conserved the number of individual–individual interactions (887). To be able to compare our results with those of Vázquez and Aizen (2004), we used the number of observed insect individuals on a plant species and the number of individuals of a visitor species as the abundance estimates in the random models. In fact, the total number of open flowers of a plant species in the observation plots during peak flowering and the number of visitor individuals per plant species were significantly positive correlated ($r_s = 0.58$, $p = 0.002$, $n = 25$). Because we wanted to estimate the extinction risk of the species, we allowed in our simulations that by chance species might get no interaction. We tested nectar holder depth and nectar holder width as size constraints in the models b and d. However, even if nectar holder width contributed significantly to the variation of the number of visitor species (Stang *et al.*, 2006), our analysis revealed that it did not contribute significantly to asymmetry in the random models *b* and *d*. In order to simplify the discussion we only present the results of the null models including nectar holder depth in this paper.

To test if the observed asymmetry is different from the asymmetry of the random models, we used a difference statistic that compares the observed value of the correlation coefficient between $n$ and $m$ with the distribution of 1,000 randomly generated values. The observed correlation was treated as significantly different from the random ones if the observed value was larger than the 25th largest random value or smaller than the 25th smallest random value. The programme Poptools (Hood, 2005) provided the shuffle algorithm and the difference statistic for the Monte Carlo simulation tests. If the asymmetry of the model was as strong as or stronger than the observed one, we considered the factors that were used to construct the model as a potential cause for the observed asymmetry.

With the correlation coefficient between $n$ and $m$ of the random models we tested the ability of the factors to produce the overall pattern of asymmetry. In order to test which of the random models was able to predict the species-specific components of asymmetry, *i.e.* the observed $n$ and $m$ of each single species, we correlated each $n_{random}$ with $n_{observed}$ and each $m_{random}$ with $m_{observed}$. We indicated the ability of the models to predict the observed $n$ and $m$ of a plant or visitor species with the mean $r_s$ and $p$ values of each of the 1,000 Spearman rank-order correlation coefficients. The higher the mean correlation coefficient is, the better is the match between the random and the observed generalization level of a certain species ($n$) and the match between the random and observed mean generalization level of its interaction partners ($m$).

### *Extinction risk*

To obtain an indication of the extinction risk of plant and flower visitor species in relation to their abundance and morphology, we counted for each species the number of zero interactions that were produced during the randomizations, which is an estimate of the short-term susceptibility to extinction by chance processes. Our definition makes the simplification that a plant can only survive if it sets seeds because of the pollination by a flower visitor and that a flower visitor species can only survive and reproduce if it can feed on nectar. We correlated nectar holder depth (plants) and proboscis length (animals) as estimators of the level of morphological specialization with the probability of observing a zero interaction for each of the four random models.

## Results

### *Interaction asymmetry and nestedness*

The interactions in our flower visitation web were significantly asymmetrical (FIGURE 3.2). Plant species that were visited by many visitor species were visited, on average, by ecologically specialized species; and plant species that were visited by few visitor species were visited, on average, by ecologically generalized species ($r_s = -0.441$, $p = 0.027$, $n = 25$). The same asymmetric relationship can be observed for the flower visitors ($r_s = -0.233$, $p = 0.014$, $n = 111$). The correlation coefficient was lower for the visitors, caused by a greater variation for visitor species that were visiting a low number of plant species (a stronger triangular relationship).

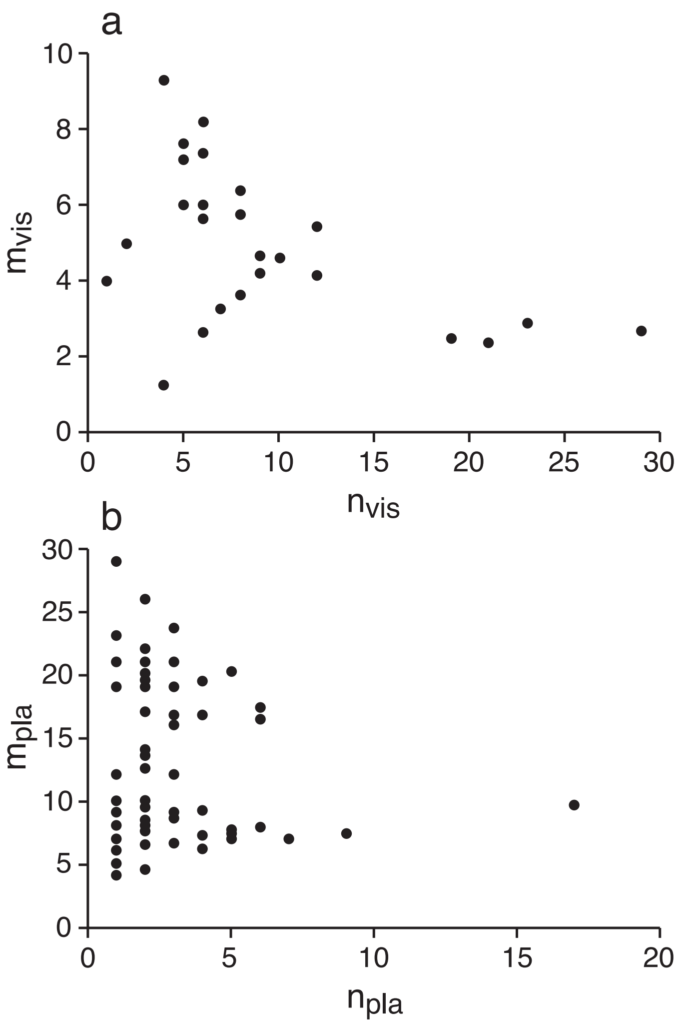

**FIGURE 3.2** – The relationship between the number of interaction partners *n* and the mean generalization level of interaction partners *m* of the observed plant–flower visitor interaction web. Each data point represents a plant (a) or flower visitor species (b).

This asymmetry of interactions was a result of a nested structure of the interaction matrix. The plant–flower visitor matrix had a size of 25 x 111 = 2775 potential interactions (without constraints) with an observed fill (connectance) of 8.3%. After ordering the species of the matrix by the number of interactions, the observed interactions (links) occur mainly in the top left corner of the matrix (FIGURE 3.S1). The observed system temperature *T* was 11.4°. This value was significantly lower than the mean of 1,000 randomizations of the matrix ($T = 25.36°$, standard deviation = 2.07°, $p < 0.001$).

### *Species traits and interaction asymmetry*

A minority of 67 (7.5%) out of the observed 887 nectar-searching insect individuals had a proboscis shorter than the nectar holder of the plants they visited (FIGURE 3.3). A random distribution of interactions throughout the individual based matrix (model *d*) gave an expected value of 272 visitations below the threshold (31%, $\chi^2 = 222.83$, $p < 0.001$).

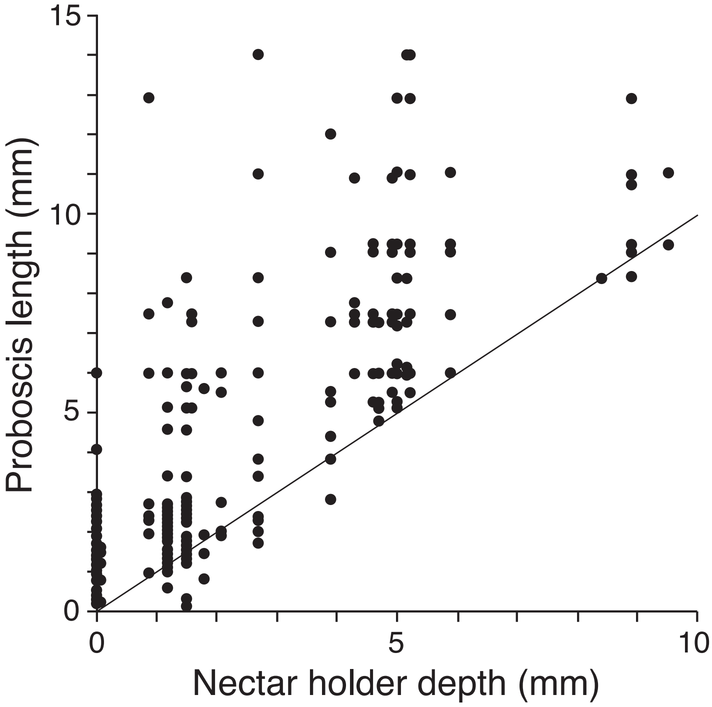

**FIGURE 3.3** – The relationship between the proboscis length of the flower visitors and the nectar holder depth of the visited plant species (minimum values (see Stang *et al.*, 2006)). Each data point represents a plant species–flower visitor species interaction. The x = y line represents the expected size threshold. The interactions occur mainly within a triangle above the threshold.

**TABLE 3.1** – The relationship between morphological traits, abundance estimates, and the number of interactions partners for the plant ($n_{vis}$, top of the table) and flower visitor species ($n_{pla}$, bottom of the table) and the mean number of interactions of these partners ($m_{vis}$ and $m_{pla}$). Spearman rank-order correlation coefficients and p values (in parentheses) are given.

| | | Number of interaction partners $n$ | Mean number of interactions of these partners $m$ |
|---|---|---|---|
| *Plants* | Nectar holder depth | –0.485 (0.014) * | +0.471 (0.018) * |
| | Nectar holder width | +0.372 (0.067) | –0.177 (0.387) |
| | Plant individuals | +0.338 (0.098) | –0.081 (0.700) |
| | Number of flowers | +0.697 (0.000) ** | –0.301 (0.143) |
| | Number of visits | +0.631 (< 0.001) ** | –0.021 (0.921) |
| *Visitors* | Proboscis length | +0.326 (< 0.001) ** | –0.455 (< 0.001) ** |
| | Proboscis diameter | +0.090 (0.348) | +0.192 (0.043) * |
| | Visitor individuals | +0.766 (< 0.001) ** | –0.336 (< 0.001) ** |

* = $p < 0.05$, ** = $p < 0.01$

The deeper the nectar holder, the fewer visitor species were observed (decreasing $n_{vis}$) and the more ecologically generalized they were (increasing $m_{vis}$, TABLE 3.1, first row). There was no significant relationship between nectar holder width and $n$ or $m$. Generalists and specialist animals visited rare plants (with few individuals) and common plants at similar rates. More flowers and more visits resulted in more visitor species (increasing $n_{vis}$) but not in visitors that were significantly more specialized (decreasing $m_{vis}$). Flower visitors showed the opposite pattern compared to plants concerning morphology but similar ones concerning abundance. The longer the proboscis, the more plant species were visited (increasing $n_{pla}$), and the more ecologically specialized the plants that were visited (decreasing $m_{pla}$). Abundant flower visitor species were found on more plant species (increasing $n_{pla}$) and visited on average more specialized plant species (decreasing $m_{pla}$) than rare ones (TABLE 3.1, last row).

The number of plant individuals was not related to nectar holder depth ($r_s = 0.074$, $p = 0.742$, $n = 25$). However, plant species with deeply hidden nectar produced fewer flowers ($r_s = –0.539$, $p = 0.005$, $n = 25$) and were visited by fewer individuals ($r_s = –0.403$, $p = 0.046$, $n = 25$) than plant species with freely accessible nectar. Abundant visitor species had longer proboscises than rare visitor species ($r_s = 0.293$, $p = 0.002$, $n = 111$).

**TABLE 3.2** – Comparison of observed interaction asymmetry of plants and their visitors with those based on Monte Carlo simulations. A negative Spearman rank-order correlation coefficient between *n* (number of interaction partners) and *m* (mean generalization level of interaction partners) means asymmetry. The mean rank-order correlation coefficients of 1,000 random runs are given. The values in parentheses are the fractions of random runs with a weaker negative correlation than the observed ones. A significantly different random *r* is indicated with an asterisk (two-sided). The observed correlation coefficients between *n* and *m* were –0.441 for the plants and –0.233 for the flower visitors (see text for further explanations).

| Model | Explanation | Plants | Flower visitors |
|---|---|---|---|
| a | Equal probability, no size threshold | –0.043 (0.983) * weaker | –0.016 (0.979) * weaker |
| b | Nectar holder depth threshold | –0.657 (0.087) equal | –0.412 (0.026) equal |
| c | Proportional visitor abundance | –0.736 (0.033) equal | –0.145 (0.796) equal |
| d | Visitor abundance, nectar holder depth threshold | –0.847 (0.001) ** stronger | –0.393 (0.061) equal |

* = $p < 0.025$, ** = $p < 0.005$

### *Null model analysis of interaction asymmetry*

The observed negative correlations between *n* and *m* for both plants and visitors were significantly stronger than those obtained from the fully random model (*a*), which were close to zero. The fraction of random runs with a weaker correlation than the observed ones were 0.983 and 0.979 (TABLE 3.2). Thus, random interactions without including size constraints and abundance did not result in a relationship between the level of specialization of plants and visitors. Including nectar holder depth and/or abundance in the null models (model *b*, *c* or *d*) always lead to a negative correlation between *n* and *m*, *i.e.* to asymmetric specialization. These randomly produced negative correlations were as strong (model *b* and *c*) as the observed ones, so that both size constraints and abundance seem to promote asymmetric specialization. The expected asymmetry for the plants was even stronger if based on both factors.

In order to assess if constraints and abundance are able to produce asymmetry on a species-specific level, we tested if they can predict which species are generalists and specialists and with which kind of species they interact. We correlated the random with the observed number of interaction partners (*n*) and the random with the observed mean

**Table 3.3** – Relationship between observed and random *n* (number of interaction partners) and observed and random *m* values (mean generalization level of interaction partners) for plants and flower visitors. The mean value of 1,000 Spearman rank-order correlation coefficients is given. The significance of the relationship is indicated as the mean *p* value (one-sided, in parentheses).

| Model | Explanation | Plants | | Flower visitors | |
|---|---|---|---|---|---|
| | | $n_{vis}$ | $m_{vis}$ | $n_{pla}$ | $m_{pla}$ |
| a | Equal probability, no size | –0.009 | 0.008 | –0.002 | –0.007 |
| | threshold | (0.260) | (0.254) | (0.242) | (0.259) |
| b | Nectar holder depth | 0.402 $^{*}$ | 0.399 | 0.199 | 0.295 $^{**}$ |
| | threshold | (0.038) | (0.051) | (0.073) | (0.003) |
| c | Proportional visitor | 0.584 $^{**}$ | –0.027 | 0.657 $^{**}$ | 0.051 |
| | abundance | (0.003) | (0.314) | (<0.001) | (0.234) |
| d | Visitor abundance, nectar | 0.551 $^{**}$ | 0.479 $^{*}$ | 0.632 $^{**}$ | 0.352 $^{**}$ |
| | holder depth threshold | (0.004) | (0.018) | (<0.001) | (0.009) |

$^{*}$ = $p < 0.05$, $^{**}$ = $p < 0.01$

level of generalization of the interaction partners (*m*), see Table 3.3. The number of visitor species on a plant ($n_{vis}$) as well as the number of plant species visited by an insect ($n_{pla}$) was best predicted by abundance (model *c*) or a combination of the size threshold and abundance (model *d*). However, the mean level of generalization of the interaction partners (*m*) could only be predicted if size thresholds were included in the model (model *b* and *d*). Thus, random interactions proportional to species abundance can predict the number of interaction partners ($n_{vis}$ and $n_{pla}$) but can not predict the mean level of generalization of these interaction partners ($m_{vis}$ and $m_{pla}$). To predict the latter we have to include the morphological threshold set by nectar holder depth. Thus, a combination of size threshold and abundance (model *d*) will give the best predictions for *n* and *m* for both plants and visitors.

### *Extinction risk*

Without size constraints and with equal probability for each species to interact with another species (model *a*), there is no differences of extinction risk between generalist or specialist species (Figure 3.4a and 3.4b). The inclusion of the size threshold (model *b*) gives an increasing extinction risk with increasing nectar holder depth (Figure 3.4c) and decreasing proboscis length (Figure 3.4d). Compared to model *a* without constraints,

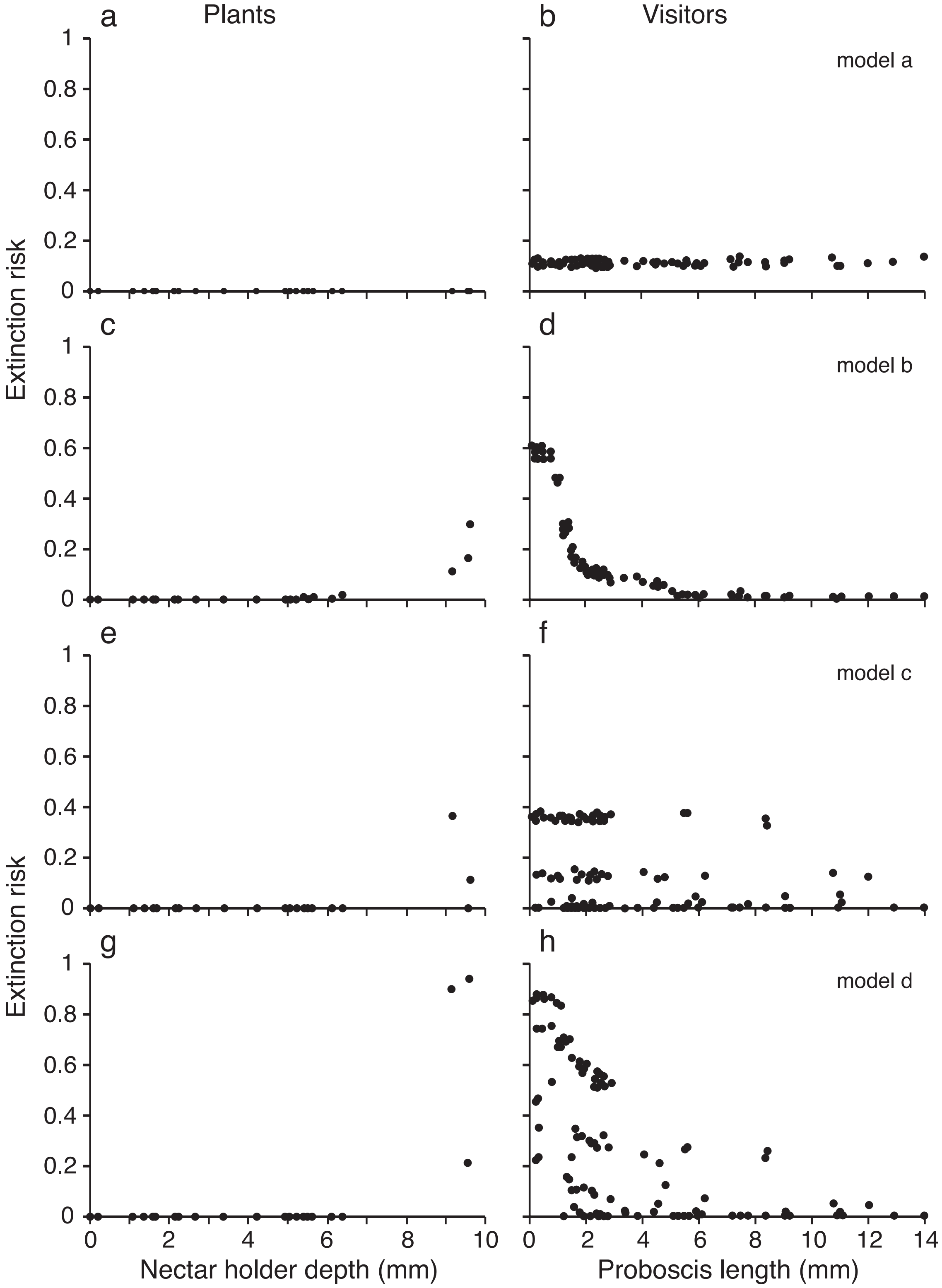

**Figure 3.4** – Extinction risk of plant and flower visitor species expressed as the probability of having no interaction partners. The probability for each model (*a*, *b*, *c* and *d*) was obtained from the Monte Carlo simulations and plotted against nectar holder depth or proboscis length. Each data point represents a plant or flower visitor species.

species with a long proboscis have now a lower extinction risk while species with deeply hidden nectar or short proboscises have a higher extinction risk. Without size constraints and with the probability to observe an interaction proportional to the abundance of the species (model *c*), the extinction risk shows a similar pattern as for model *b* (FIGURE 3.4e and 3.4f) although with a higher variation. The last model (*d*) shows that, compared to models *b* and *c*, the simultaneous inclusion of constraints and abundance increased the chance of becoming extinct especially for visitors with short proboscises (FIGURE 3.4h) and for plants with deeply hidden nectar (FIGURE 3.4g). Both groups are ecologically specialized. For many ecologically generalized visitor species (visitors with a long proboscis) the extinction risk is lower than when based on equal abundance or abundance alone (model *a*, *b* and *c*).

## Discussion

### *Asymmetric specialization and nestedness*

In the Mediterranean plant–flower visitor web we studied, specialists interact mainly with generalists and generalists mainly with specialists. This asymmetric specialization was associated with a nested structure of the interactions. Thus, generalists are not restricted to specialists but also interact with generalists. This result in itself is not new. Up to now almost all of the studied plant–flower visitor webs from all over the world showed an asymmetrical (Petanidou & Ellis, 1996; Vázquez & Aizen, 2004; Vázquez & Simberloff, 2002) and/or nested organization (Bascompte *et al.*, 2003; Dupont *et al.*, 2003; Memmott *et al.*, 2004).

### *Potential causes of asymmetric specialization*

We found that, despite the fact that both species abundance and nectar holder depth can produce asymmetry in the observed web (TABLE 3.2), only nectar holder depth was able to predict which species would be visited by specialists and which by generalists, and thus the level of asymmetry for a particular species (TABLE 3.3). In addition, the asymmetry of the random models that included both size threshold and abundance was higher than compared to those that are based on abundance alone. Vázquez et al. (2005, 2004) questioned the potential role of species traits to explain asymmetric specialization. They argued that neutrality at the individual level alone (visitor individuals distribute themselves randomly among the plant species) can account for the observed pattern. Traits that function as morphological constraints and will lead to 'forbidden interactions' (Jordano *et al.*, 2003, 2006) are in their opinion not necessary to explain the level of generalization and thus asymmetry. However, they only included species abundance in their null model analysis and in fact did not test this assertion.

We found that although abundance is able to reproduce the observed level of asymmetry, it certainly did not present the complete picture because it could not made species-specific predictions about the level of generalization of the interaction partners (*m*) (TABLE 3.3). If the probability of an interaction is only proportional to abundance, the identity of the species and thus the traits of the species that interact with each other will change with each change in abundance. However, we found that visitors with a short proboscis hardly ever try to exploit nectar from a flower with deeply hidden nectar (Stang *et al.*, 2006). If a plant species with deeply hidden nectar increases in abundance, the maximum number of potential visitor species is constrained by the size threshold. Abundance will only modify how many of the potential visitors will be actually observed. The fact that, in our simulations, abundance alone can produce the asymmetric pattern could be partly an effect of the correlation between size and abundance, *i.e.* with increasing nectar holder depth the number of flowers decreased and with increasing proboscis length the number of individuals per species increased (Stang *et al.*, 2006). If interaction asymmetry is the result of a size threshold, it will provide a biological explanation for the boundary threshold in a nested matrix and would allow us to predict were this boundary should be.

The ability of abundance to promote asymmetric specialization could be partly an effect of sampling bias, such as data aggregation, uneven sampling or insufficient sampling (Vázquez & Aizen, 2004). For our web, data aggregation can be excluded because we sampled only within a small area and a short observation period. Additionally, we used equal observation times for each plant species so that a problem of the popular transect method is avoided: common plant species are sampled more intensively than rare ones. Nevertheless, an overall insufficient sampling could have increased the influence of abundance on the degree of asymmetry in our study. Over time, rare species will be observed interacting with more and more species while abundant species are already found to interact with almost all existing potential partners at low sampling intensities. A longer sampling time will thus result in a decreasing degree of asymmetry if the total number of species remains the same. This time effect will not occur in combination with a size threshold because additional interactions will be mainly observed above the size threshold in the top left corner of the matrix. In this case, a longer sampling time will very likely increase asymmetry. This is in agreement with the observation that webs that had the same total number of species are relatively more nested if more interactions were observed (Bascompte *et al.*, 2003). Nevertheless, Vázquez and Aizen (2006) did not find an effect of sampling intensity using a sensitivity analysis. Certainly, more studies are needed to show that the effect of abundance – among the allowed interactions above the size threshold – is mainly based on visitor behaviour and not on sampling intensity or another underlying covarying biological trait.

### *Towards a functional definition of generalists and specialists*

We found that size thresholds predicted the level of ecological generalization as well as the mean level of ecological generalization of the interaction partners quite well. Thus, morphological traits will provide an essential element to characterize generalists and specialists (FIGURE 3.5). Plants that were ecological specialists had mainly deeply hidden nectar and plants that were ecological generalists mainly openly accessible nectar. Contrary to this, flower visitors that were ecological specialists had mainly a short proboscis and ecological generalists had mainly a long proboscis. Another characteristic of specialists and generalists in our study system was that specialised plants had fewer flowers and received fewer visits whereas generalized plants had many flowers and received many visits (FIGURE 3.5). The same pattern was found for the flower visitors. Ecologically and morphologically specialized flower visitors had few individuals and generalists many. However, there were more specialized visitor species than generalized ones so that all specialized visitor species together had many more individuals than generalized visitors (FIGURE 3.4).

Because of the fact that the ecological level of generalization (the number of interaction partners) is largely determined by the morphological level of generalization (the potential morphological range of interaction partners), the existence of specialist–specialist interactions among nectar producing plant species and nectar consuming visitor species is not very likely. Visitors that can use flowers with deeply hidden nectar are usually able to utilize a wide morphological range of flowers and will do this if necessary (*e.g.* because of a low abundance of flowers with deeply hidden nectar). The depth threshold is a first step towards a functional definition of generalists and specialists. A cost threshold based on energy demands of the visitors or a flight temperature threshold might complete the picture (Corbet, 2006).

### *Extinction risk of generalists and specialists*

Our analysis revealed that nectar holder depth, proboscis length and species abundance influenced the extinction risk of the species. Since asymmetric specialization was promoted by a size threshold, specialists with a small potential morphological range of interaction partners had a higher extinction risk through chance processes than generalists with a potentially broad range (FIGURE 3.5). The high number of individuals of generalist visitor species may reduce the chance of extinctions for a specialist plant species; nevertheless, the low number of flowers of these plants and the low species number of generalized visitors counterbalanced this effect. The suggestion of Ashworth et al. (2004) that asymmetric specialization is the reason that specialist and generalist plant species will show similar reproductive susceptibility to habitat fragmentation seems unlikely. In our simulations only fully randomly determined relationships between plants and flower visitors, *i.e.* relationships without constraints and without considering species abundance, resulted in equal extinction risks of specialists and generalists. Size constraints as well as abundance patterns lead to asymmetry and differences in extinction risks. As a result, it is hard to imagine that asymmetry can equalize the susceptibility to species loss. Nevertheless, asymmetric webs based on a nested organization with generalist–generalist interactions are theoretically more resistant to disturbance and species loss than asymmetric webs with a compartmentalized organization without generalist–generalist interactions (Melian & Bascompte, 2002; Memmott *et al.*, 2004).

The extinction risk of a generalist plant species is mainly lessened by the redundancy of ecologically specialized visitors. In our system these visitors were mostly beetles, flies and wasps. These are often not restricted to flowers as their food so that they may be more or less unaffected by a species loss of flowering plants. Ecologically specialized short-tongued bees, who present another important specialized visitor group of generalized plants, may be relatively more susceptible to disturbance as they depend completely on food provided by flowers. The extinction risk of generalist visitors, which were dominated by bees with long proboscises, is mainly lessened by their morphological flexibility. In fact, especially generalist visitors seem to profit from an asymmetrically structured web (see FIGURE 3.4, extinction risk of generalists of model *b* and *d* compared to model *a*). They can change their interaction partners if necessary. However, for generalized plant species these generalized

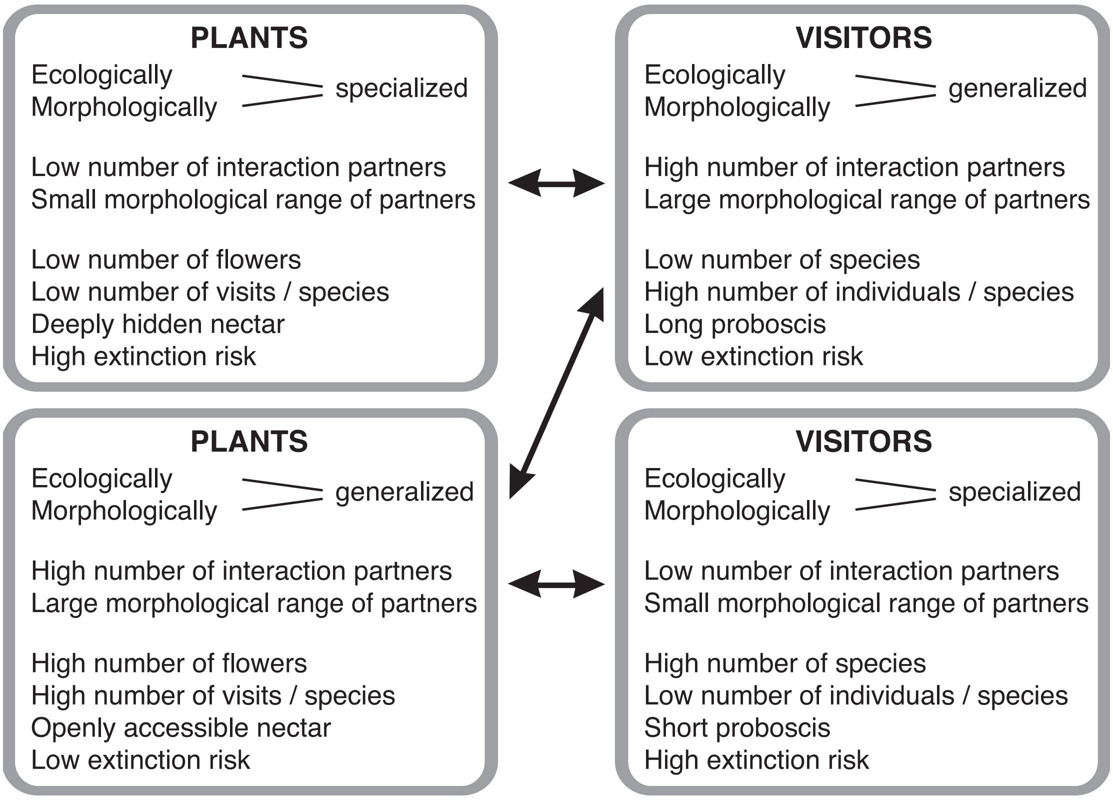

**FIGURE 3.5** – Characterization of generalist and specialist plant and visitor species based on the studied interaction web. Species traits and the two different definitions of specialization are given. The potential interactions are indicated with arrows. See text for further explanations.

visitors will have a relatively minor importance because they had, compared to specialist visitors, a small total number of individuals due to a small number of species.

Given our results, one would expect that mainly generalist–generalist interactions will remain after disturbance. Nevertheless, even generalized visitors could be more susceptible than predicted by our simulations. Generalized visitor species with a long proboscis that depend on nectar provided by flowers can not only use but often depend on a broad range of plant species. Proboscis length and body size are positively related (Stang *et al.*, 2006) and energy demands increase with body size. A combination of high energy demands and an often observed long flight time could make generalists more susceptible to plant species loss than expected. If the generalist flower visitors are threatened because of disturbance, the whole system will be less stable than through the loss of flower visitors that visit only a small number of plant species, are redundant and not obligate flower visitors. As such, generalist visitors are key species in the system (Memmott *et al.*, 2004). Specialized plants have to counterbalance the disadvantage of being specialized by attracting generalized visitors more than expected by chance, *e.g.* by providing more nectar per flower than generalized plants. Specialized visitors have to be mobile and should change the area in order to find suitable nectar plants.

Our discussion has shown that more studies are needed to fully understand the asymmetric structure of the web and the short-term extinction risk of the species in relation to the factors that promote asymmetry. One open question is the potential role of sampling intensity; another open question is the influence of other species traits such as flowering time and nectar amount of the plants, as well as flight time and energy demands of the visitors. Nonetheless, even our relatively simple simulation model (based on one size constraint and, within the allowed interactions, of abundance) was able to reproduce the observed species-specific pattern of asymmetric specialization. It revealed that specialist–specialist interactions among nectar producers and consumers might be rare because of the morphologically based intrinsic flexibility of ecological generalists, and that asymmetry alone will not equalize reproductive susceptibility and extinction risks because asymmetry is caused to a great deal by morphological constraints and abundance patterns.

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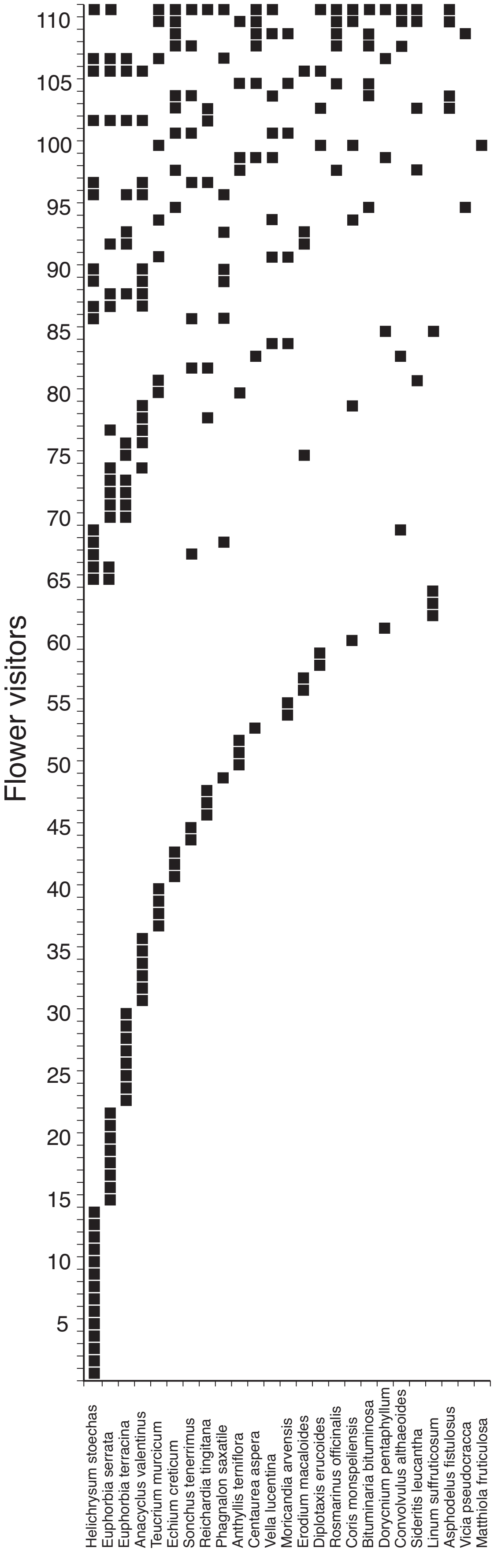
**FIGURE 3.S1** – The plant–flower visitor interaction matrix of the studied Mediterranean plant–flower visitor web of nectar producing plant species and nectar searching flower visitor species. Flower visitor species are labelled with numbers, plant species are labelled with their name. A species–species interaction is indicated with a black square. Species in the matrix were ordered by the number of interaction partners.


Flower visitors
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
Helichrysum stoechas
Euphorbia serrata
Euphorbia terracina
Anacyclus valentinus
Teucrium murcicum
Echium creticum
Sonchus tenerrimus
Reichardia tingitana
Phagnalon saxatile
Anthyllis terniflora
Centaurea aspera
Vella lucentina
Moricandia arvensis
Erodium macaloides
Diplotaxis erucoides
Rosmarinus officinalis
Coris monspeliensis
Bituminaria bituminosa
Dorycnium pentaphyllum
Convolvulus althaeoides
Sideritis leucantha
Linum suffruticosum
Asphodelus fistulosus
Vicia pseudocracca
Matthiola fruticulosa

c h a p t e r 4

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

*Martina Stang, Peter G.L. Klinkhamer & Eddy van der Meijden*

*This chapter will be submitted as:*
Martina Stang, Peter G.L. Klinkhamer and Eddy van der Meijden. Morphological matching of flowers and flower visitors: the role of size thresholds and size distributions

## 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-

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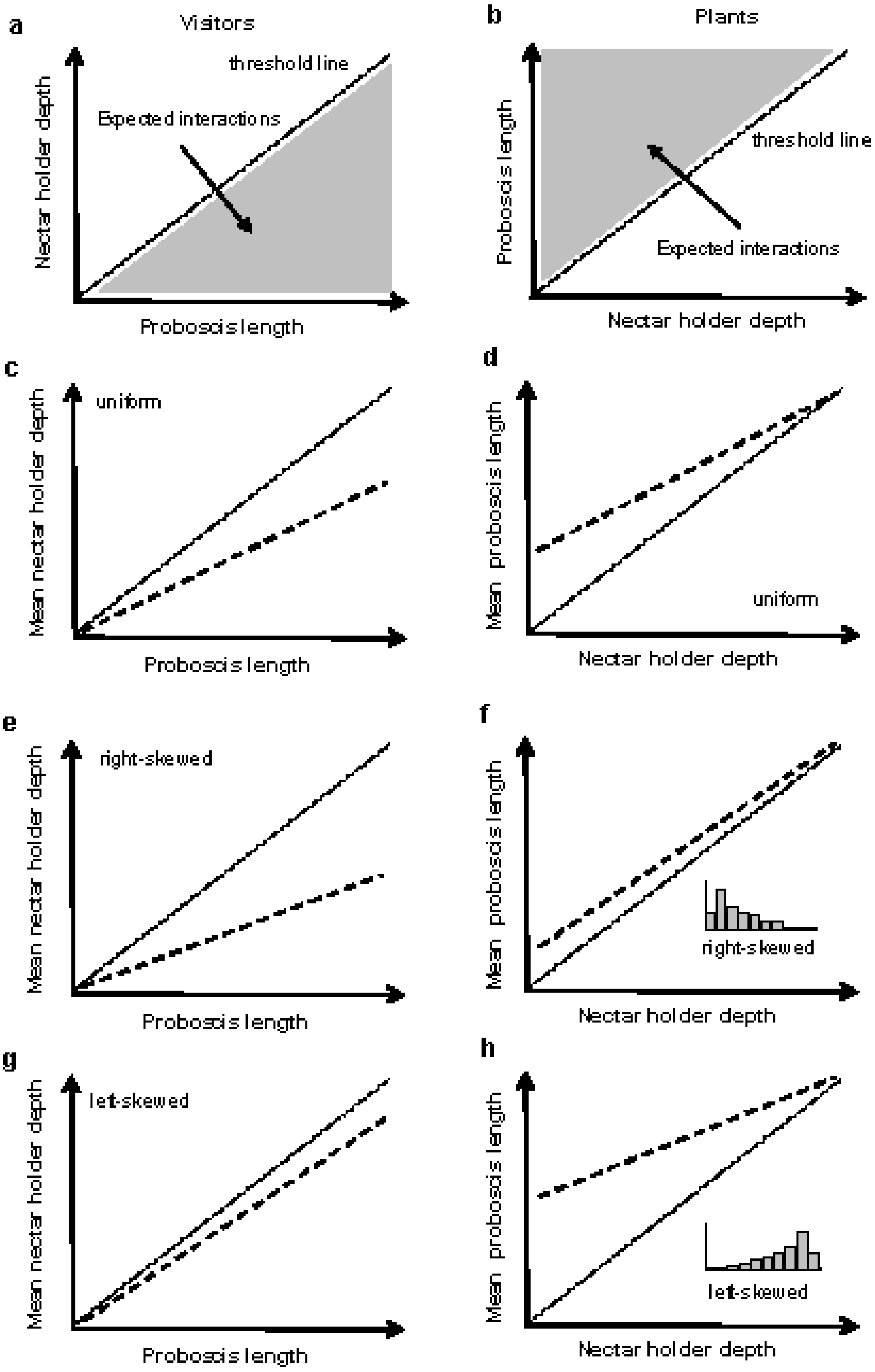
**FIGURE 4.1** – Conceptual model depicting the relationship between trait distributions and degree of size matching under the threshold rule and interactions proportional to abundance. The graphs on the left are from the visitors' and those on the right from the plants' point of view. The interactions 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
Mean nectar holder depth
Proboscis length
f
right-skewed
Mean proboscis length
Nectar holder depth
g
left-skewed
Mean nectar holder depth
Proboscis length
h
left-skewed
Mean proboscis length
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

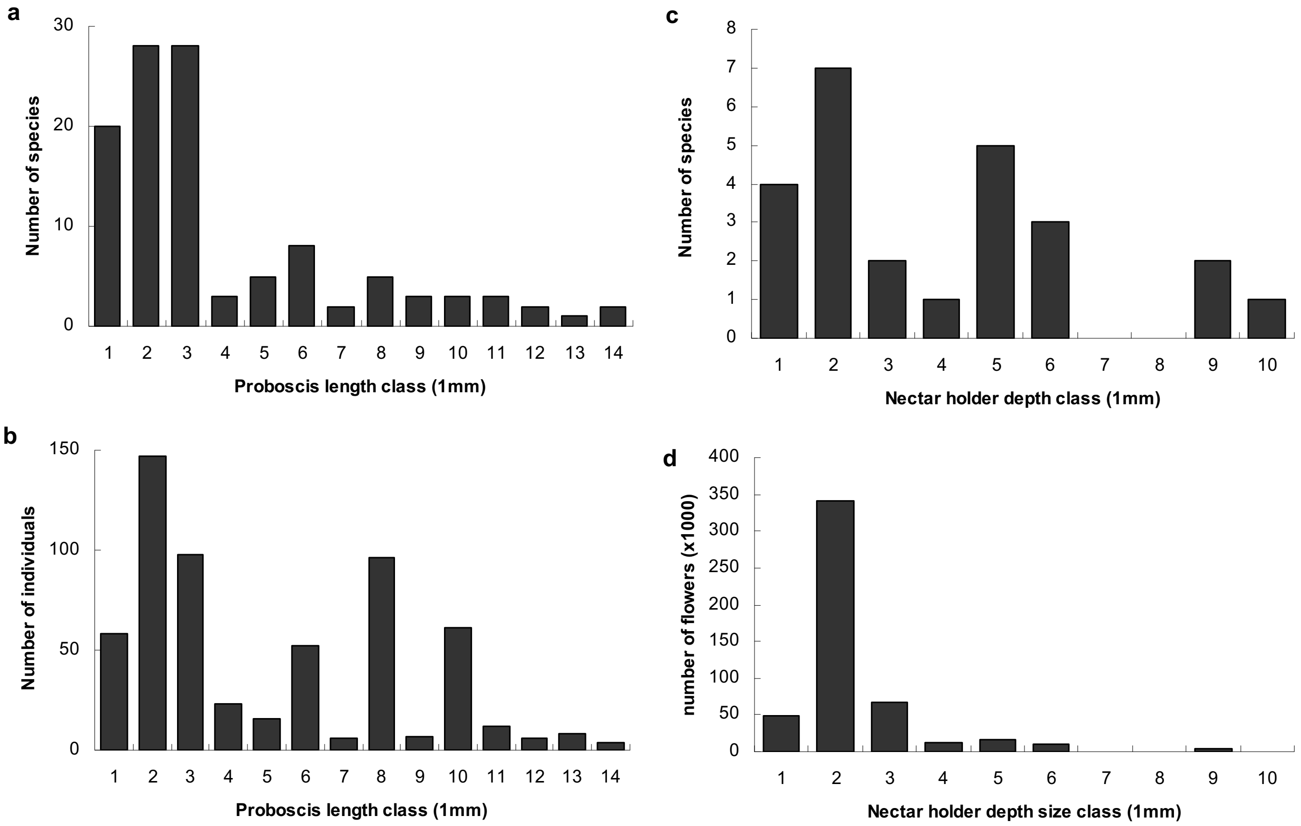


**FIGURE 4.2** – Observed proboscis length and nectar holder depth distributions. The number of species (a, c) individuals (b) or flowers (d) per size class interval of 1 mm is given. The 300 individuals of honey bees (*Apis mellifera*, proboscis length = 5.95 mm) were excluded from (b).

($z = 0.84$, $p = 0.49$, $n = 25$). 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

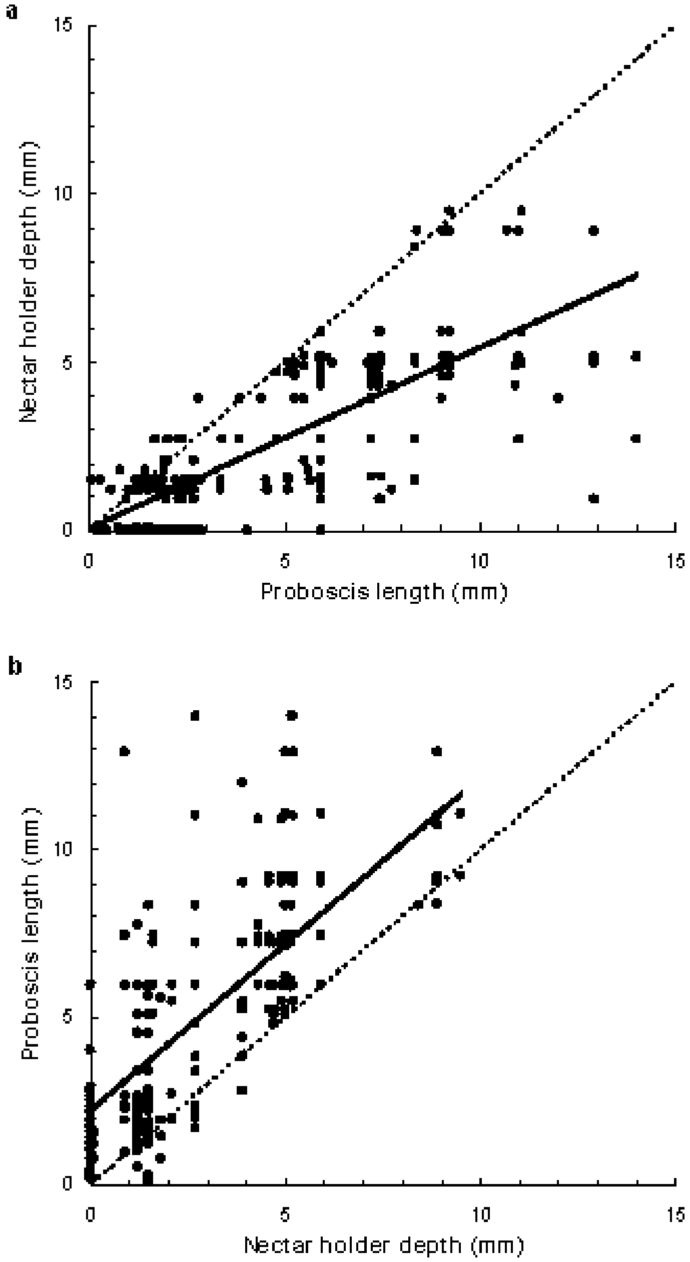


**Figure 4.3** – Observed distribution of plant–visitor interactions. The observed interactions are distributed within a triangle. In 3a the interactions were found mainly below the threshold line (visitors' point of view). In 3b the interactions occur mainly above the threshold line (plants' point of 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

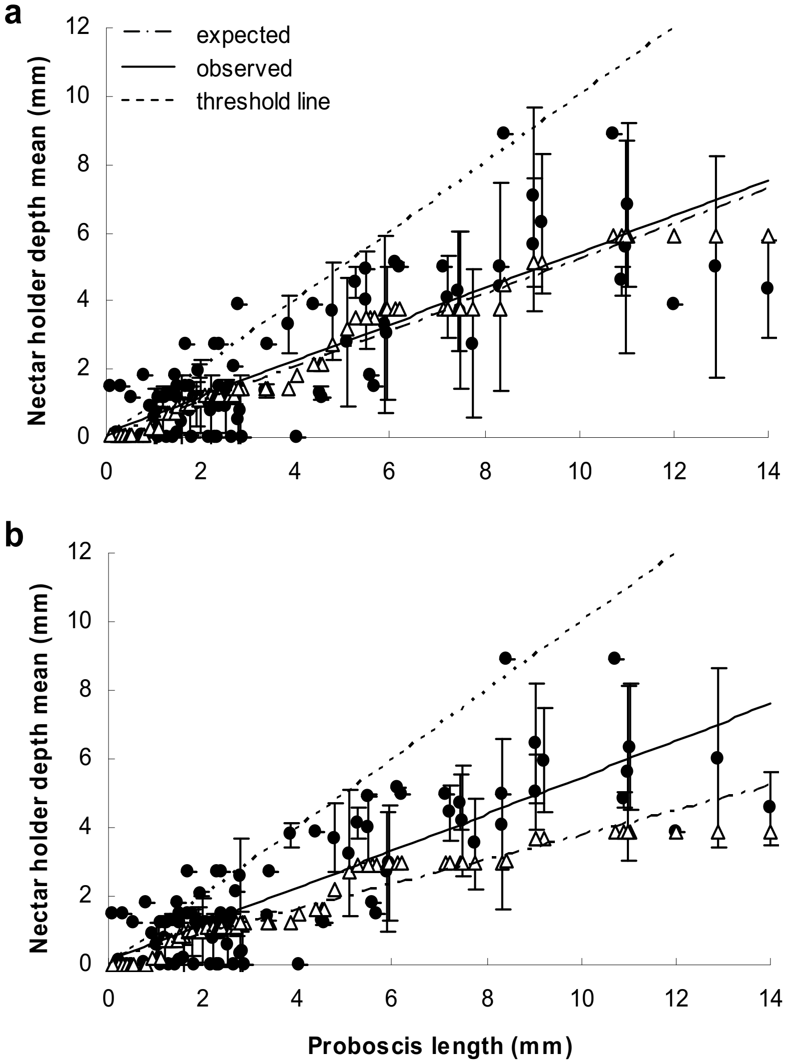


**FIGURE 4.4** – Observed and expected mean nectar holder depths in relation to proboscis length of the visitor species based on species (a) or on individuals (b). Observed values are indicated with black circles and are given with their standard deviation in a and b. Expected values are indicated with 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).

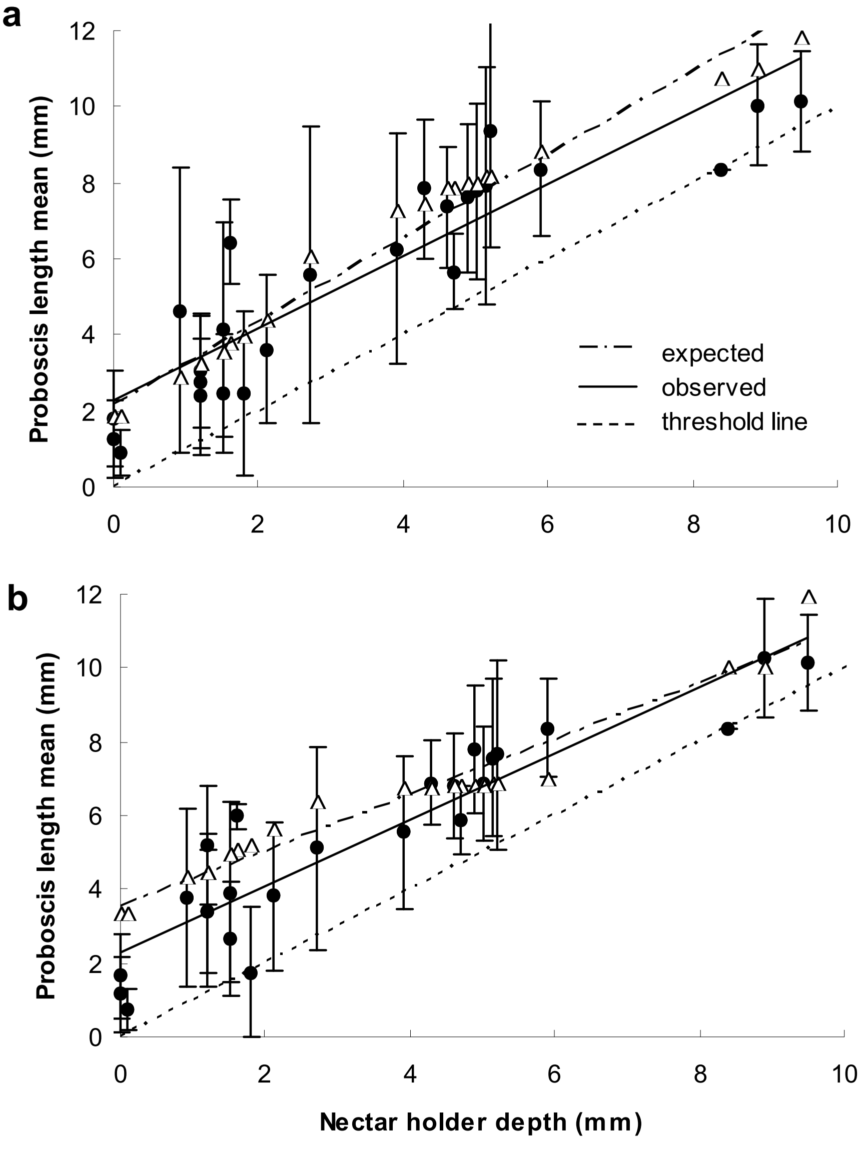


**FIGURE 4.5** – Observed and expected mean proboscis length in relation to nectar holder depth of the plant species based on species (a) or on individuals (b). Observed values are indicated with black circles and are given with their standard deviation in a and b. The linear regression line of the observed 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 tradeoffs 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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# chapter 5

## General summary

Not so long ago the relationships between plants and their pollinators were seen as mainly specialized and co-adapted. The concept of pollination syndromes, *i.e.* a certain combination of flower traits that reflects the morphology, physiology and behaviour of a certain visitor group (*e.g.* bees, butterflies or birds), represents this view. However, the idea that interactions between flowers and flower visitors are mainly specialized seems to be mostly a consequence of studying species with specialized interactions. Screening of whole flower visitation webs has shown instead that specialization in the interactions between flowers and flower visitors is rather the exception than the rule. Overall, the degree of ecological generalization varies, even locally, from one to more than hundred interaction partners. Moreover, not only does the degree of generalization across plant species or across flower visitor species differ substantially, there is also a great variation in the degree of generalization of a plant or a visitor and the degree of generalization of its interaction partners. The dominant pattern found is asymmetrical, that is, generalized plants tend to interact with generalized and specialized visitors and specialized plants tend to interact with generalized visitors, and vice versa for the animals.

The mechanisms leading to this variation in the number of interaction partners and the resulting interaction patterns found in flower visitation webs are still poorly understood. In this thesis I tested the role of two simple rules structuring interaction patterns between flowers and flower visitors. The first rule is based on the observation that rewards provided by plants to attract animals to pollinate their flowers differ greatly in their accessibility. The second rule is based on the observation

that flower visitor individuals tend to freely distribute themselves in proportion to the available recourses over patches of flowering plants. I tested the importance of these rules by comparing observed interaction patterns in a Mediterranean flower visitation web with expected patterns based on simulation models incorporating these rules. I restricted the analysis in this study to nectar producing plant species and nectar searching visitor species because of the size constraints we wanted to test.

In CHAPTER 2 I studied three possible morphological constraints: the depth and width of tubular structures hiding the nectar (nectar holder depth and width) and the size of flower parts that visitors can land on (size of the alighting place). In addition I tested the role of flower abundance for the number of visitor species. I hypothesized that the stronger the size constraints and the lower the flower abundance, the fewer visitor species on a plant species will be found. Indeed, nectar holder depth, nectar holder width and number of flowers explained a large part of the variation in the number of visitor species. The size of the alighting place did not restrict the body length of the visitors and was not related to visitor species number. The potential number of visitor species, that is the number of species in the local species pool that met the threshold criterion, was significantly positively correlated with the observed number of species. I also found that the observed visitors were a random selection out of the potential visitors. The means of the observed and expected proboscis length means were highly correlated.

In CHAPTER 3 I argued further that if size constraints and interactions proportional to abundance determine the number of interaction partners, these rules should be able to predict whether a plant species interacts with a generalized or a specialized flower visitor, *i.e.* they should also explain the degree of asymmetry in interaction patterns. The Monte Carlo simulations showed that both morphological constraints (nectar holder depth) and abundance were able to generate asymmetric patterns of specialization. However, only nectar holder depth was able to predict the level of asymmetry for an individual species. Thus, asymmetric specialization seems to be primarily the result of the depth threshold. Only when visitors meet the threshold criterion random sampling proportional to abundance plays a role.

In the second part of CHAPTER 3 I tested the potential consequences of these rules for the extinction risk of species. The simulation models provided the possibility to test if asymmetry is able to equalize extinction risks of generalized and specialized species as proposed in the literature. I hypothesized that, even if the stability of the whole web is stronger if it is asymmetrically structured, the short term extinction risk for morphologically generalized species will be lower than for morphologically specialized species. The simulations indicated that asymmetry caused by a size threshold should indeed lead to higher extinction risk of specialists. In the study system specialists were less abundant than generalists. Therefore, including abundance in the simulation models enlarges the difference in extinction risk between specialists and generalists.

In the last step (CHAPTER 4) I tested the influence of size thresholds and size distributions across species and individuals on the degree of morphological matching between proboscis lengths and nectar holder depths. The degree of morphological matching can be an important factor influencing visitation rates and per-visit pollination efficiencies of flower visitors. If it is true that a size threshold determines the degree of generalization, and generalization dominates in flower visitation webs, most plant and visitor species should show a high degree of morphological mismatching. However, the frequency distribution of traits across the potential interaction partners will also influence the average degree of matching. By using a simple analytical modeling approach based on size thresholds and interactions proportional to the frequency of traits I showed that a right-skewed distribution of proboscis lengths and a left-skewed distribution of nectar holder depths theoretically will lead on average to equal degrees of matching for all species, all other combinations will produce stronger differences. The analysis of the Mediterranean flower visitation web revealed that proboscis lengths and nectar holder depths were both right-skewed distributed across species and individuals. As predicted by the model, flower visitors with a long proboscis matched on average the nectar holder depth of the flowers less closely than those with a short proboscis, while plant species with shallow and deeply hidden nectar matched their interaction partners on average equally closely. The observed deviations from model expectations will serve as a starting point to search for additional factors that influence visitation patterns.

CHAPTER 5

# General conclusion

In this thesis I have shown that the combination of two simple rules can explain surprisingly well a great deal of the observed interaction patterns between plants and flower visitors in a Mediterranean flower visitation web: firstly, the size threshold that the nectar holder depth puts on the proboscis length of potential flower visitor; and secondly, random interactions proportional to the frequency of nectar holder depths and proboscis lengths in the local species pool. To describe the interaction patterns I used the degree of ecological generalization, the degree of interaction asymmetry and the degree of morphological matching. The size threshold and interactions proportional to abundance can explain the observed variation in the number of interaction partners, *i.e.* the degree of ecological generalization. Both rules can also generate the observed asymmetry patterns in the relationship between plants and flower visitors, *i.e.* morphologically generalized plants interact mainly with morphologically specialized visitors and morphologically specialized plants with morphologically generalized visitors. They are also sufficient to predict quite closely the overall pattern of morphological matching between nectar holder depth and proboscis length of plant and visitor species. The average degree of morphological matching for visitors and plants was strongly influenced by the shape of the frequency distributions, which was right-skewed and resemble a lognormal distribution for both nectar holder depths and proboscis lengths. Given these results, the importance of determining size constraints and measuring size distributions across species and individuals in the local species pool of plants and flower visitors has to be emphasized. Including size constraints and size distributions is essential for constructing realistic simulation models which should be used to yield a better understanding of plant–flower visitor interaction patterns at the level of the community. Knowledge about interaction patterns and the underlying causes of these patterns is essential for understanding the evolution of plants and their pollinators. It also will provide an important tool for biodiversity conservation.

# Nederlandse samenvatting

Tot voor kort werd de relatie tussen bloemplanten en hun bestuivers vooral gezien als gespecialiseerd en wederzijds sterk aangepast. Specialisatie betekent in dit geval dat één plantensoort voornamelijk door één diersoort, of een kleine groep nauw verwante diersoorten, wordt bestoven. Het concept van bestuivingsyndromen, dat is dat een bepaalde combinatie van bloemkenmerken de bouw, fysiologie en het gedrag van een bepaalde bloembezoekersgroep weerspiegelt (bijvoorbeeld dat van bijen, vlinders of vogels), hangt sterk met deze visie samen. Echter het idee dat de wisselwerkingen tussen bloemen en bloembezoekers overwegend gespecialiseerd zijn, komt doordat vooral gespecialiseerde soorten zijn onderzocht. Het onderzoek van volledige gemeenschappen van bloemplanten en bloembezoekers heeft laten zien dat gespecialiseerde relaties tussen bloemen en bloembezoekers eerder uitzondering zijn dan de regel. De mate van generalisatie varieert, ook lokaal, van één tot meer dan honderd partners. Bovendien verschilt niet alleen de mate van generalisatie van planten en van bloembezoekers aanzienlijk, ook de mate van generalisatie van de partner varieert enorm. Het algemene patroon is asymmetrisch, dat wil zeggen dat generalistische planten vooral door specialistische dieren worden bezocht en specialistische planten vooral door generalistische dieren.

De mechanismen die tot deze variatie in het aantal partners leiden en de resulterende patronen in bloemplanten-bloembezoekerwebben zijn nog steeds weinig begrepen. In dit proefschrift heb ik de rol van twee eenvoudige regels getest die de interactiepatronen tussen bloemen en bloembezoekers zouden kunnen structureren. Het eerste mechanisme is gebaseerd op de observatie dat de beloning die planten aan bezoekers aanbieden om ze naar de bloemen te lokken, sterk verschilt in hoe eenvoudig het voedsel te bereiken is. Het tweede mechanisme is gebaseerd op de observatie dat bloembezoekers ertoe neigen zich evenredig te verdelen over de beschikbare bloeiende planten. Ik heb de betekenis van deze factoren getest door in een mediterraan bloemplanten-bloembezoekerweb de gevonden patronen te vergelijken met verwachte patronen. De verwachte patronen zijn gebaseerd op simulatiemodellen die verschillende combinaties van de potentiële factoren bevatten. Ik heb de analyse in deze studie beperkt tot nectarproducerende plantensoorten en nectarzoekende bloembezoekers vanwege de morfologische beperkingen die ik wilde testen.

In HOOFDSTUK 2 heb ik drie mogelijke morfologische beperkingen bestudeerd: de diepte en de breedte van buisvormige structuren waarin de nectar zich bevindt en de afmeting van bloemdelen waarop de bloembezoeker kan landen. Bovendien heb ik de rol die het aantal bloemen voor het aantal bloembezoekers speelt, onderzocht. Ik heb de volgende hypothese getoetst: Hoe sterker de beperkingen zijn die de morfologie van de bloem aan de morfologie van de bloembezoekers oplegt en hoe kleiner het aantal bloemen, des te minder soorten bloembezoekers zullen er worden gevonden. En inderdaad, de diepte en de breedte van de nectarbuis en het aantal bloemen verklaarde voor een groot deel de verschillen in het aantal bloembezoekers. De afmeting van de landingsplaats van de bloem vormde geen beperking voor de lengte van het lichaam van de bloembezoeker en was dus ook niet gerelateerd aan het aantal soorten bloembezoekers. Het potentiële aantal soorten bloembezoekers, dat is het aantal soorten in de locale soortenpoel die aan het drempelcriterium voldoet (een tong net zo lang of langer en net zo smal of smaller dan de nectarbuis), was significant positief gecorreleerd met het aantal gevonden soorten bloembezoekers. Ik heb ook gevonden dat de waargenomen bezoekers een toevallige selectie uit de potentiële bezoekers is. De gemiddelde waargenomen en verwachte tonglengte was hoog gecorreleerd.

In HOOFDSTUK 3 beargumenteerde ik verder dat als twee simpele regels (dieptedrempel van de nectarbuis en toevallige interacties evenredig aan de hoeveelheid bloemen) het aantal interactiepartners bepalen, dat deze twee regels ook in staat zouden moeten zijn om te voorspellen of een plantensoort door generalistische of specialistische bloembezoekers wordt bezocht. Met andere woorden, deze regels zouden ook moeten kunnen voorspellen hoe groot de asymmetrie van de interactiepatronen is. Monte Carlo simulaties lieten zien dat zowel morfologische beperkingen (diepte van de nectarbuis) als de hoeveelheid bloembezoekers en bloemplanten in staat waren om asymmetrische interactiepatronen te genereren. Hoewel, alleen de diepte van de nectarbuis was in staat het niveau van asymmetrie voor iedere soort afzonderlijk te voorspellen. Daarom lijkt het erop dat asymmetrische specialisatie vooral het resultaat is van de dieptedrempel. Alleen als de bloembezoekers een voldoende lange tong hebben speelt een toevallige trekking evenredig aan de hoeveelheid van planten en dieren een rol.

In het tweede deel van HOOFDSTUK 3 heb ik de mogelijke consequenties van deze voorwaarden op de kans dat soorten uitsterven getest. De simulatiemodellen gaven de mogelijkheid om te testen of asymmetrische interactiepatronen ertoe leiden dat de kans om uit te sterven voor generalistische en specialistische soorten gelijk is, zoals in de literatuur wordt aangenomen. Ik heb de hypothese opgesteld dat, ook al is de stabiliteit van het gehele web groter wanneer het asymmetrisch is gestructureerd, de korte-termijnkans om uit te sterven kleiner is voor soorten die door hun morfologie generalistisch zijn dan voor soorten die door hun morfologie specialistisch zijn. De simulaties wezen erop dat asymmetrische interacties die door een groottedrempel worden veroorzaakt inderdaad tot een grotere kans voor gespecialiseerde soorten om uit te sterven kan leiden. In het bestudeerde systeem kwamen specialisten minder vaak voor dan generalisten. Daarom werd het verschil tussen generalisten en specialisten in de kans om uit te sterven groter waneer ook het aantal individuen in de simulatie modellen werd opgenomen.

In HOOFDSTUK 4 heb ik als laatste onderdeel van dit onderzoek de invloed van de frequentieverdeling van morfologische kenmerken in de locale soortenpoel op de mate van ‘passen’ van de tonglengte van de bloembezoeker en de nectardiepte van de bloemen getoetst. De mate waarin deze twee kenmerken qua grootte bij elkaar passen kan een belangrijke factor zijn voor de frequentie van bezoek van een bloembezoeker aan een plantensoort en de efficiëntie als bestuiver per bezoek (bijvoorbeeld hoeveel pollen wordt op de stempel gebracht). Als het waar is dat de groottedrempel en interacties evenredig aan het voorkomen van soorten de mate van generalisatie bepaalt, en als het waar is dat generalisatie in bloem–bloembezoekerwebben overheerst, dan zouden de morfologie van de meesten bloemen en de op deze bloemen waargenomen bloembezoekers vaak niet goed bij elkaar passen. Maar deze veronderstelling laat buiten beschouwing dat de frequentieverdeling van de kenmerken de mate van bij elkaar passen kan beïnvloeden. Om de potentiële invloed van deze factor te testen heb ik een eenvoudig analytisch model gebruikt dat is gebaseerd op nectardiepte, tonglengte en bloembezoek evenredig aan de frequentie van het voorkomen van deze kenmerken. Ik kon aantonen dat een scheve frequentieverdeling van tonglengtes met een staart rechts van de top van de verdeling en een scheve verdeling van nectarbuizen met een staart links van de top van de verdeling theoretisch zou leiden tot het gemiddeld goed bij elkaar passen van nectarbuizen en tonglengtes voor zowel generalistische als specialistische bloemplanten en bloembezoekers. Alle andere combinaties zouden tot grotere verschillen tussen generalistische en specialistische soorten leiden. Dit betekent dus dat generalistische dieren bloemen bezoeken die gemiddeld minder goed bij hun tonglengte passen en dat generalistische planten vooral worden bezocht door bloembezoekers die minder goed met hun tong bij de nectarbuis passen.

De analyse van het mediterrane bloembezoekerweb liet zien dat zowel tonglengtes als ook nectarbuizen een scheve frequentieverdeling met een rechte lange staart vertoonden. Een verdeling die sterk herinnert aan een log-normale verdeling van lichaamsmassa van verschillende diersoorten. De waargenomen mate van het morfologisch ‘bij elkaar passen’ van tonglengtes en nectardieptes was zoals voorspeld werd door het model op basis van de waargenomen frequentieverdelingen. Bloembezoekers met een lange tong (generalisten) pasten gemiddeld minder goed bij de nectardiepte van de bezochte bloemen dan die met een korte tong. Planten met open toegankelijke nectar en die met diep verborgen nectar verschilden gemiddeld niet veel van elkaar in de mate waarop de tongen hun waargenomen bloembezoekers pasten. Generalistische en specialistische bloemen lieten een hoge mate van matchen zien. De waargenomen afwijkingen van de theoretische verwachtingen kunnen als startpunt dienen voor de zoektocht naar verdere factoren die de bezoekpatronen beïnvloeden.

## Algemene conclusie

In dit proefschrift heb ik kunnen laten zien dat twee eenvoudige regels verrassend goed de waargenomen interactiepatronen tussen bloemplanten en hun bloembezoekers in een mediterraan bloembezoeker visitatieweb kunnen verklaren. Deze twee regels zijn de groottedrempel die de nectarbuis oplegt aan de tonglengte van de mogelijke bloembezoekers en toevallige interacties evenredig aan de frequentie van nectardieptes en tonglengtes in de locale soortenpoel. Om de interactiepatronen te beschrijven heb ik de mate van generalisatie, de mate van asymmetrie en de mate van het passen van nectardiepte en tonglengte gebruikt. De groottedrempel en interacties evenredig aan de frequentie van bloemen of individuen kan die mate van generalisatie, dit is het waargenomen aantal interactiepartners, verklaren. Beide regels kunnen ook de mate van de waargenomen asymmetrie in de relatie tussen planten en bloembezoekers genereren. Dit wil zeggen dat morfologisch generalistische planten voornamelijk door morfologisch specialistische bloembezoekers worden bezocht en morfologisch specialistische planten voornamelijk door morfologisch generalistische bloembezoekers. Deze regels zijn ook voldoende om te voorspellen hoe goed tonglengte en nectardiepte bij elkaar passen. De mate waarin deze twee kenmerken overeenkwamen werd sterk beïnvloed door de vorm van de frequentieverdeling. De waargenomen verdelingen van nectarbuizen en tonglengtes leken op een log-normale verdeling. Deze uitkomst benadrukt dat het bepalen van groottedrempels en frequentieverdelingen van morfologische kenmerken heel belangrijk zijn. Het opnemen van deze twee factoren is essentieel voor het construeren van realistische simulatiemodellen. Simulatiemodellen zouden moeten worden gebruikt voor een beter begrip van plant-bloembezoeker interactiepatronen. Kennis over de interactiepatronen en de ten grondslag liggende oorzaken van deze patronen zijn essentieel voor ons begrip over de evolutie van planten en hun bestuivers. Het zou ook kunnen dienen als een belangrijke basis voor biodiversiteitbehoud.

# Deutsche Zusammenfassung

Es ist noch nicht so lange her, dass die Beziehungen zwischen Blüten und Bestäubern als überwiegend spezialisiert und eng aneinander angepasst betrachtet wurden, das heißt, dass die Mehrzahl der von Tieren bestäubten Pflanzenarten überwiegend durch eine oder einige wenige verwandte Tierarten bestäubt wird und deutliche Anpassungen an diese zeigt. Das Konzept der blütenökologischen Stiltypen spiegelt diese Sicht wider. Unter einem Stiltyp versteht man eine charakteristische Kombination von Blütenmerkmalen, die den Bau, die Physiologie and das Verhalten einer bestimmten Blütenbesuchergruppe repräsentiert. Dabei werden die Pflanzenarten nach der Tiergruppe, an die sie Anpassungen zeigen, benannt. Beispiele sind Bienenblumen, die an die Bestäubung durch Bienen angepasst sind, Falterblumen, die an Falter angepasst sind oder Vogelblumen, die an Vögel angepasst sind. Die Vorstellung, dass die Wechselbeziehungen zwischen Blüten und Blütenbesuchern überwiegend spezialisiert sind, scheint aber vor allem eine Folge davon zu sein, dass vornehmlich Arten mit spezialisierten Wechselbeziehungen untersucht wurden. Die Analyse von vollständigen Pflanzen-Blütenbesucher-Gemeinschaften hat dagegen gezeigt, dass spezialisierte Wechselbeziehungen zwischen Blüten und Blütenbesuchern eher die Ausnahme als die Regel sind. Die Pflanzen werden oft von mehreren Tiergruppen besucht und der Grad der Spezialisierung (hier Anzahl der Arten) kann zwischen einem und mehreren Dutzend Interaktionspartnern betragen. Und nicht nur der Grad der Spezialisierung innerhalb van Pflanzenarten und Tierarten variiert deutlich, auch der Grad der Spezialisierung jener Partner, mit denen eine Art Beziehungen aufweist, kann sehr unterschiedlich sein. Das vorherrschende Muster ist asymmetrisch.

Das bedeutet, dass Pflanzen, die von vielen Tierarten besucht werden, vor allem von Tieren besucht werden, die ihrerseits wenige Pflanzenarten besuchen, und Pflanzenarten, die von wenigen Tierarten besucht werden, diese wiederum viele Pflanzenarten besuchen. Dasselbe Prinzip gilt auch aus der Sicht der Blütenbesucher.

Die Ursachen, die zu den Unterschieden in der Anzahl Interaktionspartner und den daraus folgenden Interaktionsmustern in Blütenpflanzen-Blütenbesucher-Gemeinschaften führen, sind bislang noch nicht eingehend untersucht worden. In dieser Doktorarbeit testete ich die Bedeutung von zwei einfachen Regeln, die für die Strukturierung von Interaktionsmustern zwischen Blüten und Blütenbesuchern von Bedeutung sein könnten. Die erste Regel ist auf der Beobachtung basiert, dass die Belohnung, die Blüten anbieten um potentielle Bestäuber anzulocken (z.B. Nektar), unterschiedlich tief in der Blüte verborgen ist. Die zweite Regel bezieht sich auf die Beobachtung, dass Blütenbesucherindividuen dazu tendieren, sich im Verhältnis zu den verfügbaren Blütenressourcen gleichmäßig über die Pflanzen zu verteilen. Ich testete die Bedeutung dieser Regeln, indem ich die beobachteten Interaktionsmuster in einer mediterranen Blüten-Blütenbesucher-Gemeinschaft mit Mustern verglichen habe, die entstehen, wenn diese Regeln in verschiedenen Kombinationen in Monte-Carlo Simulationsmodellen berücksichtigt werden. Auf Grund der morphologischen Merkmale, die ich testen wollte, habe ich in dieser Untersuchung die Analyse auf jene Pflanzenarten beschränkt, die Nektar produzieren sowie auf jene Insektenarten, die in der Blüte nach Nektar suchen.

In KAPITEL 2 testete ich drei Merkmale, die potentiell die Morphologie möglicher Besucher einschränken: die Tiefe und den Durchmesser von Blütenstrukturen, die den Nektar bergen, und die Größe von Blütenteilen, die potentiell als Landeplatz dienen können. Zusätzlich testete ich die Bedeutung der Blütenmenge für die Anzahl der Blütenbesucherarten. Ich stellte die Hypothese auf, dass umso stärker die Größenbeschränkung ist und umso weniger Blüten eine Pflanzenart aufweist, desto weniger Besucherarten auf dieser Pflanzenart zu finden sein werden. Ich fand tatsächlich, dass die Tiefe und der Durchmesser der Nektarröhre und die Anzahl Blüten sehr gut die Anzahl der Blütenbesucherarten zu erklären vermag. Die Größe der Blüteteile, die als

Landeplatz dienen können, beschränkte dagegen die Körpergröße der Besucher nicht und stand auch nicht im Zusammenhang mit der Anzahl der Besucherarten. Die potentielle Anzahl jener Blütenbesucherarten im lokalen Artenpool, die einen längeren und dünneren Rüssel als die Nektarröhre einer Pflanzenart haben, war signifikant positive korreliert mit der beobachteten Anzahl Besucherarten. Ich konnte auch zeigen, dass die beobachteten Besucher eine zufällige Auswahl aus der Menge der potentiellen Besucher darstellte. Die beobachtete und die erwartete mittlere Rüsselränge waren hoch korreliert.

In Kapitel 3 argumentierte ich weiter, dass sofern die Größe und die Menge der Blüten die Anzahl der Blütenbesucher beschränkt, diese Regeln auch in der Lage sein sollten vorherzusagen, ob eine Pflanzenart von spezialisierten oder unspezialisierten Blütenbesuchern besucht wird. Das bedeutet, dass diese Regeln auch die allgemein in Pflanzen-Blütenbesucher-Gemeinschaften beobachtete Asymmetrie der Interaktionsmuster erklären können sollte. Die Monte-Carlo Simulationen zeigten, dass sowohl morphologische Einschränkungen (Nektartiefe) als auch die Häufigkeit des Vorkommens dieser Arten im Artenpool in der Lage waren, asymmetrische Interaktionsmuster auf dem Niveau der Gemeinschaft zu generieren. Jedoch ermöglichte nur die Nektartiefe artspezifische Vorhersagen über den Grad der Asymmetrie zu machen. Daraus kann man schließen, dass asymmetrische Interaktionsmuster in erster Linie ein Resultat der Größenbeschränkung sind. Nur wenn die Besucher das Größenkriterium erfüllen, das heißt ihre Rüssel länger sind als die Nektarröhre, spielen wahrscheinlichkeitsbestimmte Interaktionen, welche proportional zur Häufigkeit dieser Arten im Artenpool sind, eine Rolle.

Im zweiten Teil von Kapitel 3 untersuchte ich die möglichen Konsequenzen dieser Regeln für die Aussterbewahrscheinlichkeiten von Arten. Die Simulationsmodelle gaben die Möglichkeit abzuschätzen, ob asymmetrische Wechselbeziehungen in der Lage sind, das Aussterberisiko von Spezialisten und Generalisten anzugleichen, eine Behauptung die gelegentlich in der Literatur zu finden ist. Ich stellte die Hypothese auf, dass, selbst wenn die Stabilität in asymmetrisch strukturierten Gemeinschaften höher ist als in zufällig oder symmetrisch strukturierten Gemeinschaften, das durch Zufallsprozesse bedingte kurzfristige

Aussterberisiko für morphologisch spezialisierte Arten größer sein sollte als für nicht spezialisierte Arten. Die Simulationen ließen in der Tat erkennen, dass eine durch eine Größenbeschränkung verursachte Asymmetrie das kurzfristige Aussterberisiko von Spezialisten und Generalisten nicht angleicht. Die Größenbeschränkung führte zu einem größeren Aussterberisiko für morphologisch spezialisierte Arten. In dem untersuchten System waren Spezialisten seltener als Generalisten. Deshalb führte das Einbeziehen der Häufigkeit in die Simulationsmodelle dazu, dass der Unterschied im Aussterberisiko zwischen Spezialisten und Generalisten weiter vergrößert wurde.

Als letzen Schritt (Kapitel 4) testete ich den Einfluss der Größenbeschränkung und der Frequenzverteilung auf das Maß der Übereinstimmung von Rüssellänge und Nektarröhre. Der Grad der Übereinstimmung kann ein bedeutsamer Faktor sein, der Besuchsraten und Bestäubungs-effizienz der Blütenbesucher beeinflusst. Ein vorhersagbares Maß der Übereinstimmung verschafft den Blüten die Möglichkeit, sich an die vor-herrschende Morphologie der Blütenbesucher anzupassen. Wenn es wahr ist, dass die Größenbeschränkung den Grad der Spezialisierung einer Art bestimmt, dann sollten generalistische Pflanzen von einer großen Anzahl Blütenbesuchern besucht werden, deren Rüssellänge nicht gut an die Tiefe der Nektarröhren der Blüten angepasst ist. In diesem Fall sollte also die Übereinstimmung gering sein. Diese Erwartung lässt jedoch außer Betracht, dass sobald sich die Blütenbesucher proportional zur Häufigkeit der Arten im Artenpool über die Pflanzenarten verteilen, die Frequenzverteilung der Merkmale innerhalb der Pflanzen- und Tierarten den Grad der morphologischen Übereinstimmung beeinflusst. Ich konnte anhand einfacher analytischer Modelberechnungen zeigen, dass unter der Annahme einer Größenbeschränkung und unter der Annahme von Blütenbesuchen proportional zur Frequenz der Merkmale, die Frequenzverteilung eine große Rolle spielt. Nimmt die Anzahl der Arten oder Individuen mit zunehmender Rüssellange ab (wie zum Beispiel bei einer schiefen Verteilung mit einem Schwerpunkt auf der linken Seite – vergleichbar mit einer log-normalen Verteilung), und nimmt die Anzahl der Pflanzenarten oder Individuen mit zunehmender Nektartiefe zu (wie bei einer gespiegelten log-normalen Verteilung), dann passen Rüssellangen und Nektartiefen sowohl für Generalisten als auch für Spezialisten gut zueinander. Das gilt in diesem Fall sowohl für die Pflanzen als auch für die Tiere. Alle anderen Verteilungen und Verteilungskombinationen (z.B. alle Rüssellangen oder Nektartiefen kommen gleich häufig vor, beide nehmen ab oder beide nehmen zu) führen theoretisch zu größeren Diskrepanzen für morphologisch generalistische Arten (Pflanzen mit offen dargebotenem Nektar und Blütenbesucher mit langen Rüsseln).

Die Analyse des untersuchten mediterranen Bestäubungswebs ergab, dass sowohl Rüssellängen als auch Nektarröhren log-normal verteilt waren (die meisten Arten verfügten über kurze Rüssel oder wenig tief verborgenen Nektar). Dies galt sowohl für die Verteilung basierend auf Artenzahlen als auch auf Individuenzahlen. Weiterhin konnte ich zeigen, dass Blütenbesucher mit einem langen Rüssel eine schlechtere Übereinstimmung mit den Nektarröhren der von ihnen besuchten Pflanzenarten zeigten als dies bei Blütenbesuchern mit einem kurzen Rüssel der Fall war. Dahingegen unterschieden sich Pflanzenarten mit offen dargebotenem oder tief verborgenem Nektar kaum voneinander. Für beide Pflanzengruppen galt, dass die Besucher in einem hohen Maß in ihrer Größe zu den Blüten passten. Das beobachtete Maß der Übereinstimmung entsprach sowohl für die Tiere als auch für die Pflanzen den auf Basis des analytischen Models zu erwarteten Grad der Übereinstimmung. Zusammenfassend lässt sich sagen, dass schon jene beiden einfachen Regeln zu einem hohen Maße vorhersagen konnten, inwieweit Rüssellängen und Nektarröhren zueinander passen, was nochmals die Bedeutung dieser Regeln unterstreicht. Die beobachteten Abweichungen von den Modellerwartungen können schließlich als Ausgangspunkt genommen werden, um den potentiellen Einfluss weiterer Faktoren zu untersuchen, wie zum Beispiel die Konkurrenz zwischen den Blütenbesuchern oder Präferenzen bestimmter Blütenbesucher für bestimmte Nektartiefen oder Nektarmengen.

## Allgemeine Schlussfolgerungen

In dieser Doktorarbeit habe ich zeigen können, dass die Kombination von zwei einfachen Regeln zu einem überraschend großen Maß die beobachteten Interaktionsmuster von Blüten und Blütenbesuchern erklären können. Diese Regeln waren zum einen die Größenbeschränkung, die die Tiefe der Nektarbergung den Rüssellängen der potentiellen

Blütenbesucher auferlegt, und zum anderen, dass die Blütenbesuche sich proportional zur Häufigkeit von Nektartiefen und Rüssellängen im lokalen Artenpool über die Arten verteilen. Um die Interaktionsmuster zu beschreiben, benutzte ich den Grad der Spezialisierung, den Grad der Asymmetrie der Interaktionen und den Grad der morphologischen Übereinstimmung von Rüssellängen und Nektarröhren. Größenbeschränkungen und Interaktionen proportional zur Häufigkeit der Arten konnten weitgehend die beobachteten Unterschiede in der Anzahl der Interaktionspartner erklären (den Grad der Spezialisierung). Beide Regeln waren auch in der Lage, das beobachtete asymmetrische Interaktionsmuster zu generieren: Generalistische Pflanzenarten werden vor allem von spezialistischen Blütenbesuchern besucht und spezialistische Pflanzenarten von generalistischen Blütenbesuchern. Das bedeutet, dass Pflanzenarten, die den Blütenbesuchern kaum morphologisch Beschränkungen auferlegen, überwiegend von Tieren besucht werden, die morphologisch in ihrer Blütenwahl beschränkt sind und dass Pflanzenarten, die den Blütenbesuchern deutliche morphologische Beschränkungen auferlegen, überwiegend von Tieren besucht werden, die in ihrer Blütenwahl morphologisch nicht einschränkt sind. Beide Regeln können auch relativ gut den beobachteten Grad der Übereinstimmung von Rüssellänge und Nektarröhre vorhersagen. Der Grad der morphologischen Übereinstimmung wird stark beeinflusst von den Frequenzverteilungen der Merkmale im lokalen Artenpool. Diese ähnelte sowohl bei den Nektarröhren als auch bei den Rüssellängen einer log-normalen Verteilung, wie sie charakteristisch für die Körpermassenverteilungen von Tieren ist. Diese Ergebnisse zeigen auf wie wichtig es ist, zum einen diejenigen morphologische Merkmale zu finden, die eine Einschränkung für die Interaktionen zwischen Blüten und Bestäubern darstellen und zum anderem die Verteilungsmuster dieser Merkmale im lokalen Artenpool festzustellen. Dies ist essentiell für die Konstruktion realistischer Simulationsmodelle. Diese Modelle bilden ein wichtiges Hilfsmittel, um die Interaktionsmuster zwischen Pflanzen und Bestäubern auf dem Niveau von ganzen Gemeinschaften zu verstehen. Die Kenntnis über Interaktionsmuster und der ihnen zugrunde liegenden Ursachen ist daneben unentbehrlich für das Verständnis der Evolution von Pflanzen und ihren Bestäubern und kann auch eine wichtige Basis für einen besseren Erhalt der Artenvielfalt bilden.

# Acknowledgements

Pollination ecology is a very attractive scientific field to study. It is not only fascinating to observe the interactions between flowers and flower visitors, it is also a pleasure to make other people enthusiastic about it. And this is not very difficult indeed. Most people love flowers but only a few have taken a closer look. To invite them to explore the functioning of a flower gives a lot of satisfaction. How flowers advertise and present the resources that their pollinators are keen to exploit; how flowers are able to manipulate who will visit them and how these visitors behave in a flower; and how flower visitors for their part try to trick flowers. All of this is often surprising, very ingenious and a joy to discover. With the pollination syndromes, a classification system that uses characteristic combinations of flower traits such as form, colour and scent, one gets an uncomplicated tool at hand for predicting who visits whom. It invites one to play a game. I once started this game at the beginning of my, already long lasting, interest in pollination biology. All the people who listened to my stories about this game – some of them are nature guides, others followed nature courses or attended nature excursions – I want to thank for their interest, enthusiasm and often surprising ideas and observations. Education was and is for me an important source of motivation to do science.

The system of pollination syndromes, however, has clear limitations. Looking closer at the relationship between flowers and flower visitors during different scientific studies, especially when studying whole plant – flower visitor communities, this system is not very helpful. So I began to search for alternative possibilities to describe and analyze the interaction patterns between flowering plants and their pollinators. I did this not by my own. Together with other people, with some of them I closely cooperated during a number of years, I discussed a lot of possibilities to find better ways to describe and analyze interaction patterns at the community level. I want to thank my former colleagues Christian Westerkamp and Werner Kreisch at the University of Berlin for stimulating discussions about the classification of flowers. And I want to thank in particular Bodo Schick, University of Berlin and later University of Kassel, for sharing his knowledge about the history of pollination biology and his deep insights into the functional morphology of flowers. This opened for me a new way to approach the enormous morphological diversity of flowers. The result was the index of floral complexity that I developed to make this approach operational and testable. This index uses the traits which make up the functional morphology of flowers and quantifies the ability of a flower to restrict the behaviour of the flower visitors by trying to get to the rewards. The index is inspired by the description of the structural flower types given by Faegri and van der Pijl in their famous book on pollination published in 1979 and profited much from the work of the nineteenth century pollination biologists such as Hermann Müller, Ernst Loew and Paul Knuth. The classification system of Ernst Loew, for example, is largely based on the accessibility of nectar and provided an important starting point for me. Overall, these early pollination biologists were very good observers and had a very stimulating way of describing their findings, largely inspired by the evolutionary theory of Charles Darwin.

I tested this index in real communities together with a number of students at Leiden and Groningen. I want to thank these students for their enthusiasm and their critical thoughts on the index during the ecological field courses, especially Jan de Jonge, Sietse van der Linde, Simon Maes, Anne Marieke Wagenaar, Bram van Vliet, Jeroen Zouterdijk, Marijke Langeveld and Nicolette Snijders. I also discussed the index and the results of the field studies with Manja Kwak from the University of Groningen, whom I want to thank very much for her friendship and the stimulating and encouraging discussions throughout a long number of years. And I want to thank Frank Hoffmann, also from Groningen, for the discussions about pollination biology at the level of the community. From our trips to the meetings of the Scandinavian Society of Pollination Ecologists we always went home highly motivated and with fresh ideas.

The index was the first and important step into the direction of the study of this thesis. In fact, I went a step backwards and decided to choose only part of the traits I incorporated in the index, i.e. nectar tube depth and width. I did this in order to simplify the analysis and make it even more operational than the index in its whole. I am very happy that I made this step, even this simplification is complex enough to handle in simulation models. The inspiration to do this is largely the result of doing research within the Plant Ecology Group at Leiden. It is a pleasure for me to thank the members of this group for their inspiration, their critical discussions of my community-level approach, but also the confidence in my sometimes complex ideas, and the possibility to test these ideas together with students during the field courses of ecology and my work in Spain. It was and is very pleasant and stimulating to be a researcher in this group.

Special thanks go to Kirsten Kaptein and Vera Geluk, both were in 2003 bachelor students at Leiden University and worked together with me in Spain. We did a lot of work, had not much spare time, but learned a lot. We managed to solve almost all problems and the commitment and critical thoughts of them made the beginning of my PhD project a success. And I want to thank the members of the Entomology Group of the KNNV in The Hague, especially Frank van der Meer and Alexander Deelman for sharing their huge knowledge about insects. Kirsten Leiss, University of Leiden, I want to thank for the discussions about nectar, nectar production and nectar heritability, and the nice trips together to different scientific meetings; and Tom de Jong, Frans Jabobs, Tom van Dooren and Wil Tamis, University of Leiden, and Rampal Etienne, University of Groningen, for sharing their insights into modelling or statistics.

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Last but certainly not least I want to thank my husband Ingo for all his support, his patience and his love. I enjoyed and enjoy our stimulating discussions on biology, science and philosophy. I also want to thank my parents for their support and interest in my work. Unfortunately, my father will not be able to see this book. And I want to thank my niece Zora, who joined part of my study in Spain, gathering flowers and hunting flower visitors, at that time only four years old. Every time we are together we are busy with nature and we have always nice discussions about flowers and insects. To these people, Ingo, my parents and Zora, I want to dedicate this thesis.

# Curriculum vitae

I was born on the 17th of October 1959 in Greifswald, Germany. My family moved to Berlin in 1961. In June 1978 I finished secondary school at the Sophie Scholl Gymnasium in Berlin. Between 1978 and 1986 I studied Biology at the Free University of Berlin with the main focus on morphology, physiology and ecology. During my study I was a student tutor at the Institute of Systematic Botany and Plant Geography (1983-1986). My Diploma thesis from 1986 was on the pollination biology of *Stachys recta* and *Anthericum liliago* as elements of dry meadows. In 1986 I married Ingo Stang. Between 1986 and 1997 I got two commissions at the Institute of Systematic Botany and Plant Geography. In the first one I mapped and characterized dry meadows in the Werra-Meißner Region, Hessen, Germany. The second one was on the floral biology of *Pulsatilla vulgaris* and *Scabiosa columbaria*. In 1988 and 1989 I received a scholarship at the Institute of Systematic Botany and Plant Geography for studying the flowering phenology and pollination ecology of xerothermic vegetation complexes in Central and Southern Germany. I worked as a guide in the Botanical Garden Berlin Dahlem (1984-1990) and as a lecturer at the Institute of Systematic Botany and Plant Geography (1987-1990). In 1991 my husband and I moved to the Netherlands. Since 1993 I am a member of the IVN, a non-profit nature education organisation in the Netherlands. I am involved in several nature education projects such as nature courses and the training of nature guides. In 1995 I joined the group Plant Ecology at Leiden University as a guest researcher and worked in this group on the flowering phenology of plant communities in the dunes. Between 1995 and 1999 I also collaborated with Bodo Schick, University of Kassel, Germany. We worked on two projects, the functional morphology and functional classification of flowers and the anthogram, a method to describe flowering phenology and floral diversity of plant communities. The results of this work were presented at different conferences in Germany during 1997 and 1999. In 1999 I collaborated also with Werner Kreisch, associated scientist of the Botanical Garden and Botanical Museum Berlin, Germany. We worked together on the floral biology of alpine plants in Austria. Between 2000 and 2002 I developed in Leiden an index of floral complexity in order to predict pollinator diversity. I tested this index in plant communities in the dunes. This work resulted in a publication on the relationship between plant species diversity and flower complexity along a moisture gradient in a wet dune valley. Since 2001 I am involved at the University of Leiden in field ecology courses, I give lectures on pollination ecology and community ecology and supervise bachelor students. In 2003 I started a PhD project about the structure of a flower visitation web in a Mediterranean plant–flower visitor community under the supervision of Eddy van der Meijden and Peter Klinkhamer. This work resulted in this thesis.

# Publications

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