The handle http://hdl.handle.net/1887/57797 holds various files of this Leiden University dissertation

**Author:** Klumpers, Saskia  
**Title:** Beyond random and forbidden interactions in plant-pollinator networks: how optimizing energy gain results in morphological matching among subalpine Asteraceae and their flower-visitors  
**Date:** 2017-12-15
General discussion
In recent years, plant-flower-visitor community studies have shown general interaction patterns such as asymmetry (generalist flower-visitors interact with specialist plants and vice versa) and nestedness (a core group of generalists interacts with each other and a few specialists mostly interact with generalists) (e.g. Bascompte et al. 2003; Vazquez & Aizen 2004; Bascompte, Jordano & Olesen 2006). These interaction patterns are often explained by random foraging behaviour of flower-visitors (Vazquez 2005; Vazquez et al. 2007), sometimes in combination with the existence of forbidden interactions (Stang, Klinkhamer & van der Meijden 2006; Rezende, Jordano & Bascompte 2007; Stang, Klinkhamer & van der Meijden 2007; Stang et al. 2009; Olesen et al. 2011). Forbidden interactions imply that plants are not visited by certain flower-visitor, due to a mismatch in either phenology or morphology. Surprisingly, although nectar reward and foraging efficiency is one of the most likely aspects to determine flower-visitor foraging behaviour, we lack knowledge on how these aspects affect the type and number of interaction partners and consequently plant-flower-visitor network structure (but see Junker et al. 2013; Carvalheiro et al. 2014; Tinoco et al. 2017). The main aim of this study was to investigate whether flower-visitor foraging efficiency can explain species generalization degree and plant-flower-visitor interaction patterns, such as asymmetry, nestedness and size-matching between the nectar tube depth of flowers and the average proboscis length of their flower-visitors. In addition, I used my results to investigate the ways plants can mitigate the risk that in a particular year there are insufficient numbers of flower-visitors to ensure pollination. I specifically hypothesized that plants can either be generalized on flower-visitors which are prone to temporal fluctuations or specialized on flower-visitors which are less prone to temporal fluctuations, which will result in equal visitation rates. In other words plants face a pollinator species number (SN) – species predictability (SP) trade-off.

I have analyzed the interactions of subalpine Asteraceae species, co-occurring in the Colorado Rocky Mountains, and their flower-visitors. The Asteraceae are visited by a wide diversity of flower-visitor taxa, including bumblebees, solitary bees, butterflies and flies and thus are considered to be generalists (Graenicher 1909; Lane 1996; Proctor, Yeo & Lack 1996; Torres & Galetto 2002). Therefore, they provide the unique opportunity to investigate how different flower-visitor taxa respond to variation in plant traits and how interactions are structured among generalized plant species. More important, they are an ideal study system to investigate the effect of foraging efficiency on plant-flower-visitor interaction patterns, because they have relatively shallow flowers, which do not pose strong morphological constraints on their flower-visitors. A large proportion of their potential flower-visitors, mostly bumblebees and butterflies, have a proboscis long enough to visit all plant species.

In the first experimental chapter, Chapter 2, I analyzed the interaction structure among the Asteraceae and their flower-visitors. I investigated (1) whether interactions among generalized plants and their flower-visitors were more generalized and connected and less asymmetric and nested, (2) whether plant generalization degree (both the number of flower-visitor species and Shannon diversity index) and the number of flower-visitor visits were related to local flower head density, nectar tube depth and display area and (3) whether plant species with flowers with deeper nectar tubes are visited by flower-visitor species which have a longer proboscis and how this degree of size-matching differs among flower-visitor taxa,
including bumblebees, solitary bees, butterflies and flies. In general, among generalized plant species, which have simple and easy accessible flowers, it is assumed that interactions are more generalized and connected and therefore less structured and predictable. Surprisingly, I found that the level of interaction asymmetry and nestedness was comparable to complete pollination networks (Traveset et al. 2016). These results might be explained by the (unexpected) presence of specialized flowers. However, I did find that the interactions among the Asteraceae and their flower-visitors were more generalized and connected, compared to other pollination networks (Traveset et al. 2016). This indicates that flower-visitors had a higher niche overlap and thus that species of the Asteraceae share a greater proportion of their flower-visitor species which implies that there is a high redundancy among these interactions. This indicates that plant phylogeny affects flower-visitor visitation patterns (e.g. Rezende, Jordano & Bascompte 2007).

Further, plant generalization degree (both the number of flower-visitor species and their Shannon diversity index) increased with increasing local flower head abundance and display area and decreased with increasing nectar tube depth. However, the number of visit increased with increasing display area but was unrelated to nectar tube depth or flower head abundance. In contrast to what has often been assumed (Vazquez & Aizen 2004; Vazquez 2005), these results suggest that plant-flower-visitor interactions cannot simply be explained by random encounters among flower-visitors and the flowers they visit. This is because random foraging by flower-visitors implies that more abundant plant species should not only interact with more flower-visitor species, but also receive more visits. Rather, in this community, it is likely that plant-flower-visitor interactions are influenced by proboscis length frequency distribution of flower-visitors and the negative correlation between flower density and nectar tube depth. More abundant plant species had flowers with shallow nectar tubes and therefore could be visited by a wider diversity of insect species. Moreover, because flower-visitor species with a short proboscis also visited the plants less frequently and therefore apparently were also less abundant, the number of visits was unrelated to flower head density.

Furthermore, in accordance with previous studies (Stang et al. 2009), flowers with deeper nectar tubes were, on average, visited by bumblebee-, solitary bee- and fly species with a longer proboscis. Among butterflies, I did not find these relationships. This is likely because butterflies might select flowers on different floral cues, such as nectar concentration (Watt, Hoch & Mills 1974; Pyke & Waser 1981) or floral display area. In contrast to previous studies (Stang et al. 2009), in this study system, most bumblebees species had a proboscis which was long enough to visit all plant species. Therefore, this positive relationship between nectar tube depth and bumblebee proboscis length indicates that bumblebee species with a long proboscis avoided shallow flowers, while species with a short proboscis avoided deep flowers. These results indicate that size-matching between the nectar tube depth of flowers and the average proboscis length of their flower-visitor species even occurs in the absence of size constraints.

In Chapter 3 I investigated whether flowers with deeper nectar tubes produced more nectar, as a possible explanation for the size-matching pattern. Nectar is one of the major
floral rewards for flower-visitors. Therefore, flower-visitors are expected to prefer more rewarding flowers and to visit them more frequently (Leiss & Klinkhamer 2005). I addressed the following questions: (1) Do flowers with deeper nectar tubes produce more nectar per single flower and offer more nectar per flower head, individual plant and patch? (2) Are nectar tube depth and nectar production related to the number of open flowers per head and the number of flower heads per individual or patch? Since nectar standing crop is what flower-visitors actually encounter while foraging I also tested (3) whether nectar standing crop is related to nectar production rate. In accordance with previous studies (Petanidou, Goethals & Smets 2000; Galetto & Bernardello 2004; Kaczorowski, Gardener & Holtsford 2005; Ornelas et al. 2007; Martins & Johnson 2013; Johnson et al. 2017), I found that nectar production of single flowers increased with increasing nectar tube depth. However, this relationship has not yet been studied at the level of flower heads (or inflorescence), individual plant or patch. Nevertheless, the attractiveness of a plant is likely to be determined by the total reward it offers, which is also determined by the number of open flowers and clustering of these flowers. I found that, indeed flowers with deeper nectar tubes also produce more nectar per flower head, individual plant and patch. Plant species with deeper flowers will have a stronger incentive to produce more nectar, because they need to attract flower-visitor with a long proboscis, which can potentially visit a wide diversity of flowers (Ornelas et al. 2007; Martins & Johnson 2013; Johnson et al. 2017). Further, I found that the specific scaling relationship between nectar tube depth and nectar production were remarkably similar among two years and that nectar standing crop was significantly correlated with nectar production rate. These results, together with the fact that the Asteraceae produced minute amounts of nectar, which never filled the nectar tube for more than 11%, strongly indicate that nectar may act as an underlying factor promoting flower-visitor selection for deeper flowers.

In Chapter 4 and Chapter 5, I investigated whether the time it takes flower-visitor to handle a flower and extract nectar from it (handling time) and flower-visitor foraging efficiency might be alternative explanations for why size-matching between the nectar tube depth of flowers and the average proboscis length of their flower-visitors occurs. Because a long proboscis might be of hindrance when foraging on shallow flowers, longer-tongued insects are assumed to handle these flowers less efficiently (Inouye 1980; Plowright & Plowright 1997; Kunte 2007; Karolyi et al. 2013; Bauder et al. 2015). Consequently, flower-visitors with a long proboscis may visit shallow flowers less frequently or avoid them. In Chapter 4, I analyzed how handling time was related to insect proboscis length and the nectar tube depth and nectar volume of the flowers they visit and how this affected their flower choice. I asked the following questions: (1) Do bumblebees, solitary bees, butterflies and flies differ in handling time and is their handling time related to their proboscis length and both nectar tube depth and nectar volume of the flowers they visit? And (2) do insects with a longer proboscis visit more plant species and plant species with flowers with deeper nectar tubes? Surprisingly, in contrast to what has been suggested, a longer proboscis did not appear to be of a hindrance when foraging on shallow flowers of the Asteraceae. Handling time was not related to flower-visitor proboscis length when flower-visitors foraged on relatively shallow flowers. If insects visited flowers of which the nectar tube depth matched the length of their proboscis, handling time even decreased with increasing proboscis length.
Floral traits did determine handling time of flower-visitors, but this differed among flower-visitor taxa. Among bees, handling time increased with both nectar tube depth and nectar volume, while among flies handling time only increased with increasing nectar tube depth. Among butterflies handling time only increased with increasing nectar volume. Although previous studies have examined the handling time of bumblebees (Inouye 1980; Herrera 1989), butterflies (Herrera 1989; Kunte 2007; Martins & Johnson 2013; Bauder et al. 2015) and flies (Gilbert 1981), this study provides the unique opportunity to compare handling time and flower choice of these flower-visitor taxa foraging on a diversity of flowers (but see Herrera 1989 for comparison of hymenoptera and lepidoptera). Different flower-visitor taxa had intrinsic different handling times. Bees handled flowers faster than flies while butterflies had longer handling times. Both the intrinsic differences in handling time of bees, flies and butterflies, and the different effects of floral traits on handling time among these flower-visitor taxa may be due to different nectar extraction technique and/or body mass. Bees drink nectar by lapping with their glossae (Harder 1982), while butterflies and flies both suck up the nectar actively (Kingsolver & Daniel 1979; Gilbert 1981). Some flies even spit fluid on to the nectary, most likely to produce a medium in which the nectar sugar can dissolve (Gilbert 1981). Especially when confronted with the nectar environment of Asteraceae flowers (minute amounts and concentrated nectar), lapping proboscides are able to extract nectar at a much faster rate than sucking ones of similar length (Herrera 1989). Further, butterflies are expected to have a more narrow proboscis compared to flies which makes sucking up nectar more difficult, hence the different handling times among butterflies and flies. Differences in body mass among flower-visitor taxa might also have affected nectar extraction rate. In general, bees have a greater body mass, compared to butterflies and flies and insects with a greater body mass can drink nectar at a higher rate (Harder 1986).

Although flower-visitors with a longer proboscis can potentially forage on a wider diversity of flowers (Borrell 2005), the number of plant species that solitary bees, butterflies and flies visited was not related to their proboscis length. Bumblebee species with a longer proboscis even tended to visit fewer plant species and thus were more specialized. Because bumblebees with a longer proboscis are larger and thus have higher energetic requirements (Heinrich 1983), they might be more selective in their food choice (Goulson & Darvill 2004), only selecting high rewarding flowers. Also, partitioning of floral resources among species to avoid interspecific resource competition might have made flower-visitors more selective (Pyke 1982; Stout, Allen & Goulson 1998).

In Chapter 2 of this thesis, I analyzed whether flowers with deeper nectar tubes were, on average, visited by bumblebee, solitary bee and fly species with a longer proboscis (plants’ point of view). In accordance, I found that bumblebee, solitary bee and fly species with a longer proboscis visited, on average, flowers with deeper nectar tubes (insects’ point of view). Again, I did not find this relationship among butterflies. These results are in accordance with previous studies (Brian 1957; Morse 1978; Gilbert 1981; Harder 1985; Shmida & Dukas 1990; Plowright & Plowright 1997; Borrell 2005; Stang et al. 2009; Johnson 2010). However, handling time cannot explain these patterns of size-matching, as it took flower-visitors longer to handle flowers with deeper nectar tubes.
In Chapter 5, I investigated how foraging efficiency of natural foraging bumblebees of the species *Bombus bifarius* was related to the nectar tube depth of the Asteraceae flowers they foraged on, and whether *B. bifarius* individuals visited flowers of plant species on which they experience a higher foraging efficiency more frequently. Foraging efficiency might be more important for flower-visitor flower choice than handling time, because foraging efficiency accounts not only for the time it takes to handle a flower and extract nectar, but also for the amount of nectar, the number of flowers they visit, flower abundance and clustering and the time it takes to fly from flower-to-flower. I asked the following questions: (1) is the sugar intake rate of bumblebees, while extracting nectar from a single flower and while foraging on a flower head or in a patch, related to the depth of the flowers they forage on? (2) Is the energetic expenditure of bumblebees related to the depth of the flowers they forage on? (3) Is the (rate of) net energy gain of bumblebees related to the depth of the flowers they forage on? (4) Is the (rate of) net energy gain of bumblebees related to other aspects which determine bumblebee foraging efficiency, including flower head density, sugar content per flower, number of open flowers per head, flight time between heads and the time it takes to visit a flower head? And (5) do bumblebees visit those flowers that have deeper nectar tubes, are more abundant, produce more nectar or provide a larger (rate of) net energy gain more frequently? My results show that bumblebee sugar intake rate when extracting nectar from a single flower, while visiting a flower head and while foraging in a patch increased with increasing nectar tube depth. The energy bumblebees spent was not related to the nectar tube depth of the flowers. Consequently, their (rate of) net energy gain increased with increasing sugar production and nectar tube depth, which is also shown by May (1988) for butterflies. Moreover, bumblebees indeed visited the flowers on which they experienced a higher net energy gain more frequently. Net energy gain and rate of net energy gain explained 72% and 60% of the variation in visitation rate, respectively. Visitation rate was also positively related with the sugar content of 24-hour bagged flowers and explained 55% of the variation in visitation rate. In contrast, visitation rate was not related to nectar tube depth and flower head density. These results indicate that bumblebees do not forage randomly. Rather, they choose flowers based on their nectar reward or the (rate of) net energy gain they provide, as assumed by many theoretical models (Pyke 1984), and thus that bumblebees forage optimally. Surprisingly only few previous studies have empirically tested this optimal foraging approach and only for flower-visitor foraging behaviour when foraging on a single plant species (Whitham 1977; Pyke 1981; Pyke & Waser 1981; but see Schaffer & Schaffer 1979). These results may explain why bumblebees preferentially visit flowers with deeper nectar tubes and, because these flowers are the ones of which the nectar tube depth matches the length of their proboscis, why size-matching between flower- and flower-visitor morphology occurs in plant-flower-visitor communities.

In the last chapter, Chapter 6, I tested the hypothesis that, to ensure pollination in unpredictable environments in which most flower-visitor species abundance fluctuate yearly, plants can either be generalized on those flower-visitor species which are prone to temporal fluctuations or specialized on the few flower-visitor species which are less prone to temporal fluctuations, which will result in equal visitation rates. In other words plants face a pollinator species number (SN) – species predictability (SP) trade-off. This hypothesis is based on three
assumptions. First of all, the number of flower-visitor species by which a plant is visited, is related to the nectar tube depth of its flowers. In general, plant species with shallow flowers are more generalized (Chapter 2 of this thesis) because flowers with a deep nectar tube can only be visited by flower-visitors with a long proboscis. Secondly, plants species with flowers with deeper nectar tubes produce more nectar (Chapter 3 of this thesis). And third, flower-visitors which are less prone to temporal fluctuations are those species which are large, generalized, abundant and/or are social and consequently may have higher energetic requirements. Thus, for plants to be generalized, their flowers need to be relatively shallow. However, these shallow flowers may not produce enough nectar to fulfill the energetic requirements of flower-visitors which are less prone to temporal fluctuations. Therefore, generalized plant species either may not attract flower-visitor species which are less prone to temporal fluctuations, or if they do attract these flower-visitors, these flower-visitors will not visit them frequently. In turn, plant species which have deep-tubed flowers are able to produce enough nectar to attract flower-visitors which are less prone to temporal fluctuations. However due to their deep nectar tubes, these flowers cannot attract a wide diversity of flower-visitor species and thus are specialized. To test this hypothesis I asked the following questions: (1) Is the fluctuation of flower-visitor species abundance related to their generalization degree and proboscis length? (2) Does nectar tube depth determine plant generalization degree and how does this relationship fluctuate across years? (3) Do specialist plant species remain more specialized across years? (4) Are specialized plants visited by species that are temporarily more stable? And (5) is flower visitation rate and its fluctuation among years related to plant species generalization degree and its fluctuation among years? My results indicate that indeed a pollinator species number (SN) – species predictability (SP) trade-off may exist. The majority of flower-visitor species was only observed in one of the three years, and thus experienced high fluctuation in abundance across years. However, specialized plant species attract those flower-visitors which were less prone to temporal fluctuations. Their flower-visitors were observed in the community in all three years. Although generalized plants were also visited by flower-visitor species which were less prone to temporal fluctuations, these flower-visitors visited them less frequently. Consequently, flower visitation rate was unrelated to plant generalization degree. In one year, flower visitation rate even tended to decrease with increasing visitation rate.

Flower-visitors which were less prone to temporal fluctuation where those species which were on average more generalized. As in our study, Olesen, Stefanescu and Traveset (2011) found that for butterflies, most specialists were sporadic, only occurring in one or two years, while the temporally stable species varied in their generalization degree although they all had more than one interaction partner. Moreover, our results indicate that among bees and flies, species with a longer proboscis tended to be less prone to temporal fluctuation. We did not find such a relationship among butterflies. The abundance of bee- and fly species with a short proboscis might fluctuate stronger among years, compared to species with a long proboscis because species with a short proboscis occur more locally. As species with a short proboscis are smaller, they fly shorter distances and thus have a smaller foraging range, compared to species with a long proboscis. Higher year-to-year fluctuation in species abundance does not necessarily imply that these species are also less prone to extinction.
Studies by Biesmeijer et al. (2006); Burkle, Marlin and Knight (2013) and Burkle, Marlin and Knight (2013) both show that a higher proportion of flower-visitor species with a longer proboscis went extinct in the past few decades. However, it does indicate that, for plants, in the short term, these species are less reliable as flower-visitors. Overall, our results show that in unpredictable environments, where most flower-visitor species abundances fluctuate from year-to-year, being generalized on those species which are more prone to temporal variations, or being specialized on those species which are less prone to temporal variations are two strategies with which plants might cope with the yearly fluctuation of flower-visitors and ensure flower-visitor visitation. An important next step is to investigate how these different strategies affect (conspecific) pollen transfer and with that reproductive success and plant fitness.

Overall, my results provide a better understanding of how plant- and flower-visitor morphological traits affect plant-flower-visitor interaction patterns. My results indicate that flower abundance is less important to determine plant-flower-visitor interactions than floral traits. In contrast to what has been assumed by many network studies (Vazquez & Aizen 2004; Vazquez 2005; Vazquez et al. 2007), flower-visitors do not distribute themselves randomly among flowers. Rather, plant-flower-visitor interactions are structured by species traits, their frequency distributions and correlations among them. First of all, although, more abundant plant species are visited by a wide array of flower-visitor species, the number of visits was unrelated to flower head density. Plant species with more dense flower heads had shallow tubed flowers and there were relatively few flower-visitor species with a short proboscis. These two aspects are likely to affect the positive relationship between flower head density and plant generalization degree, rather than random foraging behaviour. Secondly, if flower-visitors forage randomly, flower-visitors would distribute themselves among plant species proportional to plant species abundance. Consequently, all plant species would experience comparable visitation rates. In contrast, I found that flower heads of plant species on which bumblebees foraged more efficiently (a higher (rate of) net energetic gain), where more frequently visited by bumblebees.

Furthermore, this study emphasizes the importance of nectar production and flower-visitor foraging efficiency for plant-flower-visitor interaction patterns, such as size-matching (between the nectar tube depth of flowers and the average proboscis length of their flower-visitors), and even the temporal stability of plant-flower-visitor interactions. Larger- and longer-tongued flower-visitors have higher energetic requirements and therefore might prefer plant species with a deeper nectar tube, because these plant species produce more nectar and because flower-visitors forage more efficiently on these flowers. Specialized plants might be less vulnerable to yearly fluctuations than previously thought because they are visited by longer-tongued and generalized flower-visitors which are less prone to local temporal fluctuations. Therefore these interactions are more predictable. Deeper- and specialized flowers are likely to attract these longer-tongued and generalized flower-visitors, because they produce more nectar or because flower-visitors forage more efficiently on these flowers. Therefore, to predict the response of plants to yearly fluctuations in plant-flower-visitor communities, it is important to, not only, have insight in species generalization degree, but also an in-depth understanding of the mechanisms behind plant-flower-visitor interactions.
Therefore, the importance of species traits, especially nectar production and flower-visitor foraging efficiency as mechanisms behind plant-flower-visitor interaction patterns should receive more attention in plant-flower-visitor network studies.

References


