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Flower-visiting preferences of bumble bees (Apidae: *Bombus* spp.) in grasslands of the Velyka Dobron' Game Reserve (Transcarpathia, Ukraine)

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Abstract. Frequency of flower visitations of six bumble bee (*Bombus*) species was surveyed in Transcarpathia (Ukraine). In four areas of the Game Reserve of Nagydobrony and surroundings five-five sampling quadrates were designated. The frequency of flower visitations of 16 dominant plant species (7 spp. in Fabaceae, 3 spp. in Asteraceae, 3 spp. in Lamiaceae, and 3 species from other families) were registered in four repetitions in mid-summer. The most frequent large bumble bee species, *B. terrestris*, exploited the widest spectrum of flower sources, with insignificant preference for *Lotus corniculatus*, *Prunella vulgaris* and *Lythrum salicaria.* The flower visitation of *B. lapidarius* was characterised by the high frequency of *L. salicaria* and *Medicago sativa.* The small bumble bee species, *B. pascuorum, B. sylvarum* and *B. humilis*, also showed significantly different preferences. Generally, the three most frequently visited plant species belonged to Fabaceae, followed by *Symphytum officinale* (Boraginaceae). We did not observe any significant difference between large *vs*. small bumble bees, either concerning the nectar source families or in length and shape of the visited flower corollas. Significant influence of flower colours, both as they are experienced by bumble bees (blue, bluish-green) and according to the visible colours (purple), was found on the flower visitation by nearly all bumble bee species. Since practically all species have shown somewhat different preferences for nectar sources, we could not observe any genuine communities of bumble bees; however, accidental feeding assemblages can exist based on high preferences of species in Fabaceae.

Key words: colour preferences, corolla length, Fabaceae, large and small species.

Introduction

The majority of angiosperm plant species needs animal pollination for sexual reproduction. The estimations of Kearns and Inouye (1997) show that approximately 67% of flowering plant species are pollinated by insects, which ratio can even be higher in temperate zones. The fertilization of ca. 80% of European plant species depends on their pollinators (Williams 1994; Klein et al. 2007; Ollerton et al. 2008). A recent worldwide survey (Ollerton et al. 2011) shows an even higher figure (87.5%) in this connection. Bees (Hymenoptera: Apoidea) are known as the most efficient, specialized and important pollinator insect group (Steffan-Dewenter & Tscharntke 1999, Kremen et al. 2002). Based on European and North American studies, however, the number of pollinator insects significantly decreased during the last years, especially those of wild bees (Williams 1982, Buschman & Nabhan 1996, Westrich 1996, Goulson et al. 2005, 2008, Biesmeijer et al. 2006, Winfree et al. 2008, Szabó et al. 2012). One of the possible reasons of this decline is the excessive use of pesticides in rural zones (Kearns et al. 1998, Brittain et al. 2010). This has reduced the population of herbs, especially species in the Fabaceae and Lamiaceae, which constitute essential food resources for bees (Carvell et al. 2001, Goulson et al. 2008). The decreased amount of available pollen and nectar results in considerable reduction in the number and diversity of pollinating insects (bees, butterflies, hover flies, etc.), which may reduce the probability of cross-pollination and lead to decline of agricultural production (Corbet et al. 1991, Biesmeijer et al. 2006). Therefore, the disruption of plant-pollinator interactions leads to a self-destructive circle, where due to the loss of key pollinators of plants, the whole natural community may experience dramatic changes (Kearns et al. 1998, Potts et al. 2010).

The most well-studied wild bees of the Carpathian Basin are the bumble bees (Apidae, Bombini). Based on previous assumptions, bumble bees can optimally adapt to changing nectar sources (Fontaine et al. 2008). Morphologically, bumble bee species are characterised by different body size and tongue length that makes them able to pollinate a diverse range of flowering plant species (Inouye 1980, Williams 1986, Raine & Chittka 2007). The importance of bumble bees is partly based on the effective pollination of tubular flower types requiring large body size and/or long mouth parts. Furthermore, pollination of some plants such as flowers of species in Solanaceae (e.g. *Solanum lycopersicum*) is only possible by hard and long-term vibration of wing muscles of bumble bees ('buzz pollination'), which frequency is necessary to open the antheridium (Corbet 1996, Kearns & Thomson 2001, Osborn & Williams 1996). Therefore, conservation of *Bombus* spp. is one of the important objectives of nature protection and has also important economic aspects (Goulson et al. 2008).

Up to now, most of the studies paid attention to the ecology and ethology of the six most widespread European bumble bee species (*Bombus terrestris*, *B. lapidarius*, *B. hortorum, B. lucorum*, *B. pascuorum*, *B. pratorum*), while pollen and nectar usage and other ecological traits of the remaining almost 40 European species remained less known (Goulson et al. 2005, Goulson et al. 2008, Fontaine et al. 2008). Numerous studies focusing on host plant preferences of bumble bee species mostly used pollen analysis. Significant data have been collected by pollen analytical investigations of larvae faeces from the nest (Anasiewicz & Warakomska 1977, Warakomska & Anasiewicz 1991). Further studies significantly extended the list of known foraging plants by analysing pollen collected from the body of the flower-visitor bumble bees. It was shown that *B. terrestris* and *B. lapidarius* play an important role *inter alia* in the pollination of *Lotus* spp., *Trifolium* spp. and species in Rosaceae (Teper 2004, 2005). Anasiewicz & Warakomska (1969) showed that *B. terrestris* is one of the main pollinators of *Medicago* spp.. The same authors (1976) reported that *B. hortorum* is one of the main pollinators of *Trifolium pratense*. The results of other studies also confirmed that bumble bees play the most significant role in the pollination of Fabaceae, which constitute their most important pollen and nectar sources (Ruszkowski & Bilinski 1969, Ruszkowski 1971, Goulson et al. 2005).

The colour perception of bumble bees proved to be a further important aspect of pollinator surveys. Numerous publications have shown that the colour perception of bees and humans (mammals) substantially differs (Chittka et al. 1993, Spaethe et al. 2001, Raine & Chittka 2007, Dyer et al. 2011), including sensitivity to different wavelength ranges. Bees, as most other insects, typically have photoreceptors that respond to ultraviolet, blue and green light (Peitsch et al. 1992, Briscoe & Chittka 2001). For better comparisons, the Floral Reflectance Database (FreD, see: Arnold et al. 2010) was developed, which provides free access to reflectance spectra of a large number of flowers. Numerous field and experimental surveys were carried out focusing on the innate vs. learning capacity of bees in the colour preferences (e. g. Gumbert 2000, Raine & Chittka 2005, Orbán & Plowright 2014).

We aimed to investigate bumble bee visits on flowers of dominant dicotyledonous plant species in meadows within a Transcarpathian forest reserve. According to former observations (Szanyi 2013), the presence of six bumble bee species was known from the selected sample areas. Based on our surveys, we intended to answer the following questions: (1) Can we observe differences in the flower visitation frequency of the plant species? (2) Is there some kind of resource partitioning between the two most common large bumble bee species (*B. terrestris*, *B. lapidarius*), or more generally, between the large vs. small bumble bee species? Or more simply: does body size of bumble bees and species identity have an influence on the visited plant species/families? (3) Can we find higher frequency of visitations of species in Fabaceae than other plants? (4) Can we find any regularity in the frequency of flower visitations of bumble bees according to the colours as perceived by the bees vs. humans? (5) Can we observe differences in the frequency of visitations on plants with short and long corolla tubes? (6) Can we detect some bumble bee assemblages, i.e. species groups with similar preferences in the frequented plant species?

Materials and methods

Study areas

Four meadows were selected in Velyka Dobron' Wildlife Reserve.

- ″Körerdő″ – (48°25′50.21″ N; 22°24′12.36″ E, ~1.1 ha) grassland between a mixed hardwood forest and a monocultural agriculture land, surrounded by an artificial channel. The forest fringe was especially rich in flowering tall forbs and polycormon-forming plants. As a result, *Melampyrum nemorosum* was the most frequent plant species on the shaded parts, while Asteraceae (*Cirsium arvense, Taraxacum officinale*) and Fabaceae (*Vicia cracca, Trifolium repens*) were dominant on the sunnier and drier patches.
- ″Kismakkos″ – (48°25′59.08″ N; 22°24′43.14″ E, ~1.9 ha) was a wet meadow partly dominated by tussock-forming tall grasses, completely surrounded by mixed hardwood forest and black locust (*Robinia*) plantations. *Rubus fruticosus* was frequent on the edges; *Betonica officinalis* was abundant on the mesic parts, while some Fabaceae (*Medicago sativa, Galega officinalis*) were dominant on the drier parts.
- ″Felső-erdő″ – (48°25′44.80″ N; 22°25′07.47″ E, ~5.3 ha) was a tall grass meadow, completely surrounded by a hardwood forest. *Rubus fruticosus* and *R. caesius* were frequent on the edges. The mesic patches were dominated by Fabaceae, and on the drier sunny parts, species of Asteraceae were abundant.
- ″Rezervátum″ – (48°25′13.53″ N; 22°25′48.93″ E, ~3.6 ha) was a tall grass meadow, surrounded by mixed hardwood forest and scrubby forest fringe. On the edges, *Rubus fruticosus* and *Melampyrum nemorosum* were frequent. The drier patches were dominated by species of Fabaceae and Asteraceae.

Sampling methods

Samples were made four times by netting in 2013 (16-17/07; 24-25/07; 9-10/08; 19-21/08). All samplings were carried out between 10 am and 4 pm in sunny and nearly windless weather conditions (the temperature was usually >20°C according to the suggestion of Goulson & Darwill (2004)). The vegetation of the study areas was surveyed with the standard Braun-Blanquet method in 2012 (Szanyi et al. 2015a). Those patches were selected in which the cover of dicotyledonous plants was over 50%. Five 3×3 m quadrats were randomly designated in each sampling area. Samples were collected during 20 minutes in each quadrate. All flower-visiting bumble bees were captured by butterfly net and released after identification and registration of locality, time and visited plant species. We used the Identification Chart of the Bumble Bee Conservation Trust (https://bumblebeeconservation.org) for field identification. Some problematic individuals were preserved for later identification. We used the keys of Móczár et al. (1985) and the website Atlas Hymenoptera (Rasmont & Iserbyt, 2014; http://www.atlashymenoptera.net/). Among the preserved voucher specimens, we did not found any individuals of *B. lucorum* and *B. muscorum*, respectively. According the average body-size data, *B. hortorum, B. terrestris* and B. *lapidarius* were considered as large species, and all others as small ones. The plant species were identified by the keys of Király et al. (2009). The data on actino- vs. zygomorphic shape of flowers were also taken from the same source. The colour preferences of bees were analysed by trichromatic model of colour perception of bees (reviewed by Dyer et al. 2011). The colour of flowers (see Table 1) as perceived by bees was specified according to Dyer et al. (2011) and the Floral Database (Arnold et al. 2010; http://reflectance.co.uk/advance.php). Data of corolla length were taken from the pocket-book, Exkursionsflora (Rothmaler et al. 1972), and the home page, www.luontoporti.com/suomi/en.

Statistical methods

To characterize host plant preferences, the relative frequencies of visitations (number of individuals visited a given plant species/total number of individuals) on different plant species by samples were calculated for each bumble bee species. If we would use the number of individuals, it could bias the results because of the different abundances of the species in different sites and sampling times. Data of the five 3×3 m quadrates per study site and per sampling occasion were summarised. During the analysis, sampling site and sampling time were used as repetitions. These frequencies were also calculated in the case of overall bumble bees and groups of large (*B. terrestris*, *B. lapidarius* and *B. hortorum*) and small (*B. pascuorum*, *B. sylvarum* and *B. humilis*) species formed based on their body size. Differences in host-plant visitations among the different plant species, families and flower types (form, colour) were analysed. Flowers were typified by their visible colour and colour precepting by bees, and corolla length (long, short) (Table 1.)

Table 1. List of the plant species visited by bumble bees during the study with their abbreviated names, visible colour, colour precepting by bees (Colour-Bee), corolla length and flower type. (PV: purplish-violaceous, WY, whiteish-yellowish, long: mean >10 mm; short: <10 mm).

	Family	Abbr. name	Colour-Bee	Colour	Flower type	Corolla length (mm)
Betonica officinalis	Lamiaceae	betoff	UV-blue	PV	long	12-15
Cirsium arvense	Asteraceae	cirarv	blue-green	PV	short	4-5
Cirsium vulgare	Asteraceae	cirvul	blue	PV	short	5-6
Galega officinalis	Fabaceae	galoff	blue-green	PV	long	10-15
Lotus corniculatus	Fabaceae	lotcor	green	WY	short	8-10
Lythrum salicaria	Lythraceae	lytsal	UV-blue	PV	long	10-12
Medicago sativa	Fabaceae	medsat	blue	PV	short	5-8
Melampyrum nemorosum	Scrophulariaceae	melnem	blue-green	WY	long	15-20
Mentha arvensis	Lamiaceae	menarv	blue	PV	short	4-7
Mentha pulegium	Lamiaceae	menpul	blue	PV	short	4-5
Prunella vulgaris	Lamiaceae	pruvul	blue	PV	long	8-16
Symphytum officinale	Boraginaceae	symoff	blue	PV	long	15-18
Taraxacum officinale	Asteraceae	taroff	green	WY	short	4-6
Trifolium pratense	Fabaceae	tripra	blue	PV	long	12-15
Trifolium repens	Fabaceae	trirep	blue-green	WY	short	8-10
Vicia cracca	Fabaceae	viccra	blue	PV	long	10-12
Vicia grandiflora	Fabaceae	vicgra	blue-green	PV	long	10-14

As our data did not meet the assumptions of parametric tests (i.e. normal distribution, homogeneity of variances), the non-parametric Kruskal-Wallis test was used in multiple comparisons. To compare two groups and pairs, the Mann-Whitney U-test was used (Reiczigel et al., 2007). Visitation preferences of plant species by the different bumble bee species was also surveyed by Principal Component Analysis (PCA) (Podani 1997a). Statistical analyses were performed by SPSS 21.0, and SynTax statistical softwares (Ketskeméty et al., 2011, Podani 1997b).

Results

During the samplings, 692 individuals of six bumble bee species (*B. terrestris, B. lapidarius, B. hortorum, B. pascuorum, B. sylvarum and B. humilis*) were observed. These species are generally distributed and most frequent in the Carpathian Basin (Móczár et al. 1985). The most frequent species was *B. terrestris*, which occurred together with the other frequent large species, *B. lapidarius*, in all sampling areas. *B. pascuorum* also belonged to the most frequent species group.

Bumble bees visited various resource-plant species with different frequencies (K-W: H=48.51 n=173, d=16, p<0.001). Three species in Fabaceae were the most frequently visited plants: *Lotus corniculatus, Trifolium pratense,* and *Vicia cracca.* Each of these species was chosen by, on average, more than 15% of all bumble bees. Other species with more than 10% of flower visitation included *Lythrum salicaria, Medicago sativa, Melampyrum nemorosum, Prunella vulgaris* and *Trifolium repens* (Table 2).

We found significant differences in the frequency of flower visitations of different plant species in the large *Bombus* species (K-W: H=46.25, n=173, d=16, p<0.001). In the case of *B. terrestris*, the most frequently visited plant species were *L. corniculatus*, *P. vulgaris* and *L. salicaria* (K-W: H=31.87, n=173, d=16, p=0.010). The flower visitation frequency of *B. lapidarius* was significantly different both from the other large species (*B. terrestris* and *B. hortorum*) and from the small species, too. It was characterised by the highest frequencies of visitation to flowers of *L. salicaria*, *M. sativa* and *L. corniculatus*, however, the frequency of visitations was over 20% in the case of *T. pratense* and *V. cracca*, too (K-W: H=29.03, n=150, d=16, p=0.024). The third large species, *B. hortorum*, preferred some other flowers (K-W: H=41.11, n=156, d=16, p<0.001); the highest frequency was observed in the case of *M. nemorosum*, *P. vulgaris, Symphytum officinale*, and *T. repens. Mentha pulegium* was completely neglected by the large bumble bee species (Table 2).

Considering overall small *Bombus* species, there were no significant differences among plants on the basis of visitation frequencies (K-W: H=24.96, n=173, d=16, p=0.071). Among them, *B. pascuorum* showed the highest visitation frequency on *Betonica officinalis*, *L. corniculatus* and *Cirsium arvense* (K-W: H=44.36, n=173, d=16, p<0.001), while *B. humilis* showed preference for *Mentha arvense*, *M. sativa* and *T. pratense* (K-W: H=36.52, n=162, d=16, p=0.002). *B. sylvarum* did not show significant differences in the frequency of flower visitations (K-W: H=24.30, n=121, d=16, p=0.083). Further, frequently (>10%) visited plant species by small bumble bee species included *M. nemorosum, P. vulgaris, S. officinale, Taraxacum officinale and V. cracca,* while *L. salicaria* was nearly completely neglected (Table 2).

According to the results of PCA, only the visitation of *M. pulegium* was clearly separated from all other plant species, since this plant species was only preferred by *B. humilis* and mostly neglected by other species. Some separation was also shown in the case of *C. vulgare, L. salicaria* and *M. sativa* due to the strong preferences by *B. lapidarius* and *B. sylvarum* (Fig. 1.).

Significant differences were found also in the frequency of visitations of the flowers belonging to different plant families, in case of bumble bees generally (K-W: H=28.27, n=66, d=5, p<0.001) and both large (H=31.348 n=66, d=5, p<0.001) and small species (H=11.17, n=66, d=5, p=0.048) respectively (Fig. 2). Considering all bumble bee species, the highest visitation frequencies were found in Fabaceae (>40 %) and Lamiaceae (>25%).

The two largest and common bumble bee species, *B. terrestris* and *B. lapidarius*, showed also significant differences in

Table 2. Number of total sampled individuals (N_{sum}) and mean relative frequencies of host plant visitations (RF%(±SD)) per samples per bumble bee species, big-small categories of species (BIG, SMALL) and at the whole sample level (SUM) with the results of Kruskal-Wallis test (Sign: significantly differed at p<0.05 level, NS: did not differ significant). For abbreviations of plant species, see Table 1.

	B. terrestris	*B. lapidarius*	*B. hortorum*	*B. pascuorum*	*B. sylvarum*	*B. humilis*	BIG	SMALL	SUM
N_{sum}	253	56	112	107	79	85	309	383	692
K-W	Sign	Sign	Sign	Sign	NS	Sign	Sign	NS	Sign
betoff	9.43 (9.68)	2.38 (8.91)	0.00 (0.00)	**29.83 (32.84)**	4.55 (15.08)	0.00 (0.00)	6.04 (5.77)	**16.48 (21.78)**	8.95 (7.8)
cirarv	9.56 (9.42)	2.78 (9.62)	0.00 (0.00)	**17.07 (19.39)**	0.00 (0.00)	0.00 (0.00)	6.40 (6.62)	8.30 (10.01)	6.81 (5.47)
cirvul	0.67 (2.31)	6.14 (13.76)	0.00 (0.00)	8.33 (28.87)	4.81 (9.88)	1.85 (6.42)	2.09 (3.80)	4.74 (10.51)	2.67 (4.11)
galoff	5.64 (13.96)	13.00 (31.99)	11.00 (31.43	5.19 (17.23)	**20.00 (38.3)**	0.00 (0.00)	7.05 (14.24)	5.76 (10.96)	6.31 (12.56)
lotcor	**23.92 (15.67)**	**31.25 (47.32)**	10.00 (22.36	**16.00 (35.78)**	6.25 (12.5)	0.00 (0.00)	**21.35 (16.57)**	**17.43 (35.11)**	**18.43 (19.59)**
lytsal	**18.29 (22.74)**	**50.00 (70.71)**	0.00 (0.00)	0.00 (0.00)	5.56 (7.86)	0.00 (0.00)	14.78 (14.80)	2.22 (3.85)	11.98 (12.09)
medsat	8.24 (11.97)	**35.23 (45.70)**	1.28 (4.44)	4.62 (13.91)	**25.75 (34.52)**	**22.22 (32.97)**	8.56 (10.51)	11.99 (13.37)	10.26 (9.90)
melnem	7.87 (12.55)	5.33 (11.67	**29.31 (33.55)**	2.78 (9.62)	5.71 (15.12)	**31.21 (35.7)**	12.39 (13.27)	10.42 (13.10)	12.18 (9.87)
menarv	4.27 (11.67)	0.00 (0.00)	2.70 (8.00)	1.56 (6.25)	0.61 (2.01)	4.54 (14.48)	3.09 (6.90)	1.79 (6.25)	2.61 (6.38)
menpul	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.56 (5.41)	0.00 (0.00)	0.60 (2.06)	0.31 (1.07)
pruvul	**18.59 (24.28)**	0.00 (0.00)	**21.34 (29.20)**	0.89 (3.57)	1.52 (5.03)	19.00 (36.43)	**17.22 (16.43)**	8.04 (18.00)	13.74 (14.28)
symoff	6.73 (10.86)	0.00 (0.00)	21.33 (44.07)	13.10 (21.39)	10.00 (13.69)	13.19 (17.31)	6.21 (6.84)	11.45 (15.41)	8.91 (9.95)
taroff	7.14 (12.20)	0.00 (0.00)	6.67 (16.33)	11.84 (20.23)	**20.00 (44.72)**	19.05 (37.80)	5.60 (7.97)	13.69 (24.85)	8.65 (12.26)
tripra	14.63 (19.16)	25.00 (46.29)	13.62 (23.30)	13.17 (20.48)	8.33 (20.41)	**20.63 (30.05)**	16.46 (15.56)	15.74 (18.59)	**16.73 (15.58)**
trirep	12.54 (20.77	10.75 (23.63)	**23.37 (31.45)**	12.18 (22.88)	15.60 (23.48)	4.29 (9.57)	15.77 (16.33)	10.98 (16.78)	13.94 (15.39
viccra	16.15 (21.40)	25.00 (41.83)	12.78 (21.13)	14.29 (20.20)	**35.48 (36.5)**	10.18 (17.57)	**20.53 (21.36)**	**16.27 (22.25)**	**20.37 (14.99)**
vicgra	10.14 (17.57)	6.25 (8.84)	5.13 (8.88)	0.00 (0.00)	16.67 (23.57)	0.00 (0.00)	11.21 (13.32)	6.67 (11.55)	8.89 (8.01)

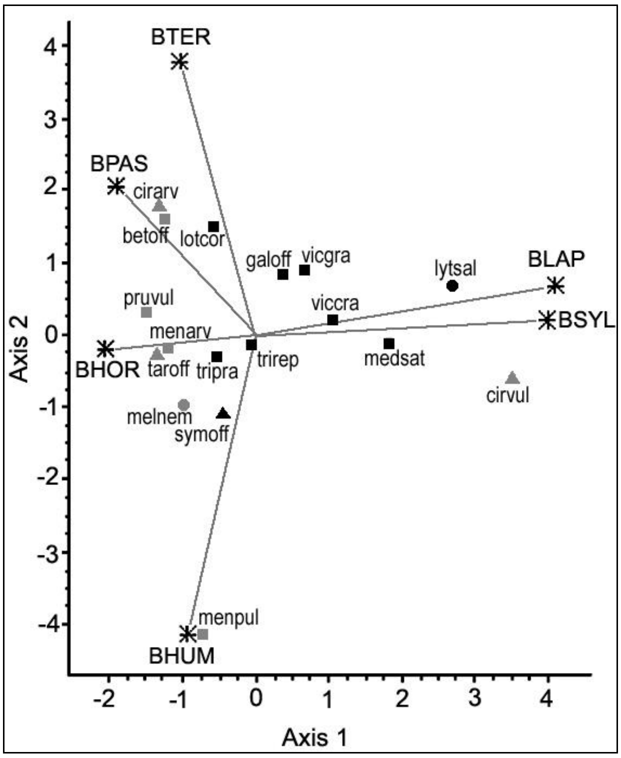

Figure 1. Principal component analysis biplot (PCA) of host plants and the studied bumble bee species in Nagydobrony (West Ukraine) (Axis 1: 37% and Axis 2: 31% explained var.). black square: *Fabaceae*, black circle: *Lythraceae*, black triangle: *Boraginaceae*, gray square: *Lamiaceae*, gray circle: *Scrophulariaceae*, grey triangle: *Asteraceae*; BHOR: *B. hortorum*, BHUM: *B. humilis*, BLAP: *B. lapidarius*, BPAS: *B. pascuorum*, BSYL: *B. sylvarum*, BTER: *B. terrestris*. For abbreviations of plant species, see Table 1.

the frequency of visitations at the level of plant families (K-W: H=20.38, N=66, d=5, p=0.001; H=34.79, N=53, d=5, p<0.001). The bulk of the large bumble bees chose species in the Fabaceae, and individuals of *B. lapidarius* visited this family significantly more times than all other families. We found a high preference for Fabaceae also in the case of the third large species, *B. hortorum* (K-W: H=20.38, N=58, d=5, p=0.001) (Figs. 2 and 3).

The visitation frequencies of plant families also differed significantly in the case of *B. pascuorum*, with nearly equally high frequencies of Fabaceae, Lamiaceae and Asteraceae (K-W: H=15.69, N=66, d=5, p=0.008). The frequency of visitations of plant families also differed significantly in the case of *B. sylvarum* (K-W: H=18.47, n=45, d=5, p=0.002), with a preference for Fabaceae, while in the case of *B. humilis*, there were no differences among plant families (K-W: H=8.73, n=61, d=5, p=0.120) (Fig. 4).

We found significant differences in the visitations of different coloured flowers in the case of bumble bees generally (K-W: H=34.47, n=58, d=3, p<0.001) and both groups of large and small *Bombus* species (K-W: H=34.41, n=58, d=3, p<0.001; H=8.84, n=58, d=3, p=0.032) (Table 3). Bumble bees more often chose „blue” flowers, while the frequency of visitations was gradually decreasing in bluish-green, green and UV-blue direction. These differences were also observed when we separately considered the three large species, *B. terrestris*, *B. lapidarius* and *B. hortorum* (K-W: H=25.12, n=58, d=3, p<0.001; H=11.21, n=50, d=3, p=0.011, K-W: H=22.21, n=51, d=3). Although the frequentation of the blue colour was the highest for each case, in the case of *B. hortorum*, flowers with blue and bluish-green colours were most often frequented parallelly. Among small species, both *B. sylvarum* and *B. humilis* showed preferences for blue flowers (K-W: H=10.78, n=40, d=3, p=0.013; H= 25.08, n=54, d=3, p<0.001), while in the case of *B. pascuorum*, there were no significant differences in emerged preferences (K-W: H=2.47, n=58, d=3, p=0.481) (Table 4).

Concerning the visible spectrum, a higher frequentation of purplish-violaceous flowers was shown for bumble bees

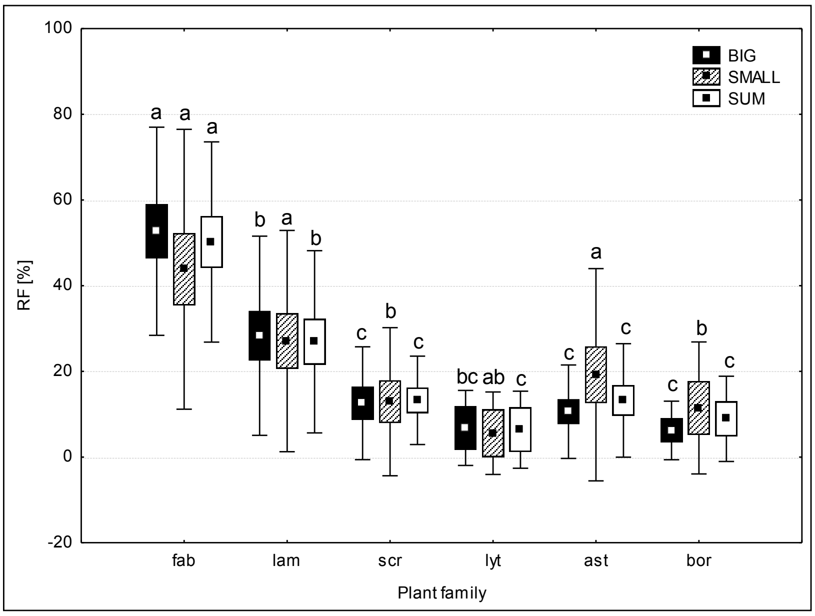

Figure 2. Mean relative frequencies of visitations (RF%) of plant families (mean/SE/SD) per samples in large and small bumble bee groups and the whole sample (SUM). The lowercase letters refer to the results of the Mann-Whitney U test (p<0.05). fab: Fabaceae, lam: Lamiaceae, scr: Scrophulariaceae, lyt: Lytraceae, ast: Asteraceae, bor: Boraginaceae.

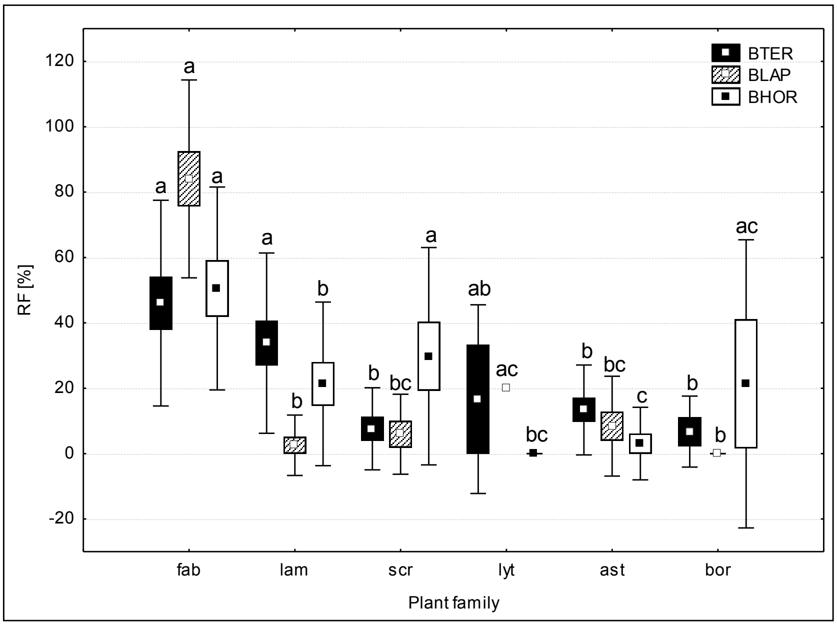

Figure 3. Mean relative frequencies of visitations (RF%) of plant families (mean/SE/SD) per samples in large bumble bees: *Bombus terrestris* (BTER) and *Bombus lapidarius* (BLAP). The lowercase letters refer to the results of the Man-Whitney U test (p<0.05). Abbreviations of plant families are same as in Fig. 2.

generally. In the case of bumble bees and groups of large and small species, the same preferences could be found (Table 3). In this respect, the otherwise rather different big species showed different preferences for purplish flowers, because we could not detect differences in the frequency of visitations of purplish-violaceous vs. whitish-yellowish flowers in *B. hortorum* (U-test: p=0.366) (Table 4). The small *B. pascuorum* has also shown a significant preference for purplish flowers and similar pattern was detected in *B. sylvarum* and *B. humilis* (U-test: p<0.05).

In contrast to earlier expectations, we could not find any significant differences between the visitations of actino- vs. zygomorphic and of flowers with short- vs. deep corolla tubes (see: details in Appendix). The only exception was found in *B. hortorum*, which showed a significant preference for flowers with deep corolla tubes (U-test: p=0.029) (Table 2 and 3).

Discussion

Our surveys were carried out in the northeastern part of the Carpathian Basin, which is a traditional lowland agricultural region with significant contribution of natural woodland vegetation and mostly abandoned semi-natural meadows. In consequence, this area belongs to the floristically richest parts of the large Pannonian Plain. Although it is faunistically still undersurveyed, the high species diversity of some invertebrate groups (land snails, ground beetles, orthopterans, butterflies and moths) was already noticed (Deli et al., 1997, Ködöböcz & Magura, 1999, Szanyi et al. 2015b, 2015c). Therefore, a survey of pollinators and nectar sources could fill some information gaps here. However, we should be cautious in generalisations, since our surveys were carried out in a single summer period. Therefore, only few conclusions can be drawn, mostly on the expectations formulated in the Introduction.

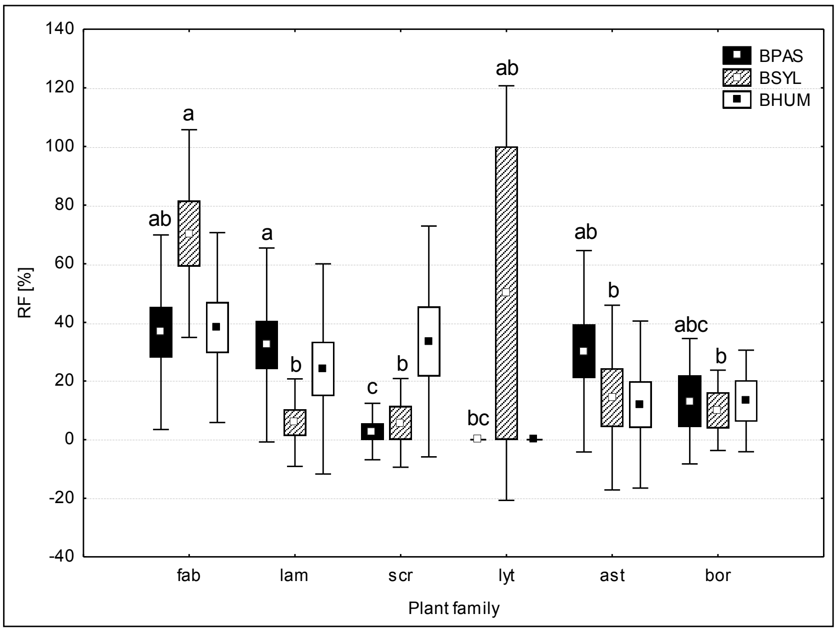

Figure 4. Mean relative frequencies of visitations (RF%) of plant families (mean/SE/SD) per samples in small bumble bees: *Bombus pascuorum* (BPAS), *Bombus sylvarum* (BLAP) and *Bombus humilis* (BHUM). The lowercase letters refer to the results of the Man-Whitney U test ($p<0.05$). Abbreviations of plant families are same as in Fig. 2.

Table 4. Mean (±SD) relative frequencies of visitations of different groups of host plants by six studied bumble bee species according to flower colour (Colour-Bee: according perception of bees; Colour: visible colour, BL purplish-violaceous, FS: whitish-yellowish). The lowercase letters refer to the results of the Mann-Whitney U test ($p<0.05$).

	B. terrestris	*B. lapidarius*	*B. hortorum*	*B. pascuorum*	*B. sylvarum*	*B. humilis*
Colour-Bee						
blue	50.61 (18.82) a	57.50 (45.40) a	46.99 (39.24) a	31.03 (34.62)	52.34 (38.50) a	65.07 (36.49) a
bluegreen	28.67 (22.70) b	24.05 (36.36) b	46.58 (34.06) a	28.97 (26.75)	30.74 (35.95) ab	26.04 (33.45) b
green	16.96 (19.63) bc	15.63 (35.20) b	10.00 (20.00) b	16.29 (28.25)	17.86 (37.40) bc	14.81 (33.79) bc
UVblue	10.13 (10.19) c	9.52 (27.51) b	0.00 (0.00) b	29.83 (32.84)	5.56 (15.11) c	0.00 (0.00) c
Colour						
PV	74.88 (20.88) a	79.58 (31.96) a	55.94 (39.04)	79.37 (27.08) a	73.66 (32.24) a	65.07 (36.49) a
WY	25.12 (20.88) b	20.42 (31.96) b	44.06 (39.04)	20.63 (27.08) b	26.34 (32.24) b	34.93 (36.49) b
Flower type						
long	52.72 (32.31)	34.23 (42.25)	64.33 (34.93)	47.54 (38.33)	47.14 (42.17)	51.92 (41.66) a
short	47.28 (32.31)	65.77 (42.25)	35.67 (34.93)	52.46 (38.33)	52.86 (42.17)	48.08 (41.66) b

Table 3. Mean (±SD) relative frequencies of visitations of different groups of host plants by big and small bumble bee species and whole sample (SUM) according to flower colour (Colour-Bee: according perception of bees; Colour: visible colour, PV: purplish-violaceous, WY: whitish-yellowish). The lowercase letters refer to the results of the Mann-Whitney U test ($p<0.05$).

	BIG	SMALL	SUM
Colour-Bee			
blue	52.52 (20.9) a	47.33 (31.12) a	50.21 (14.97) a
bluegreen	29.05 (22.08) c	30.31 (19.75) ac	30.68 (15.94) b
green	15.24 (20.14) bc	15.61 (22.26) bc	15.27 (18.55) c
UVblue	8.91 (7.65) b	12.60 (20.07) b	9.57 (8.00) c
Colour			
PV	75.49 (20.73) a	69.52 (24.56) a	71.74 (17.72) a
WY	24.51 (20.73) b	30.48 (24.56) b	28.26 (17.72) b
Flower type			
long	50.80 (32.54)	54.27 (30.13)	52.33 (27.48)
short	49.20 (32.54)	45.73 (30.13)	47.67 (27.48)

We found eight plant species which were visited with high frequency, out of which five belonged to Fabaceae, including the three most highly visited species. This issue clearly corresponds with the results of several former studies (Anasiewicz & Warakomska, 1969, 1976, Ruszkowski & Bilinski, 1969, Ruszkowski, 1971, Carvell et al. 2001, Goulson et al., 2005). In this respect, the colour, size and shape of flowers seem to have a secondary importance. The yellow *Lotus corniculatus* and the purplish *Medicago sativa, Trifolium pratense* and *Vicia cracca* similarly belonged to the most frequented species both for small and large bumble bees (see: details in Appendix). It also means that the diversity of bumble bee assemblages can be important for the effective pollination of species in Fabaceae.

At least some of the most frequented plant species were visited by all the six bumble bee species. Additionally, the effect of the summer season can be detected in our findings, showing that the relatively late flowering *Betonica officinalis, Melampyrum nemorosum* and *Lythrum salicaria* also belonged to the most often-visited plant species. Considering possible resource partitioning, we only found some evidence for it in the case of the two commonest large species, *B. terrestris* and *B. lapidarius*. For example, *Betonica officinalis* and *Prunella vulgaris* were frequently visited by *B. terrestris*, but almost completely neglected by *B. lapidarius*, while *Lythrum salicaria* and *Medicago sativa* were more often visited by the latter species. Some differentiation was also found in the visitation frequencies among the small species, mostly between *B. pascuorum* and *B. humilis*. Surprisingly, the nectar source preferences of the large *B. lapidarius* and the small *B. sylvarum* showed similar patterns. Even some „rare species," e. g. *B. humilis*, which was mentioned in references as a specialist (Goulson & Darvill, 2004), seem to be more generalist and not rare at the same time (162 observations, 3 plant species that were visited >20%) according to our data.

No significant differentiation was shown, however, between large vs. small bumble bees, neither concerning the food source families, nor in the length and shape of the corollas of flowers. Significant influence of flower colours, both as they are experienced by bumble bees (see: Dyer et al. 2011) and according to the visible colours, was found in nearly all cases. A relatively recent publication (Raine & Chittka 2007) contains a large amount of data on preferences of bumble bees related to the nectar production of the visited flowers. This work included information on a number of species in Fabaceae, e.g. *Trifolium repens, T. hybridum, Lathyrus pratensis* and *Lotus corniculatus*, as important nectar resources, as well as *Lythrum salicaria* and *Symphytum officinale*. These species also belong to the most visited plants according to our surveys. Although we did not collect data on the nectar production of the most frequently visited plants, our data seem to support the connection of the frequentation of flowers with the level of their nectar production.

Since practically all species have shown different preferences for nectar sources to some degree, we could not observe any communities of bumble bees, however, accidental feeding „assemblages" can exist based on high preferences of species in Fabaceae. Therefore, the high diversity of bumble bee assemblages can improve the efficiency of the pollination of Fabaceae.

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