Flower visitation by hoverflies (Diptera: Syrphidae) in a temperate plant-pollinator network

Jan Klecka[1], **Jiří Hadrava**[1,2], **Paolo Biella**[1,3], **and Asma Akter**[1,3]

[1]**Czech Academy of Sciences, Biology Centre, Institute of Entomology, České Budějovice, Czech Republic**
[2]**Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic**
[3]**Department of Zoology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic**

Corresponding author:
Jan Klecka

Email address: jan.klecka@entu.cas.cz

ABSTRACT

Hoverflies (Diptera: Syrphidae) are among the most important pollinators, although they attract less attention than bees. They are usually thought to be rather opportunistic flower visitors, although previous studied demonstrated that they show colour preferences and their nectar feeding is affected by morphological constraints related to flower morphology. Despite the growing appreciation of hoverflies and other non-bee insects as pollinators, there is a lack of community-wide studies of flower visitation by syprhids. The aim of this paper is to provide a detailed analysis of flower visitation patterns in a species rich community of syrphids in a Central European grassland and to evaluate how species traits shape the structure of the plant-hoverfly flower visitation network. We found that different species varied in the level of specialisation, and while some species visited a similar spectre of flowers, others partitioned resources more strongly. There was a consistent difference in both specialisation and flower preferences between three syrphid subfamilies. Eristalinae and Pipizinae were more specialised than Syrphinae. Trait-based analyses showed that relative flower visitation i) increased with plant height, but most strongly in Eristalinae; ii) increased with inflorescence size in small species from all three subfamilies, but was independent of inflorescence size in large species of Eristalinae and Syrphinae; and iii) depended on flower colour, but in a subfamily-specific way. Eristalinae showed the strongest flower colour preferences for white flowers, Pipizinae visited mostly white and yellow flowers, while Syrphinae were less affected by flower colour. Exploration of the structure of the plant-hoverfly flower visitation network showed that the network was both modular and nested. We also found that there were almost no differences in specialisation and relative visitation frequency between males and females. Overall, we showed that flower visitation in syrphids was affected by phylogenetic relatedness, body size of syrphids and several plant traits.

INTRODUCTION

Hoverflies (Diptera: Syrphidae) are one of the most abundant groups of flower visiting insects. Together with other families of flies, their role in plant-pollinator interactions is often underappreciated (Inouye et al., 2015). However, Diptera often make up a similar proportion of flower visitors as Hymenoptera and are even the dominant group of pollinators in some habitats, e.g. in higher altitudes and latitudes (Kanstrup and Olesen, 2000). Apart from being important pollinators of many wild plants (Orford et al., 2015; Sakurai and Takahashi, 2017; Moquet et al., 2018), hoverflies play an important role also in pollination of numerous crops (Ssymank et al., 2008; Inouye et al., 2015; Rader et al., 2016). For example, hoverflies of a genus *Eristalis* have been successfully used for pollination of peppers in greenhouses (Jarlan et al., 1997), and are pollinators of rapeseed (Ohsawa and Namai, 1987, 1988; Jauker and Wolters, 2008; Rader et al., 2009), apple trees (Solomon and Kendall, 1970), strawberries (Kendall et al., 1971), etc. (Inouye et al., 2015). Hoverflies are thus an important group of pollinators not only from the perspective of biodiversity conservation, but also for pollination of crops in agricultural settings. The interest in the role of flies in general and Syrphidae in particular as pollinators has been increasing (Ssymank et al., 2008). However, our knowledge of their preferences for different flowers and their partitioning of floral resources is still limited.

Adults of all known syrphid species feed almost exclusively on pollen and nectar or honeydew (Rotheray and Gilbert, 2011) and are usually considered as generalist flower visitors (Branquart and Hemptinne, 2000). However, individual species cover a broad range from generalists to species with strong preferences for a small number of plants (Branquart and Hemptinne, 2000; Colley and Luna, 2000). Their flower preferences may, however, shift depending on local flower availability and plant phenology (Cowgill et al., 1993; Colley and Luna, 2000). Several studies reported that selectivity of some hoverfly species depends on certain plant traits. Overall, hoverflies seem to visit mostly open bowl-shaped flowers (Branquart and Hemptinne, 2000), where they feed on both nectar and pollen (Gilbert, 1981), but some of them have relatively long proboscises which allow them to reach nectar even in flowers with relatively long spurs (Gilbert, 1981; Vlašánková et al., 2017). Additional plant traits affecting flower visitation by at least some species are inflorescence height (Gervasi and Schiestl, 2017; Klecka et al., 2017). It has been recently demonstrated that selective flower visitation by hoverflies can exert a selection pressure strong enough to cause rapid evolutionary shifts in multiple plant traits (Gervasi and Schiestl, 2017; Zu and Schiestl, 2017). However, there are still very few studies focusing on flower visitation patterns of entire local assemblages of syrphids.

Ecological and evolutionary processes which are responsible for structuring of communities of interacting insects and plants discussed above in the specific case of syrphids also lead to distinct structural features of entire plant-flower visitor networks. Most of these networks are usually strongly nested (Bascompte et al., 2003), modular (Olesen et al., 2007), or both nested and modular at the same time (Fortuna et al., 2010). Modules pack species connected by numerous interactions and can be linked to trait complementarity (Olesen et al., 2007). On the other hand, nested structure means that specialised insects tend to interact with plants also visited by more generalised insects and vice versa. In this case, mutual specialisation is rare, which has a stabilising effect on the structure of plant-flower visitor networks (Burgos et al., 2007; Bastolla et al., 2009).

The aim of this paper is to advance our understanding of flower visitation by hoverflies by a thorough analysis of flower visitation in a species-rich community of plants in a Central European grassland. We focus on the level of specialisation of different species, their overlap in resource use, and the structure of the entire plant-hoverfly flower visitation network. Our analyses show that hoverflies in our study area varied in their level of specialisation, partitioned floral resources depending on their relatedness, body size and several plant traits, and formed a plant-hoverfly flower visitation network which was nested and modular at the same time.

METHODS

We conducted sampling of plant-flower visitor interactions in a small area in the southern part of the Czech Republic between the northern edge of the city of Český Krumlov and nearby villages Vyšný and Lazec in June-August 2015. We gathered observations from eight flower-rich grassland patches between 48°49’29.5”N, 14°18’59.5”E in the South, 48°49’42.6”N, 14°19’24.4”E in the East, and 48°50’7.0”N, 14°15’36.5”E in the North-West. Geographic coordinates of individual sites are given in Supplementary Table 1. Sampling was conducted on public land and did not involve any protected species. For this reason, we did not need to obtain any permits for this project.

We sampled flower-visiting insects by transect walks and collected all visitors to flowers of all herbaceous flowering plants except grasses, although some syrphids are known to feed on pollen of grasses and sedges (Ssymank and Gilbert, 1993). Sampling was carried out during sunny days with no rain between 08:00 and 17:00 hours. In this paper, we focus on hoverflies (Diptera: Syrphidae), which were among the most abundant groups of flower visiting insects in the study area. All collected individuals were killed by ethyl-acetate, transported to the lab, pinned, and identified using keys of van Veen (2010) and Speight and Sarthou (2014) to the species level with the exception of hoverflies of the genus *Pipizella* and a few damaged specimens. We also identified the sex of all individuals. Several individuals per species were photographed using a stereomicroscope Olympus SZX7 and a DSLR camera Canon 700D controlled from a computer by digiCamControl software. We measured body length, thorax

Table 1. The list of species of Syrphidae collected during this study. The number of observations and the number of plant species visited is provided for each species and sex. Species-level identification was not possible in *Pipizella*, *Sphegina*, and in a few damaged individuals of *Platycheirus*.

	No. of observations			**No. of plant species visited**		
	Total	**Females**	**Males**	**Total**	**Females**	**Males**
Eristalinae						
Arctophila bombiformis	2	0	2	1	0	1
Cheilosia proxima	1	0	1	1	0	1
Cheilosia ruficollis	2	2	0	2	2	0
Chrysogaster basalis	1	1	0	1	1	0
Chrysogaster coemiteriorum	3	3	0	2	2	0
Chrysogaster solstitialis	32	20	12	4	2	3
Eristalis arbustorum	16	5	11	4	2	3
Eristalis horticola	2	2	0	2	2	0
Eristalis interruptus	56	32	24	12	10	8
Eristalis intricarius	1	0	1	1	0	1
Eristalis pertinax	2	1	1	2	1	1
Eristalis sepulchralis	1	0	1	1	0	1
Eristalis similis	1	0	1	1	0	1
Eristalis tenax	10	8	2	9	8	2
Helophilus pendulus	3	1	2	3	1	2
Myathropa florea	5	3	2	4	2	2
Neoascia podagrica	4	4	0	2	2	0
Orthonevra nobilis	2	1	1	1	1	1
Rhingia campestris	11	4	7	6	3	6
Rhingia rostrata	2	0	2	2	0	2
Sericomyia silentis	11	6	5	4	3	3
Sphegina sp.	1	1	0	1	1	0
Syritta pipiens	124	72	52	24	18	16
Vollucela bombylans	4	4	0	2	2	0
Vollucela pellucens	2	2	0	2	2	0
Pipizinae						
Heringia pubescens	1	1	0	1	1	0
Pipiza nocticula	5	5	0	4	4	0
Pipizella sp.	84	41	43	16	9	10
Syrphinae						
Chrysotoxum bicinctum	4	3	1	4	3	1
Chrysotoxum cautum	4	4	0	1	1	0
Chrysotoxum fasciatum	1	1	0	1	1	0
Chrysotoxum vernale	1	1	0	1	1	0
Chrysotoxum verralli	1	0	1	1	0	1
Didea alneti	4	1	3	2	1	2
Episyrphus balteatus	194	91	103	29	23	22
Eupeodes bucculatus	4	1	3	4	1	3
Lapposyrphus lapponicus	69	23	46	22	12	17
Melanostoma mellinum	18	8	10	10	6	7
Melanostoma scalare	1	0	1	1	0	1
Meliscaeva cinctella	1	0	1	1	0	1
Paragus haemorrhous	3	2	1	2	2	1
Parasyrphus lineolus	4	2	2	3	2	2
Platycheirus albimanus	2	2	0	2	2	0
Platycheirus peltatus	1	0	1	1	0	1
Platycheirus scambus	1	1	0	1	1	0
Platycheirus sp.	2	0	2	2	0	2
Scaeva pyrastri	18	6	12	10	6	7
Sphaerophoria scripta	237	143	94	47	38	34
Syrphus ribesii	58	35	23	16	14	10
Syrphus torvus	159	71	88	28	21	21
Syrphus vitripennis	18	12	6	13	9	6
Xanthogramma pedissequum	1	1	0	1	1	0

width, and head width of at least 8 individuals, or all individuals in species where less individuals were collected, using the Fiji distribution (Schindelin et al., 2012) of ImageJ (Rueden et al., 2017) and a plugin Microscope Measurement Tools. All specimens are deposited in Jan Klecka's collection at the Institute of Entomology, Biology Centre of the Czech Academy of Sciences.

We measured a set of three traits of all plant species visited by hoverflies to test their effect on flower visitation (Supplementary Table 4). Specifically, we measured plant height (the height of the top flower above ground), inflorescence size as the largest distance between any two open flowers within an inflorescence, and classified flower colour into four categories (blue, purple, white, and yellow), similar to previous studies (Haslett, 1989a). We conducted plant trait measurements in several patches for each species, measuring at lest 10 plant individuals per site, except in very rare species.

For data analysis, we pooled observations from the entire study area, because most sampled patches were close to each other, often <1 km apart, well within dispersal range of most hoverflies (Rader et al., 2011; Moquet et al., 2018). We performed all analyses in R 3.2.3 (R Core Team, 2015). We visualised the structure of plant-hoverfly flower visitation network using the package bipartite 2.08 (Dormann et al., 2009; Dormann, 2011). We used generalised linear models (GLM) with either a Poisson distribution with overdispersion (quasipoisson), a Binomial distribution with overdispersion (quasibinomial), or Normal distribution depending on the properties of the response variable. We used non-metric multidimensional scaling implemented in vegan 2.4-4 package for R (Oksanen et al., 2017) to visualise diet overlap between syrphid species. Diet overlap was calculated using Pianka's overlap index Pianka (1973) using the plant-hoverfly flower visitation matrix (Supplementary Table 2) to estimate pairwise diet overlap values between all pairs of hoverfly species. We performed the diet overlap analysis using EcoSimR 0.1.0 package for R (Gotelli et al., 2015). Finally, we performed analyses of modularity and nestedness of the plant-hoverfly flower visitation network using the package bipartite 2.08 for R (Dormann et al., 2009; Dormann, 2011).

RESULTS

We observed 1195 interactions between a total of 51 species of syrphids from three subfamilies (Eristalinae, Pipizinae, and Syrphinae) and 57 plant species from 20 families (Table 1). The network of plant-syrphid flower visiting interactions is shown in Fig. 1, raw data are available in Supplementary Tables 1 and 2.

The number of plant species visited by individual syrphid species increased with the number of observations linearly on a log-log scale ($F_{1,49} = 873.41, P < 1*10^{-6}$) and differed significantly between subfamilies, with Syrphinae visiting more plant species than Eristalinae and Pipizinae after accounting for the number of observations ($F_{2,48} = 4.16, P = 0.0216$; Fig. 2A.). Species from the subfamily Syrphinae were more generalised than the other two subfamilies also according to our calculation of a specialisation index d' ($F_{2,15} = 4.81, P = 0.0243$; Fig. 2B.), which was restricted to species with at least five observations. Body length had no effect on the number of plant species visited ($F_{1,47} = 0.47, P = 0.4986$), nor on the value of the specialisation index d' ($F_{1,14} = 1.28, P = 0.2764$). The same results were obtained using head width and thorax width as alternative measures of body size. Comparison of the specialisation index d' revealed no consistent differences in specialisation between males and females (linear mixed effects model with species as a random factor; $\chi^2_1 = 1.99, P = 0.1586$) (Table 2).

There was a clear differentiation between Syrphinae and Eristalinae in their flower preferences based on nonmetric multidimensional scaling (NMDS) with values of pairwise diet overlap between all pairs of species of syrhids (Fig. 3). Values of Pianka's overlap index of individual syrphid species pairs ranged from 0 to 0.988 (mean = 0.363, Supplementary Table 3), i.e. from completely different to almost identical pattern of visitation of flowers of individual plant species. We included only syrphid species with at least five observations in this analysis. The two species of Pipizinae included in the analysis did not cluster together, although they appeared distinct from the other two subfamilies (Fig. 3). Additional insight into differences between the three subfamilies can be gained from a comparison of visitation frequency on plants from different families shown in Fig. 4.

Males and females showed only minor differences in their preferences for flowers of different plant species. Comparison of the frequency of flower visits of males and females in individual plant species revealed a significant difference only in *Eristalis interruptus* (χ^2 test for contingency tables; $\chi^2 = 21.76, P = 0.0048$; P estimated by 10000 Monte Carlo simulations). Males of *Eristalis interruptus* visited mostly *Daucus carota*, while females visited mostly *Centaurea scabiosa* and *Achillea millefolium* (Supplementary File 1). Other abundant species showed only minor differences in flower visitation, but

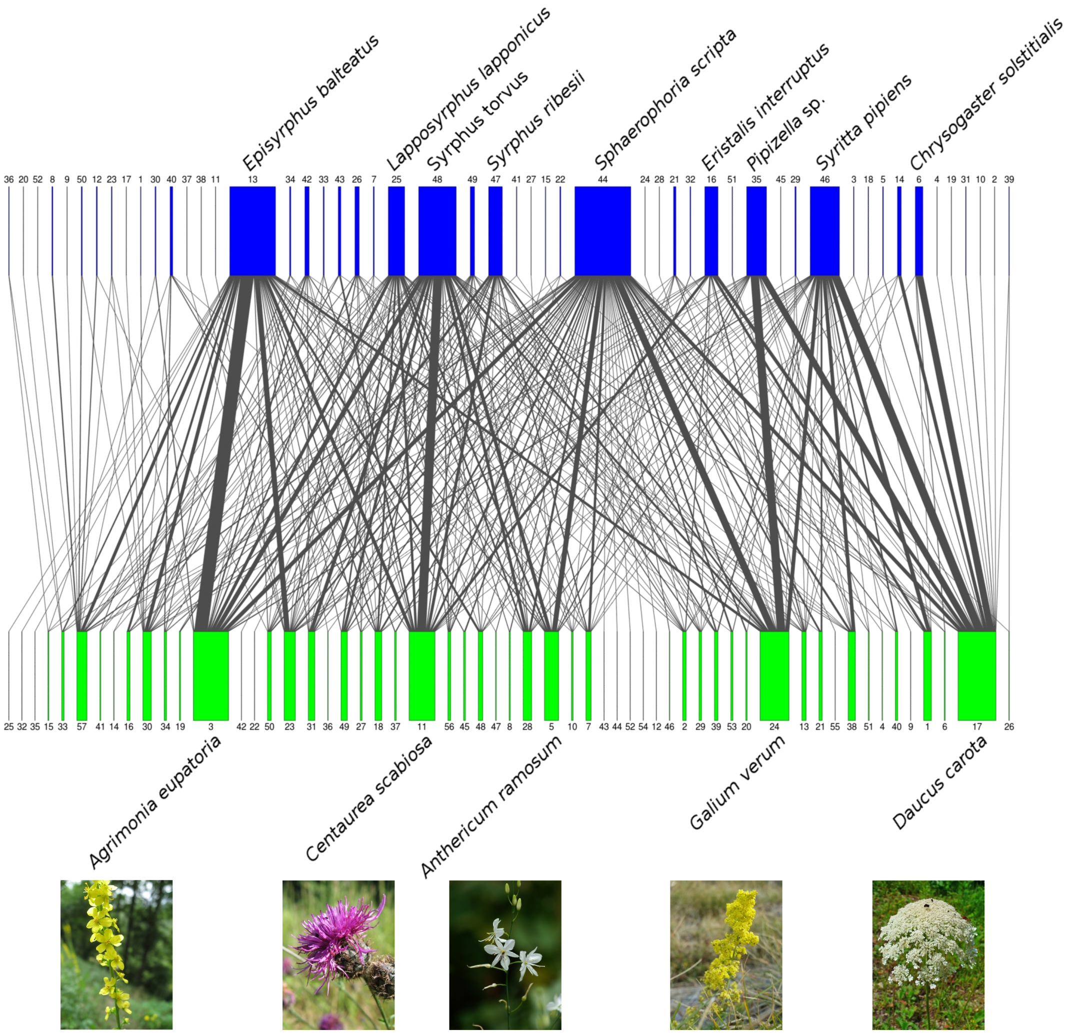

Figure 1. Flower visitation network of plants and hoverflies. Hoverflies are displayed in the upper row with blue boxes. The width of the boxes is proportional to the number of individuals observed. Plants are displayed in the lower row as green boxes whose width is proportional to the number of observations made at the individual plant species. The width of the connecting lines is proportional to the number of interactions observed between each plant-syrphid pair. The most abundant species are named, all species are identified by numbers - see Supplementary Table 2 for legend.

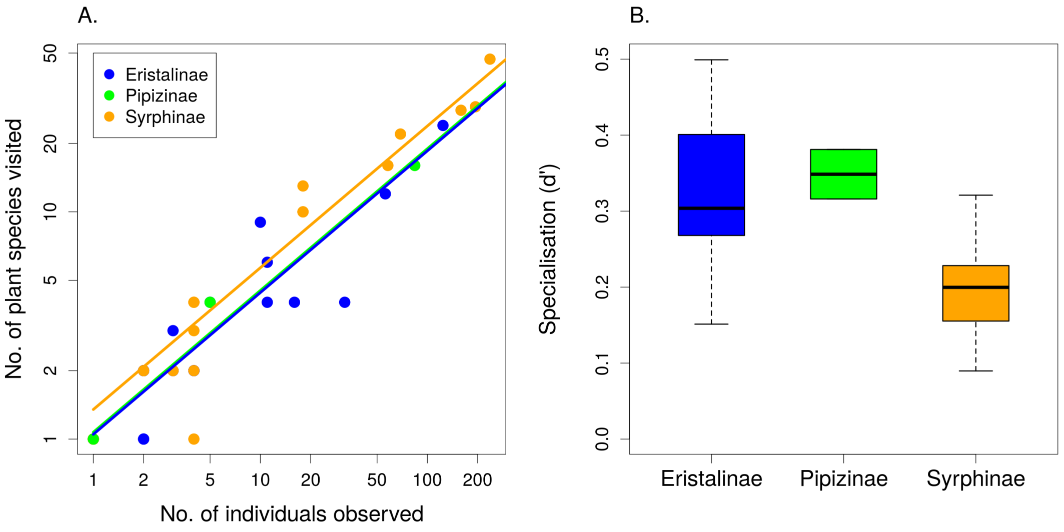

Figure 2. The level of resource specialisation of syrphids. (A.) The number of plant species visited depended on the number of observations. Variation around the regression line shows that species below the line were more specialised than expected and species above the line were more generalised. The green line is mostly hidden by the blue line. (B.) Syrphinae were more generalised than Eristalinae and Pipizinae.

the number of observations was low in many cases (Supplementary File 1); species with <10 observations per sex were not included in these analyses.

Relative visitation rate of plants by individual syrphid species significantly increased with plant height, but with a different slope in syrphids from different subfamilies and different size classes (Figure 5A., Table 3). The effect of inflorescence size also differed between small and large syrphids and between species from different subfamilies (Figure 5B., Table 3). Specifically, relative visitation increased with inflorescence size in small syrphids from all three subfamilies, but was independent of inflorescence size in large syrphids (Figure 5B.). Flower colour also had a significant effect on visitation by syrphids, but this effect varied between the three syrphid subfamilies (Figure 5C.-E., Table 3). Eristalinae clearly visited mostly white flowers (Figure 5C.), Pipizinae had a similar visitation rate to yellow and white flowers (Figure 5D.), while Syrphinae showed only minor differences in visitation of flowers of different colours (Figure 5E.). When we look at the syrphid community as a whole, plants with yellow and white flowers were overall most frequently visited, with 37.7% of visits to yellow flowers and 31.4% to white flowers. Purple flowers received 26.1% visits, while blue flowers received only 4.9% of visits. In contrast to subfamily, syrphid size class did not affect the dependence of visitation on flower colour (flower colour x syrphid size class interaction, $F = 1.25, P = 0.2921$; Table 3).

Analysis of the structure of the plant-syrphid flower visitation network showed that the network was both modular and nested at the same time. Modularity analysis detected four modules, with most Eristalinae clustered in one module, while Syrphinae dominated two other modules, and the most generalised species, *Sphaerophoria scripta*, was classified separately (Fig. 6). The network was not only modular, but also nested (Fig. 7). Nestedness index was significantly different from random expectation ($NODF = 29.08, P < 0.001$, based on 999 simulations). However, comparison of nestedness calculated for syrphids and plants separately showed that only the syrphids had significantly nested pattern of interactions ($NODF = 18.02, P < 0.001$), while the pattern for plants was not significantly different from random expectation ($NODF = 38.27, P = 0.221$).

DISCUSSION

Flower visitation by Syrphidae was characterised by a variable degree of specialisation at the species level. Syrphids have been traditionally considered as generalised flower visitors. We showed that not only different species fell in different positions along a gradient from more specialised to truly generalised

Table 2. Values of the specialisation index d' of male and female syrphids. Larger values of the d' index correspond to more specialised flower visitation. Species where one or both sexes had <5 observations were excluded from the analysis.

	Specialisation (d')	
Species	**Females**	**Males**
Chrysogaster solstitialis	0.428	0.405
Episyrphus balteatus	0.265	0.253
Eristalis arbustorum	0.286	0.275
Eristalis interruptus	0.273	0.262
Lapposyrphus lapponicus	0.171	0.231
Melanostoma mellinum	0.248	0.166
Pipizella sp.	0.272	0.282
Scaeva pyrastri	0.186	0.247
Sericomyia silentis	0.341	0.287
Sphaerophoria scripta	0.126	0.171
Syritta pipiens	0.234	0.271
Syrphus ribesii	0.163	0.214
Syrphus torvus	0.158	0.258
Syrphus vitripennis	0.067	0.249

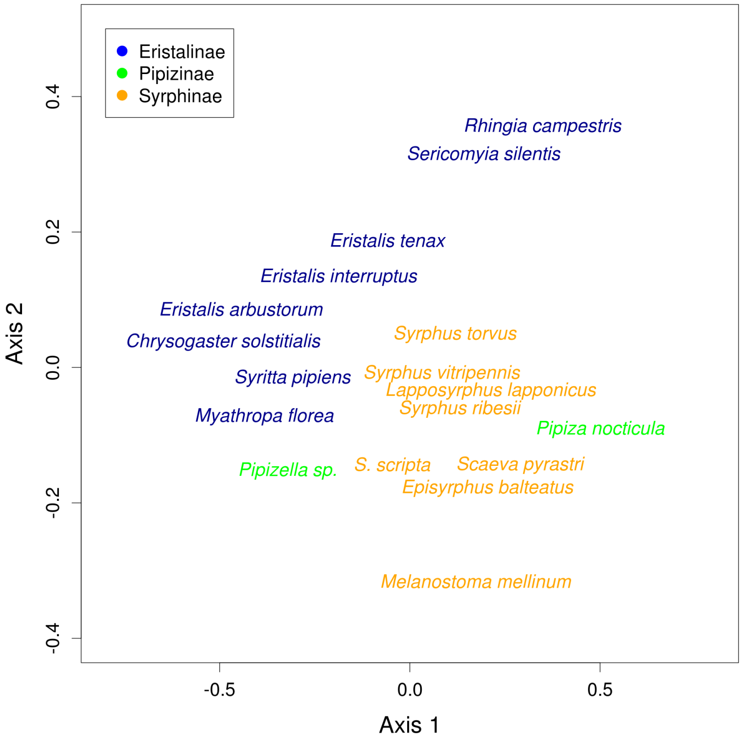

Figure 3. Results of Nonmetric multidimensional scaling (NMDS) show differences in flower preferences in Syrphinae and Eristalinae. NMDS analysis was run with a matrix of dissimilarities of the relative frequency of flower visitation on different plants by individual species of syrphids. The position of individual species in the plot corresponds to the center of the species label. *S. scripta* = *Sphaerophoria scripta*.

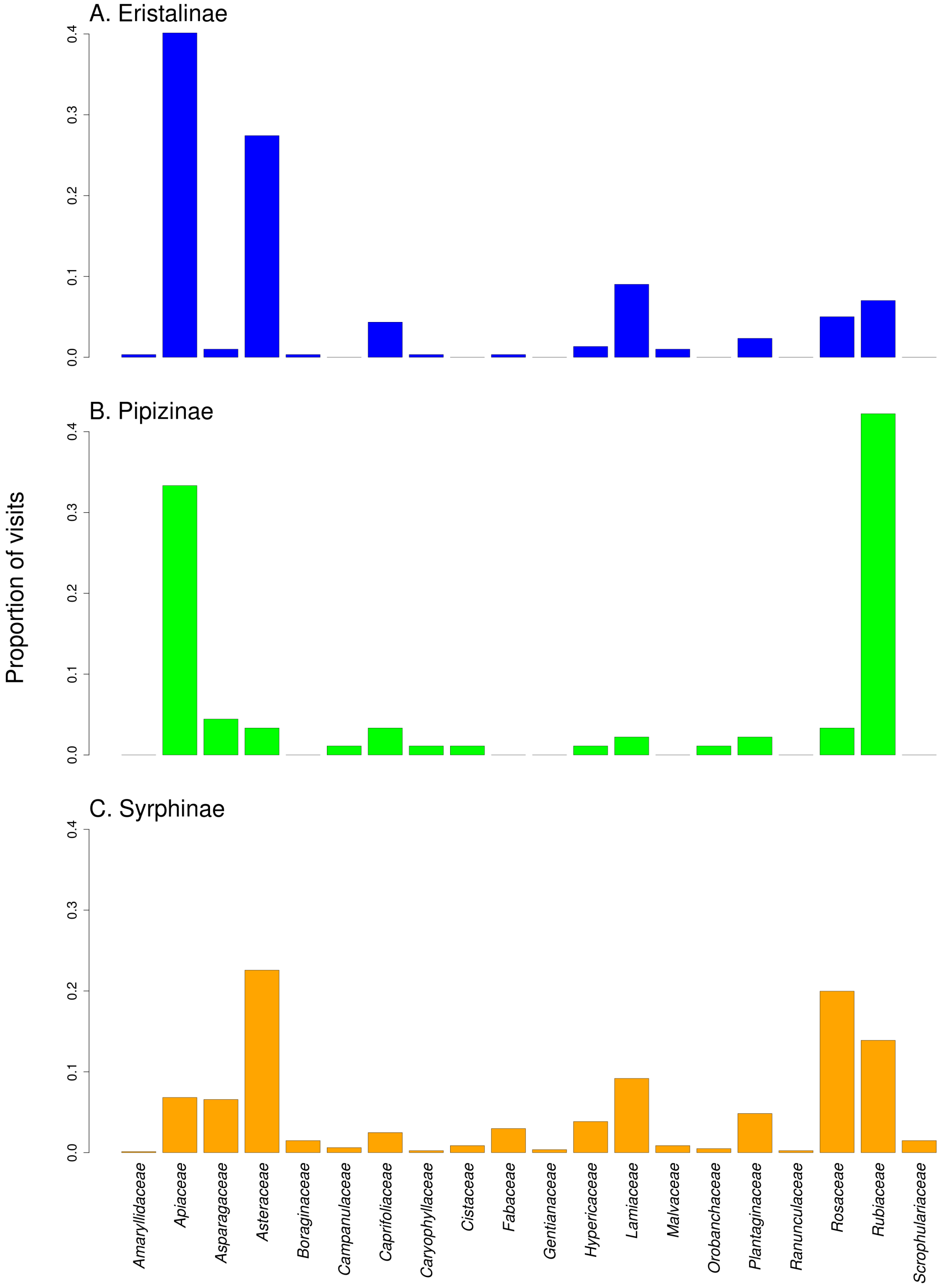

Figure 4. **Comparison of the proportion of flower visits by the three subfamilies of Syrphidae to individual plant families.** The bars show the proportion of observations of flower visits depending on plant family.

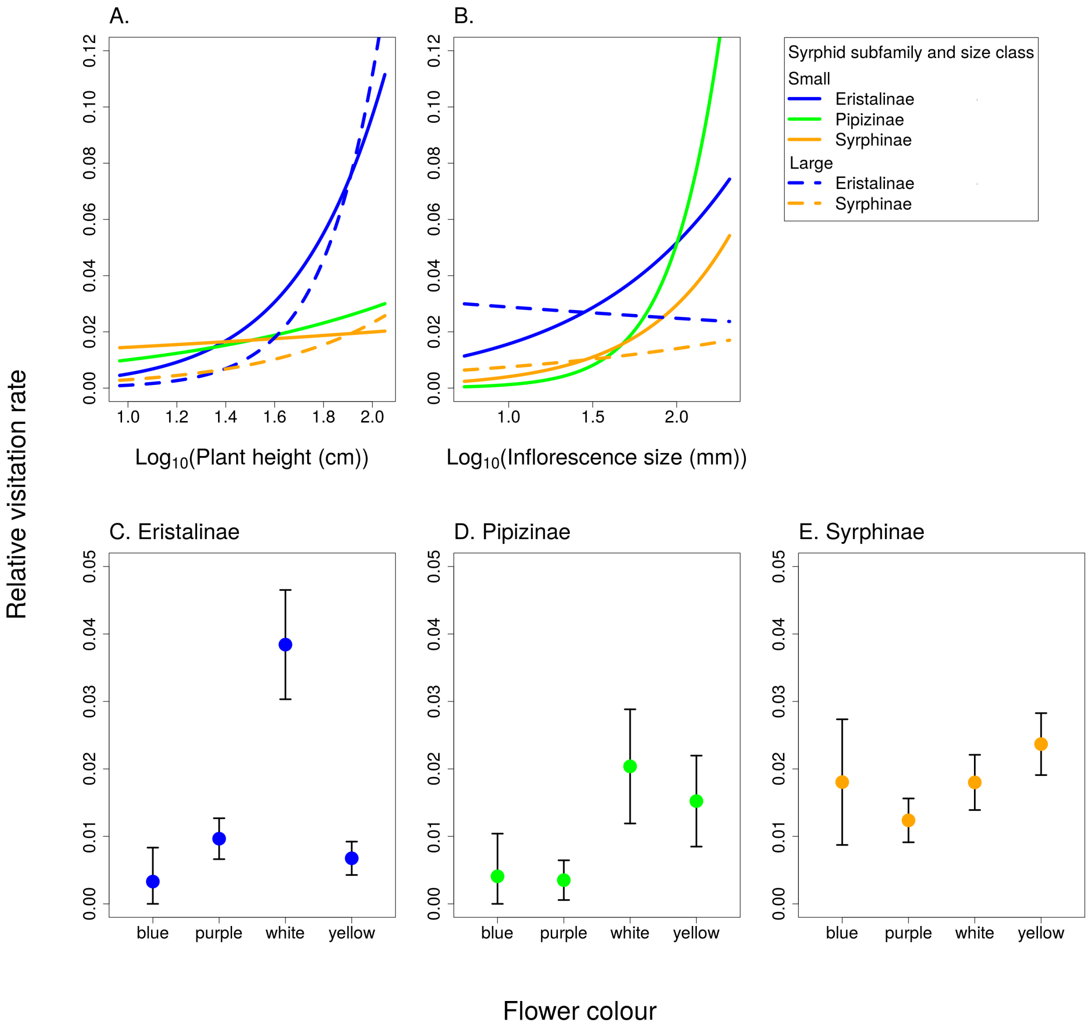

Figure 5. **The effect of species traits on flower visitation by Syrphidae.** A. Taller plants were visited more frequently by syrphids with a slope dependent on their body size and subfamily. B. Small syrphids visited more often plants with large inflorescences, while visitation by large syrphids was not affected by inflorescence size. C.-E. Flower colour affected visitation by the three subfamilies of syrphids differently.

Table 3. **The effect of species traits on flower visitation by syrphids.** Results of a GLM testing the dependence of relative visitation rate on species traits. Significance of all interaction terms in the model is shown.

Model term	***df***	***F***	***P***
Log_{10}(plant height) x syrphid subfamily	2	4.09	0.0170
Log_{10}(plant height) x syrphid size class	1	4.39	0.0365
Log_{10}(inflorescence size) x syrphid subfamily	2	3.11	0.0449
Log_{10}(inflorescence size) x syrphid size class	1	7.31	0.0070
Flower colour x syrphid subfamily	6	4.07	0.0005
Flower colour x syrphid size class	3	1.25	0.2921

flower visitors, but that there were also significant differences in average specialisation between the three syrphid subfamilies. The pattern of higher specialisation of Eristalinae and Pipizinae compared to the more generalised Syrphinae was clear, although in the case of Pipizinae, we have to note that our observations included only three species of Pipizinae (counting individuals of *Pipizella* sp., which could be identified only to genus, as one species).

We also found pronounced differences in relative flower preferences both at a coarse level between subfamilies, and at a finer level between species. Results of the NMDS showed that Eristalinae and Syrphinae were nicely separated in the diagram, but also that species from the same genus clustered together, e.g. the three species of each of the genera *Eristalis* and *Syrphus* (Fig. 3). Pairwise diet overlap values were as high as 0.98 in *Eristalis arbustorum* and *Chrysogaster solstitialis* (the maximum possible value is 1.0), and the three species of the genus *Syrphus* had diet overlap values between 0.84 and 0.88, which suggests that they had almost identical diets. On the other hand, many species showed clear diet partitioning evidenced by small values of diet overlap (Supplementary Table 3). This is noteworthy in relation to ongoing debates about mechanisms of species coexistence. Conflicting theoretical explanations of species coexistence showed that species can coexist only if they are sufficiently different according to classic theory of limiting similarity (Hardin, 1960; MacArthur and Levins, 1967), or alternatively if they are sufficiently similar as proposed by Hubbell's neutral theory (Hubbell, 2001). An emerging consensus is that both explanations are correct, i.e. that species can coexist if they are either sufficiently different or sufficiently similar (Scheffer and van Nes, 2006; Sakavara et al., 2018; Scheffer et al., 2018). Indeed, there are examples of closely related coexisting species with different trophic niches, as well as examples where they have a very similar niche (Goulson et al., 2008). In our case, we observed local coexistence of some closely related species with very high diet overlaps, which is consistent with the argument that similar species can coexist. Coexistence may be facilitated by differences in flower-visiting behaviour, such as microhabitat choice (Janovský et al., 2013) or timing of activity during the day (Gilbert, 1980).

Trait-based analysis of relative visitation rate of flowers by individual syrphid species showed that phylogenetic relatedness, i.e. belonging to the same or different subfamily, affected flower colour preferences. We did not measure plant abundance in sufficient detail to test whether relative visitation rates to different flower colour categories significantly deviated from a random pattern, but we can gain insight into flower colour preferences from comparison of different species collected in the same study area. This comparison revealed differences in the relative preference for white, yellow, purple, and blue flowers between the three syrphid subfamilies. So, we can say that Eristalinae appeared to strongly prefer white flowers, while Syrphinae were relatively indiscriminate in the colour of flowers they visited (Fig. 5). Previous studies on the effect of flower colour in hoverflies suggested that they visit mostly white or yellow flowers (Haslett, 1989a; Sutherland et al., 1999), with some exceptions, such as *Rhingia campestris* with a preference for blue flowers (Haslett, 1989a), but previous studies were restricted to a small set of species. We show that flower colour preferences varied between different syrphid subfamilies. Interestingly, Haslett (1989a) observed that out of a group of six species, *Episyrphus balteatus* from the subfamily Syrphinae was the least selective species towards flower colour, while several species from the subfamily Eristalinae were more selective. Our data with a larger set of species provide compelling evidence of this difference between subfamilies. There is not enough know about the visual system of different species, but it is likely that interspecific differences in visitation of flowers of different colours represent foraging preferences rather than differences in the visual system which seems to be quite uniform among flies (Lunau, 2014). The dominant flower colour represents probably a relatively long-range visual signal, while other cues may be used when the hoverfly approaches the flower. For example, small yellow spots are known to elicit an innate proboscis extension response and serve as cues visually guiding hoverflies towards pollen, which is usually yellow (Lunau and Wacht, 1994). Not only anthers with exposed pollen, but also other small yellow structures may thus guide the hoverfly towards pollen once it reached the flower (Lunau and Wacht, 1994; Lunau, 2014).

Based on our results, Eristalinae and Pipizinae showed a stronger response to all plant traits, i.e. flower colour, plant height and inflorescence size, compared to Syrphinae. Taken together, these results highlight the differences in average specialisation level between the generalised Syrphinae on one side and more specialised Eristalinae and Pipizinae on the other side. Interestingly, Moquet et al. (2018) found that they could split hoverflies of Belgian heathlands according to an analysis of several life-history and ecological traits into two distinct groups roughly corresponding to the two dominant subfamilies, Eristalinae and Syrphinae. Our detailed analysis of flower visitation provides additional evidence of important ecological differences between the syrphid subfamilies.

Apart from phylogenetic relatedness at the subfamily level, we found body size to be an important trait modifying the responses of syrphid relative visitation rate to selected plant traits. Flower colour was related mostly to phylogenetic relatedness, while plant height and inflorescence size were related also to syrphid body size. Small syrphids preferred large inflorescences, which may be advantageous because they could exploit resources clustered in one place (Akter et al., 2017). Another trait that has been evaluated previously is the relationship between corolla depth and proboscis length. Some previous studies showed a positive correlation between the average depths of flowers and proboscis length or length/width ratio in bees (Stang et al., 2006, 2009) as well as hoverflies (Gilbert, 1981; Branquart and Hemptinne, 2000). We did not test this relationship mostly because we did not distinguish nectar and pollen feeding. Even species with a short proboscis are regularly visiting long-spurred flowers to feed on pollen and can even lick nectar at the entrance to the spur without being able to reach deep inside (Vlašánková et al., 2017). Proper analysis of a morphological fit between the flowers and flower visitors would thus require a more detailed data on mechanisms of feeding by individual species and on morphology of both the insects and the flowers.

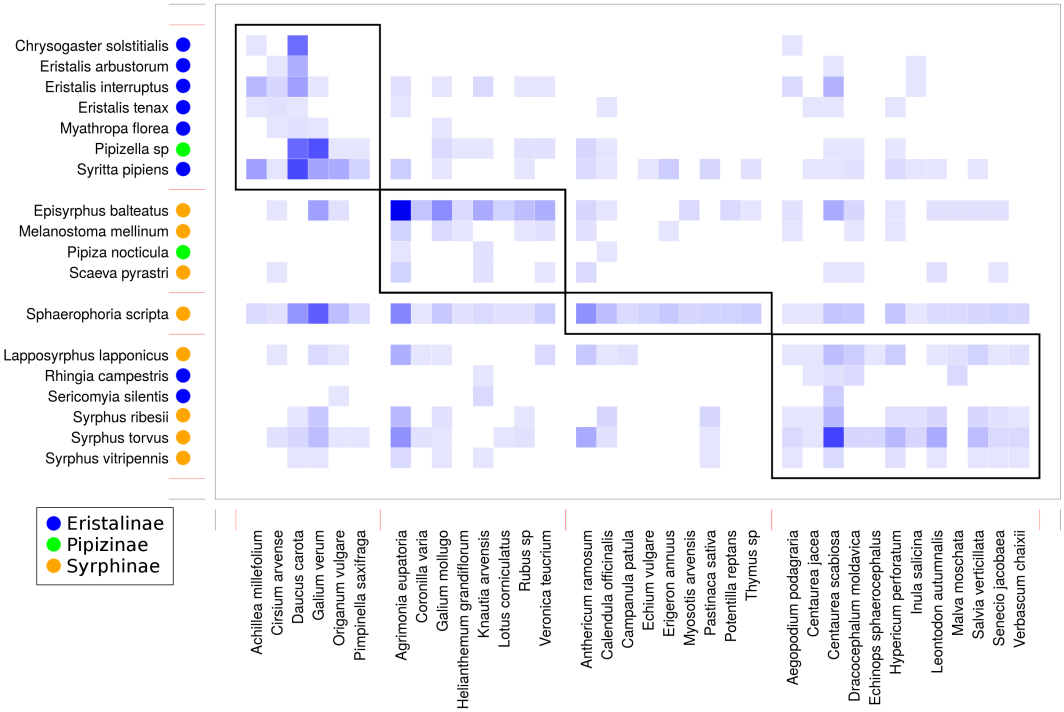

Figure 6. **Modules detected in the plant-syrphid flower visitation network.** Results of modularity analysis restricted to syrphid and plant species with at least 5 observations. Syprhidae are displayed in rows and plants in columns. The blue rectangles show observed interactions with more frequent interactions shown by darker colour The three syrphid subfamilies are distinguished by coloured circles next to the species names (see legend).

Network modularity (Olesen et al., 2007) partly reflected these patterns, because we found that the plant-hoverfly flower visitation network could be partitioned into four modules; most Eristalinae were clustered in one of them. Nestedness analysis showed that the syrphid flower visitation was significantly nested, i.e. that more specialised species visited mostly flowers of plants which were a subset of those visited by more generalised species, which is a typical pattern in plant-flower visitor networks (Bascompte et al., 2003; Fortuna et al., 2010). However, nestedness of the plants did not differ from a random expectation, so the nestedness pattern was asymmetric. This is likely because the plants were visited by a range of other insects, not only hoverflies, so the network as we analysed it here was incomplete from the plants' point of view.

Despite the clear patterns we found at the interspecific level, we detected very little differences in flower visitation by males and females of species sufficiently abundant to allow such comparison. Both the level of specialisation and the relative visitation rates to individual plant species were very similar in males and females in most cases. Similarly, Sutherland et al. (1999) found that males and females of *Episyrphus balteatus* showed very similar flower colour preferences. However, we did not distinguish nectar and pollen consumption during our observations, so we cannot rule out a possible difference between sexes in pollen vs. nectar feeding. Indeed, several previous studies reported that females of hoverflies feed on pollen more frequently than males (Gilbert, 1981; Haslett, 1989b; Hickman et al., 1995), probably because proteins from pollen are necessary for egg development. Males thus often feed less on pollen and more on nectar which serves mostly as a source of energy for their active lifestyle, because they are usually more active than females and spend a large amount of time by hovering (Haslett, 1989b). However, no significant difference in pollen consumption between males and females was found in a few other species, so the generality of this patterns is unclear (Irvin et al., 1999).

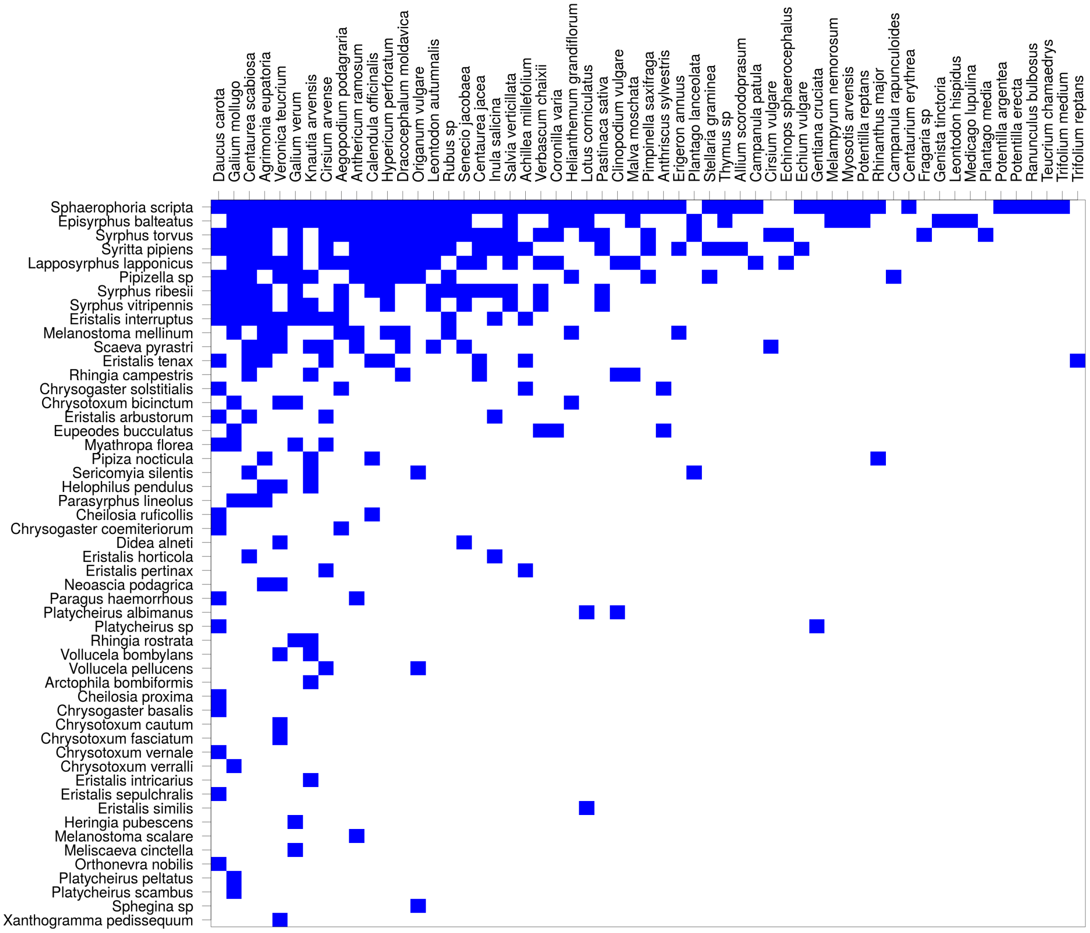

Figure 7. The plant-hoverfly flower visitation network was significantly nested. Syprhidae are displayed in rows and plants in columns. The blue rectangles show observed interactions.

Conclusions

Hoverflies and other dipterans are important pollinators, but they have been neglected compared to bees (Ssymank et al., 2008; Orford et al., 2015), and we need to learn more about they foraging biology, flower preferences, and pollination efficiency. We provided insights into some of these issues. However, there are questions we did not consider, such as feeding on pollen of wind-pollinated plants either by visiting flowers of grasses, sedges, trees, etc., or by eating pollen accumulated on the surface of leaves (Ssymank and Gilbert, 1993; Saunders, 2017). Apart from the need for more detailed understanding of foraging biology of hoverflies and other flower-visiting flies, there is a lot of unknowns about their pollination efficiency, although some studies demonstrated that hoverflies and other dipterans may provide pollination service comparable to bees (Kearns and Inouye, 1994; Inouye et al., 2015). Filling these knowledge gaps is urgently needed given the reported widespread declines of many native pollinators around the world (Potts et al., 2010).

ACKNOWLEDGMENTS

We would like to thank Dagmar Hucková, Tomáš Gregor, Eliška Konečná, Jan Buriánek, Michal Rindoš, and Samane Sakaki for help with sampling in the field or processing the samples. This study was supported by the Czech Science Foundation (projects GP14-10035P and GJ17-24795Y awarded to JK).

REFERENCES

Akter, A., Biella, P., and Klecka, J. (2017). Effects of small-scale clustering of flowers on pollinator foraging behaviour and flower visitation rate. *PLoS ONE*, 12(11):e0187976.

Bascompte, J., Jordano, P., Melián, C. J., and Olesen, J. M. (2003). The nested assembly of plant–animal mutualistic networks. *Proceedings of the National Academy of Sciences*, 100(16):9383–9387.

Bastolla, U., Fortuna, M. A., Pascual-Garcia, A., Ferrera, A., Luque, B., and Bascompte, J. (2009). The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature*, 458(7241):1018.

Branquart, E. and Hemptinne, J.-L. (2000). Selectivity in the exploitation of floral resources by hoverflies (Diptera: Syrphinae). *Ecography*, 23(6):732–742.

Burgos, E., Ceva, H., Perazzo, R. P., Devoto, M., Medan, D., Zimmermann, M., and Delbue, A. M. (2007). Why nestedness in mutualistic networks? *Journal of Theoretical Biology*, 249(2):307–313.

Colley, M. and Luna, J. (2000). Relative attractiveness of potential beneficial insectary plants to aphidophagous hoverflies (Diptera: Syrphidae). *Environmental Entomology*, 29(5):1054–1059.

Cowgill, S., Sotherton, N., and Wratten, S. (1993). The selective use of floral resources by the hoverfly *Episyrphus balteatus* (Diptera: Syrphidae) on farmland. *Annals of Applied Biology*, 122(2):223–231.

Dormann, C. F. (2011). How to be a specialist? Quantifying specialisation in pollination networks. *Network Biology*, 1(1):1–20.

Dormann, C. F., Fründ, J., Blüthgen, N., and Gruber, B. (2009). Indices, graphs and null models: analyzing bipartite ecological networks. *The Open Ecology Journal*, 2:7–24.

Fortuna, M. A., Stouffer, D. B., Olesen, J. M., Jordano, P., Mouillot, D., Krasnov, B. R., Poulin, R., and Bascompte, J. (2010). Nestedness versus modularity in ecological networks: two sides of the same coin? *Journal of Animal Ecology*, 79(4):811–817.

Gervasi, D. D. and Schiestl, F. P. (2017). Real-time divergent evolution in plants driven by pollinators. *Nature Communications*, 8:14691.

Gilbert, F. (1980). Flower visiting by hoverflies (Syrphidae). *Journal of Biological Education*, 14(1):70–74.

Gilbert, F. S. (1981). Foraging ecology of hoverflies: morphology of the mouthparts in relation to feeding on nectar and pollen in some common urban species. *Ecological Entomology*, 6(3):245–262.

Gotelli, N. J., Hart, E. M., and Ellison, A. M. (2015). *EcoSimR: Null model analysis for ecological data*. R package version 0.1.0.

Goulson, D., Lye, G. C., and Darvill, B. (2008). Diet breadth, coexistence and rarity in bumblebees. *Biodiversity and Conservation*, 17(13):3269–3288.

Hardin, G. (1960). The competitive exclusion principle. *Science*, 131(3409):1292–1297.

Haslett, J. (1989a). Interpreting patterns of resource utilization: randomness and selectivity in pollen feeding by adult hoverflies. *Oecologia*, 78(4):433–442.

Haslett, J. R. (1989b). Adult feeding by holometabolous insects: pollen and nectar as complementary nutrient sources for *Rhingia campestris* (Diptera: Syrphidae). *Oecologia*, 81(3):361–363.

Hickman, J. M., Lövei, G. L., and Wratten, S. D. (1995). Pollen feeding by adults of the hoverfly *Melanostoma fasciatum* (Diptera: Syrphidae). *New Zealand Journal of Zoology*, 22(4):387–392.

Hubbell, S. P. (2001). *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press.

Inouye, D., Larson, B. M., Ssymank, A., and Kevan, P. G. (2015). Flies and flowers III: ecology of foraging and pollination. *Journal of Pollination Ecology*, 16(16):115–133.

Irvin, N., Wratten, S., Frampton, C., Bowie, M., Evans, A., and Moar, N. (1999). The phenology and pollen feeding of three hover fly (Diptera: Syrphidae) species in Canterbury, New Zealand. *New Zealand Journal of Zoology*, 26(2):105–115.

Janovský, Z., Mikát, M., Hadrava, J., Horčičková, E., Kmecová, K., Požárová, D., Smyčka, J., and Herben, T. (2013). Conspecific and heterospecific plant densities at small-scale can drive plant-pollinator interactions. *PLoS ONE*, 8(10):e77361.

Jarlan, A., De Oliveira, D., and Gingras, J. (1997). Pollination by *Eristalis tenax* (Diptera: Syrphidae) and seed set of greenhouse sweet pepper. *Journal of Economic Entomology*, 90(6):1646–1649.

Jauker, F. and Wolters, V. (2008). Hover flies are efficient pollinators of oilseed rape. *Oecologia*, 156(4):819–823.

Kanstrup, J. and Olesen, J. M. (2000). Plant-flower visitor interactions in a Neotropical rain forest canopy: community structure and generalisation level. *The Scandinavian Association for Pollination Ecology honours Knut Fægri*, pages 33–42.

Kearns, C. A. and Inouye, D. W. (1994). Fly pollination of *Linum lewisii* (Linaceae). *American Journal of Botany*, 81(9):1091–1095.

Kendall, D., Wilson, D., Guttridge, C., and Anderson, H. (1971). Testing *Eristalis* as a pollinator of covered crops. *Long Ashton Research Station Reports*, 1971:120–121.

Klecka, J., Hadrava, J., and Koloušková, P. (2017). Vertical stratification of plant-pollinator interactions in a temperate grassland. *PeerJ PrePrints*.

Lunau, K. (2014). Visual ecology of flies with particular reference to colour vision and colour preferences. *Journal of Comparative Physiology A*, 200(6):497–512.

Lunau, K. and Wacht, S. (1994). Optical releasers of the innate proboscis extension in the hoverfly *Eristalis tenax* L.(Syrphidae, Diptera). *Journal of Comparative Physiology A*, 174(5):575–579.

MacArthur, R. and Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *American Naturalist*, 101(921):377–385.

Moquet, L., Laurent, E., Bacchetta, R., and Jacquemart, A.-L. (2018). Conservation of hoverflies (Diptera, Syrphidae) requires complementary resources at the landscape and local scales. *Insect Conservation and Diversity*, 11(1):72–87.

Ohsawa, R. and Namai, H. (1987). The effect of insect pollinators on pollination and seed setting in *Brassica campestris* cv. nozawana and *Brassica juncea* cv. kikarashina. *Japanese Journal of Breeding*, 37(4):453–463.

Ohsawa, R. and Namai, H. (1988). Cross-pollination efficiency of insect pollinators (Shimahanaabu, *Eristalis cerealis*) in rapeseed, *Brassica napus* L. *Japanese Journal of Breeding*, 38(1):91–102.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H. (2017). *vegan: Community Ecology Package*. R package version 2.4-4.

Olesen, J. M., Bascompte, J., Dupont, Y. L., and Jordano, P. (2007). The modularity of pollination networks. *Proceedings of the National Academy of Sciences*, 104(50):19891–19896.

Orford, K. A., Vaughan, I. P., and Memmott, J. (2015). The forgotten flies: the importance of non-syrphid Diptera as pollinators. *Proceedings of the Royal Society B: Biological Sciences*, 282:20142934.

Pianka, E. R. (1973). The structure of lizard communities. *Annual Review of Ecology and Systematics*, 4(1):53–74.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6):345–353.

R Core Team (2015). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rader, R., Bartomeus, I., Garibaldi, L. A., Garratt, M. P., Howlett, B. G., Winfree, R., Cunningham, S. A., Mayfield, M. M., Arthur, A. D., Andersson, G. K., et al. (2016). Non-bee insects are important contributors to global crop pollination. *Proceedings of the National Academy of Sciences*, 113(1):146–151.

Rader, R., Edwards, W., Westcott, D. A., Cunningham, S. A., and Howlett, B. G. (2011). Pollen transport differs among bees and flies in a human-modified landscape. *Diversity and Distributions*, 17(3):519–529.

Rader, R., Howlett, B. G., Cunningham, S. A., Westcott, D. A., Newstrom-Lloyd, L. E., Walker, M. K., Teulon, D. A., and Edwards, W. (2009). Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. *Journal of Applied Ecology*, 46(5):1080–1087.

Rotheray, G. E. and Gilbert, F. (2011). *The natural history of hoverflies*. Forrest Text.

Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., and Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, 18(1):529.

Sakavara, A., Tsirtsis, G., Roelke, D. L., Mancy, R., and Spatharis, S. (2018). Lumpy species coexistence arises robustly in fluctuating resource environments. *Proceedings of the National Academy of Sciences*, 115(4):738–743.

Sakurai, A. and Takahashi, K. (2017). Flowering phenology and reproduction of the *Solidago virgaurea* L. complex along an elevational gradient on Mt Norikura, central Japan. *Plant Species Biology*, 32(4):270–278.

Saunders, M. E. (2017). Insect pollinators collect pollen from wind-pollinated plants: implications for pollination ecology and sustainable agriculture. *Insect Conservation and Diversity*, 11(1):13–31.

Scheffer, M. and van Nes, E. H. (2006). Self-organized similarity, the evolutionary emergence of groups of similar species. *Proceedings of the National Academy of Sciences*, 103(16):6230–6235.

Scheffer, M., van Nes, E. H., and Vergnon, R. (2018). Toward a unifying theory of biodiversity. *Proceedings of the National Academy of Sciences*, in press:201721114.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7):676–682.

Solomon, M. and Kendall, D. (1970). Pollination by the syrphid fly, *Eristalis tenax*. *Long Ashton Research Station Reports*, 1971:101–102.

Speight, M. and Sarthou, J. (2014). StN keys for the identification of the European species of various genera of Syrphidae (Diptera) 2014. *Syrph the Net, the database of European Syrphidae*, 80:1–125.

Ssymank, A. and Gilbert, F. (1993). Anemophilous pollen in the diet of Syrphid flies with special reference to the leaf feeding strategy occurring in Xylotini (Diptera, Syrphidae). *Deutsche Entomologische Zeitschrift*, 40(2):245–258.

Ssymank, A., Kearns, C. A., Pape, T., and Thompson, F. C. (2008). Pollinating flies (Diptera): a major contribution to plant diversity and agricultural production. *Biodiversity*, 9(1-2):86–89.

Stang, M., Klinkhamer, P. G., and Van Der Meijden, E. (2006). Size constraints and flower abundance determine the number of interactions in a plant–flower visitor web. *Oikos*, 112(1):111–121.

Stang, M., Klinkhamer, P. G., Waser, N. M., Stang, I., and van der Meijden, E. (2009). Size-specific interaction patterns and size matching in a plant–pollinator interaction web. *Annals of Botany*, 103(9):1459–1469.

Sutherland, J. P., Sullivan, M. S., and Poppy, G. M. (1999). The influence of floral character on the foraging behaviour of the hoverfly, *Episyrphus balteatus*. *Entomologia Experimentalis et Applicata*, 93(2):157–164.

van Veen, M. (2010). *Hoverflies of Northwest Europe: Identification keys to the Syrphidae*. KNNV Publishing, Zeist, Netherlands, 2 edition.

Vlašánková, A., Padyšáková, E., Bartoš, M., Mengual, X., Janečková, P., and Janeček, Š. (2017). The nectar spur is not only a simple specialization for long-proboscid pollinators. *New Phytologist*, 215(4):1574–1581.

Zu, P. and Schiestl, F. P. (2017). The effects of becoming taller: direct and pleiotropic effects of artificial selection on plant height in *Brassica rapa*. *The Plant Journal*, 89(5):1009–1019.