

POLLINATORS OF *PULSATILLA GRANDIS* WENDER. IN SOUTHERN BAKONY (HUNGARY)

MÉSZÁROS, T.^{1*} – JÓZÁN, ZS.²

¹*Department of Plant Sciences and Biotechnology, Georgikon Faculty, University of Pannonia
Keszthely, Festetics u. 7, 8360 Hungary
(phone: +36-83-545-095; fax +36-83-545-058)*

²*Mernye, Rákóczi Ferenc u. 5, 7453 Hungary*

**Corresponding author
e-mail: meszarost773@gmail.com*

(Received 23rd Jul 2018; accepted 5th Oct 2018)

Abstract. Sexual reproduction plays an important role in the maintenance of the genetic variability of plant species. In the case of entomophilous species (like *Pulsatilla grandis*) pollinators are needed to this way of propagation. It is important to have complete knowledge on the pollinators to understand the biology of plant species. Our aim was to collect information about the pollinators of *P. grandis*, as there is no available data on the pollinators of this endangered species. We studied the flower-visiting species in 2017 and 2018 on the Csatár Hill near Veszprém (Hungary) in a population of about 2000 individuals of *P. grandis*. The number of collected *Apis mellifera* individuals (239) was the highest followed by *Bombus* (31 individuals) and *Andrena* (15 individuals) species. *Lasioglossum*, *Polistes* and *Priocnemis* species were collected as well. We collected 5 male insects which visit the flowers only for nectar but they can help the self-pollination as they are searching for nectar in the flower. We recorded the most intense flower visitation between 10-11 a.m. Our new results can help to develop the conservation strategy of *P. grandis*.

Keywords: *flower-visiting insects, Apis mellifera, Bombus, pollen, nectar*

Introduction

The frequency and intensity of grazing is decreasing due to the decline in livestock farming. As a result the diversity of semi-natural grasslands is decreasing as well. The species number of plants and insects is dwindling in these habitats (Walker and Pinches, 2011). Biodiversity influences ecosystem services in some semi-natural landscapes (Lowenstein et al., 2015). Having enough information about the pollination strategy of rare and endangered species is a key factor to develop protection and management strategies. However, our knowledge is imperfect even in the case of intensively studied species (Denisow et al., 2014a; Gostin, 2011). In the case of metapopulations it is especially important to know the plant-pollinator interactions and the requirements of effective gene flow to maintain healthy populations of rare and endangered species (Schemske et al., 1994). Sexual reproduction is essential from genetic and evolutionary aspects. It contributes to the inter- and intrapopulation genetic variability of individuals (Charlesworth and Charlesworth, 1987). Fructification rate and seed set depends on fertilization which is the result of pollination, so the effectiveness of pollination is a key factor in the reproduction success of entomophilous species (Larson and Barrett, 2000). The plant-pollinator interactions are very complex and can be influenced by many factors in addition to environmental ones (Denisow et al., 2014a). In the temperate climate zone about 80% of plant species is pollinated by insects. Both cultivated and wild species are important elements of pollination systems (Strzałkowska et al., 2016). In

the case of early spring flowering species the number of pollinators is low. The number of flower-visitations by insects is limited due to unfavourable weather conditions (Kratochwil, 1988). In early spring pollen is an especially important protein source for insects (Moquet et al., 2015). The demand for nectar and pollen increases quickly in early spring by bee-like insects (Denisow, 2006). The pollen is needed to forage the brood to reach the proper family size. *Pulsatilla grandis* is a valuable and threatened species of semi-dry grasslands. This species is a significant pollen source for bees as it is flowering in early spring. The morphology and ecology of *Pulsatilla* species also promote the pollination by insects (Zimmermann, 1935). They attract insects with their bell-shaped, actinomorphic flowers and lots of yellow stamens (Walker and Pinches, 2011; Essl, 2005). In the case of partly opened flowers the pollinators first touch the pistils so they can be fertilized with the pollen carried from another plant. While in the case of older, fully opened flowers the bees land on tepals, so they first touch the stamens and after that the pistils (Zimmermann, 1935). Some authors classified *Pulsatilla* species as pollen producing plants, having a lot of stamens but producing no nectar, similar to other species of the *Ranunculaceae* family. Although Weryszko-Chmielewska and Sulborska (2011) proved that the staminodes of *Pulsatilla* flowers produce nectars, so they function as nectaries. Staminodes are much smaller than stamens and have a simple structure. They can be found on the basis of the androecium. The nectar is produced when the pistil is ready for fertilization. Insects have to reach the basis of androecium to reach the nectar. Meanwhile they support fertilization as they touch the stigma. The pollen production of *Pulsatilla* species is 4.22-9.16 g/m², so they are significant pollen sources. These species produce more pollen than other early spring perennials (Strzałkowska-Abramek et al., 2016). *Pulsatilla grandis* has different strategies to ensure the efficient pollination. It can close its flowers in the case of unfavourable weather conditions (rain, wind) until the flowers are not pollinated (Sauberer and Panrok, 2015). Most anthers of *Pulsatilla* flowers open in the warmest hours of the day, which is typical of many other early spring species as well (Denisow et al., 2014b, 2015). Pollen emission round midday is advantageous for early spring plants and increases the number of insect visitations (Strzałkowska et al., 2016). The anthers open one after the other. So the flower produces pollen during a long period of time and the ratio of fertilization is higher. This long period of pollen emission is important from population genetic aspect. The number of pollinators, carrying pollen from other individuals, increases as well, so the genetic variability of the next generation multiplies (Kratochwil, 1988). Because the flying distance of pollinator insects is about 10 km, the gene flow between distant populations is not probable (Walker, 2011). *Pulsatilla vulgaris* Mill. is a related species occurring in Western Europe. The success of its reproduction is considerably influenced by the pollination of insects and the spread of pollen depends on the flying distance of pollinators. There is a positive correlation between the foraging distance and the size of pollinator species. Bumblebee species which also visit its flowers has longer foraging range and provide gene flow between distant populations as well (Hensen et al., 2005; Walther-Hellwig and Frankl, 2000; Osborne et al., 2008). It was observed that besides bumblebees honey bees have longer flying distances as well (Steffan-Dewenter and Kuhn, 2003), therefore these insects have significant role in the pollination of populations living in fragmented habitats.

Insects visit *Pulsatilla* species both for nectar and pollen (Table 1). However, the visiting *Apoidea* species do not fertilize the flowers in every cases, some of them are nectar robbers (Kratochwil, 1988).

Our aim was the collect information about the pollinators of *P. grandis*, as there is no available data on the pollinators of this endangered species.

Table 1. Pollinators of the *Pulsatilla* genus and *Pulsatilla vulgaris*

Taxon	Pollinator	Comment	Reference
<i>Pulsatilla</i> genus	<i>Apis mellifera</i> Linnaeus, 1758 <i>Bombus terrestris</i> (Linnaeus, 1758)	Mainly visited by honey bees (<i>Apis mellifera</i> Linnaeus, 1758) for pollen and nectar	Zimmermann (1935)
<i>Pulsatilla</i> genus	Honey bees and bumble bee species	Pollen and nectar collection	Weryszko-Chmielewska and Sulborska (2011)
<i>P. vulgaris</i>		Nectar collecting bees	Weryszko-Chmielewska and Sulborska (2011)
<i>P. vulgaris</i>	<i>Lasioglossum lineare</i> (Schenck, 1869), <i>Andrena bicolor</i> Fabricius, 1775	Solitary bees are the main pollinators	Kratochwil (1988)
<i>P. vulgaris</i>	<i>Halictidae</i> family, <i>Andrena</i> genus		Sauberer and Panrok (2015)
<i>P. vulgaris</i>	<i>Apis</i> and <i>Bombus</i> species		Walker (2011), Walker and Pinches (2011)
<i>P. vulgaris</i>	<i>Halictus fulvicornis</i> (Kirby, 1802), <i>H. tumulorum</i> (L. 1758), <i>H. albipes</i> (Fab. 1781), <i>H. flavipes</i> (Fab. 1787), <i>H. leucopus</i> (Kirby, 1802), <i>Osmia bicolor</i> (Schrank, 1781), and <i>Bombus agrorum</i> (Fab. 1787) [= <i>B. pascuorum</i> (Scopoli, 1763)]	Great Britain	Wells and Barling (1971)

Materials and methods

Study species

Pulsatilla grandis Wender. is a Natura 2000 species, which is listed in the Annex II and Annex IV of the Habitats Directive of the European Commission (Council Directive 92/43/EEC) (Randić et al., 2013). Its range is Pannonian type, i.e. distributed from Western Ukraine to Austria and the Czech Republic (Sauberer and Panrok, 2015). It is listed as a threatened species, e.g. in Germany, Czech Republic, Slovenia, Slovakia and Ukraine (Dostalova and Király, 2013).

This perennial species of dry grasslands is flowering in early spring. Its attractive flowers are pollinated by insects.

Its flowers open in late February or early March. The leaves develop at the end of flowering. After flowering the peduncle elongates bearing the fruit over the layer of other plants which helps the dispersion of seeds by wind (Kaligarić et al., 2006; Sauberer and Panrok, 2015).

Flowers are mainly cross-pollinated but in the lack of allogamy self-pollination is also possible (Walker and Pinches, 2011; Zimmermann, 1935; Lindell, 1998).

Study area

The pollinators of *P. grandis* were studied on the calcareous rocky steppes of the Csatár Hill (N47.107830, E17.848507) (Figs. 1, 2 and 3), near Veszprém in Southern Bakony (Hungary). The summit of this dolomite hill is 340 metres above sea level. This population of *P. grandis* contains more thousand individuals. The botanical values of the area are threatened by the expansion of weekend houses.

Measuring pollinators

The pollinators were studied in the spring of 2017 and 2018. The methods were different in the two years. On the 25 March 2017 we observed three fully-opened flowers of an individual and one fully-opened flower of another one. On the 28th March 2017 three fully-opened flowers of an individual were studied (no other flower visitation was recorded). The flower-visitations were recorded through 18 h from between 9 a.m. and 5 p.m. on both days. The weather was sunny and windless on both days.

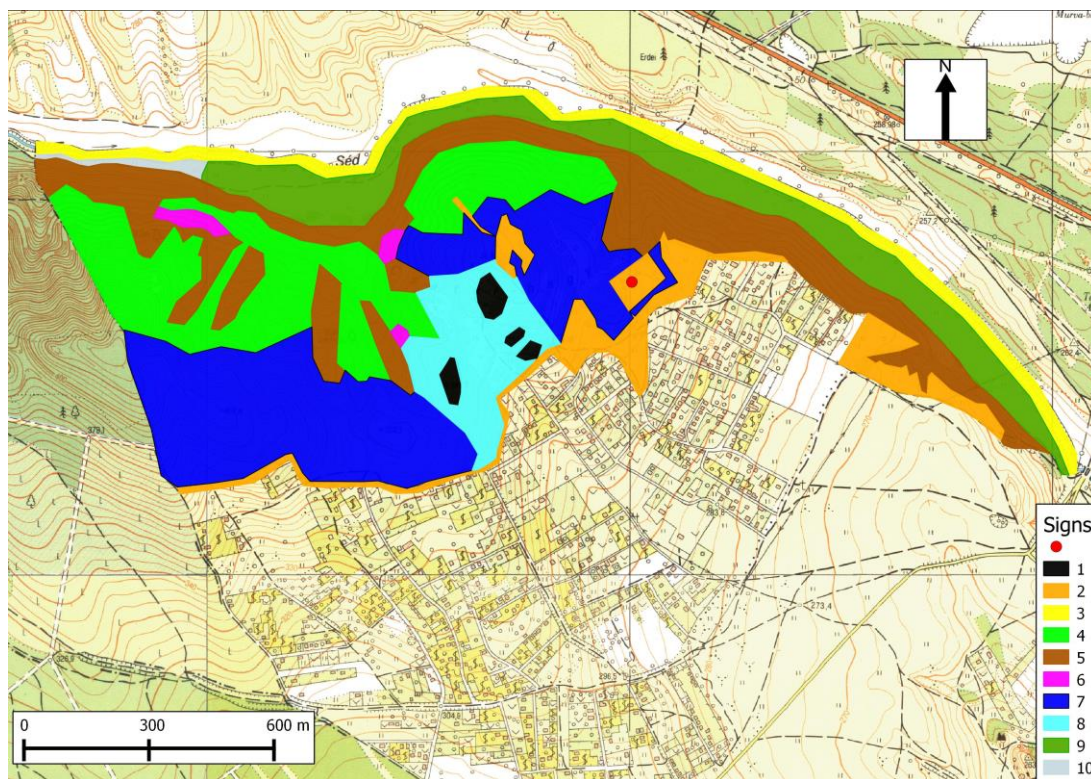


Figure 1. Vegetation map of the Csatár Hill (vegetation types were classified according to Borhidi (2003)). Legend: red point: experimental site, 1: *Seselio leucospermi-Festucetum pallentis* Zólyomi (1936) 1958, 2: *Chrysopogono-Caricetum humilis* Zólyomi (1950) 1958, 3: *Aegopodio-Alnetum* V. Kárpáti, I. Kárpáti & Jurko 1961, 4: *Mercuriali-Tilietum* Zólyomi & Jakucs in Zólyomi 1958, 5: *Daphno laureolae-Fagetum* (Isépy 1970) Borhidi in Borhidi & Kevey 1996, 6: *Fago ornetum* Zólyomi (1950) 1958, 7: *Vicio sparsiflorae-Quercetum pubescentis* Zólyomi ex Borhidi et Kevey 1996, 8: *Cotino-Quercetum pubescentis* Soó (1931) 1932, 9: *Arum et ager incultum*, 10: *Urtico-Convolutetum* Görs & T. Müll. 1969



Figure 2. *Experimental site. (Photo: T. Mészáros, date: 11.03.2017)*



Figure 3. *Experimental site. (Photo: T. Mészáros, date: 10.05.2018)*

Photos were taken about the flower visiting insects to identify them and record the time of visitation. With this method it was possible for pollinators to visit the flowers more times. However this method was not effective enough. Only a little ratio of visitations was recorded and identified. At the same time only these results show that some pollinators visit the same plant individuals repeatedly. Those individuals that were not identified reliably are not included in the results of this study. In 2018 we studied a 3800 square metres area. The number of *P. grandis* individuals was about 2600. The pollinators were collected in glasses every hour. An insect collecting nest (30 cm in diameter) was used to collect the insects individually and then put them in the glasses. We studied the area through 20 h on 2-10 April (1-3 people collected simultaneously). The length of observation (25 h) and sampling (20 h) were different as there were periods when no pollinators visited the plants (*Table 2*). The study days were sunny and windless, excepting 4 April which was a sunny but very windy day. The individuals were identified with a binocular microscope by Zs. Józán. The collected individuals were identified according to following literature: Andrenidae species: Schmid-Egger and Scheuckl (1977), *Apis mellifera* and *Bombus* species: Móczár (1957), Halictidae species: Móczár (1967) and Ebmer (1970), *Osmia* species: Móczár (1958), Vespidae species: Móczár (1995), Pompilidae species: Wolf (1972) and for all species: Schmiedeknecht (1930).

Table 2. Flower visitations of *Pulsatilla grandis* (2018)

Date	Length of observation (h)	Length of sampling (h)
2 April	3 p.m.-6 p.m.	3 p.m.-6 p.m.
3 April	9 a.m.-4 p.m.	10 a.m.-4 p.m.
4 April	9 a.m.-4 p.m.	10 a.m.-3 p.m.
8 April	9 a.m.-4 p.m.	10 a.m.-3 p.m.
10 April	11 a.m.-12 a.m.	11 a.m.-12 a.m.
Total:	25	20

a.m. = before noon, p.m. = after noon

Besides the cited publications distribution data of species are based on the unpublished studies of Zs. Józán who studied and collected pollinators through 5 decades in the Bakony Hills, Western and Southern Transdanubia. Flower-visitation data was collected by the author and Jenő Papp. The specimens of collected insects are preserved in the Rippl-Rónai Museum (Kaposvár, Hungary) and in the Natural History Museum of the Bakony Hills (Zirc, Hungary).

Results

In 2017 we recorded 31 flower-visitations of *Aculeata* (6 species) species (*Table 3*). *Apis mellifera* was the most frequent (80% of all visitations) pollinator of *P. grandis*. The *Bombus* genus was the second most frequent with 12%. The flower-visitations of *Andrena* and *Lasioglossum* species were not significant, both representing 3%.

We recorded the most visitations between 10-11 a.m., followed by the period between 11-12 a.m. *Apis* species visited the flowers between 9 a.m and 3 p.m., the *Bombus* species between 10 a.m. and 3 p.m., the *Andrena* species between 11-12 a.m.,

while the *Lasioglossum* species between 12-1 p.m. There was no visitation between 1-2 p.m.

Table 3. Aculeata pollinators of *Pulsatilla grandis* in decreasing frequency (2017)

Species	Sex	Number of flower-visitations (during 18 h)	Time of flower visitation
<i>Apis mellifera</i> Linnaeus, 1758	Worker	25	9 a.m.-1 p.m., 2-3 p.m.
<i>Bombus lapidarius</i> (Linnaeus, 1758)	♀	2	11-12 a.m., 2-3 p.m.
<i>Bombus pascuorum</i> (Scopoli, 1763)	♀	1	10-11 a.m.
<i>Andrena bicolor</i> (Fabricius, 1775)	♀	1	11-12 a.m.
<i>Lasioglossum fulvicorne</i> (Kirby, 1802)	♀	1	12-1 p.m.
<i>Bombus haematurus</i> (Kriechbaumer, 1870)	♀	1	12-1 p.m.
Total:		31	

♀ = female, a.m. = before noon, p.m. = after noon

In 2018 23 species (299 individuals) were collected (Table 4). *Apis mellifera* was the most frequent (80% of all visitations) pollinator of *P. grandis*. It was followed by the *Bombus* genus with 10%. The ratio of *Andrena* species was only 5% (Table 5).

Table 4. Aculeata pollinators of *Pulsatilla grandis* in decreasing frequency (2018)

Species	Number of individuals	Sex	Time of flower visitation
<i>Apis mellifera</i> (Linnaeus, 1758)	239	Worker	10 a.m.-6 p.m.
<i>Bombus lapidarius</i> (Linnaeus, 1758)	8	♀	11 a.m.-1 p.m., 2-3 p.m.
<i>Andrena bicolor</i> (Fabricius, 1775)	6	5 ♀ + 1 ♂	10-11 a.m., 12-2 p.m.
<i>Bombus pratorum</i> (Linnaeus, 1761)	6	♀	12-5 p.m.
<i>Bombus haematurus</i> (Kriechbaumer, 1870)	5	♀	10-12 a.m., 3-4 p.m., 5-6 p.m.
<i>Bombus hypnorum</i> (Linnaeus, 1758)	5	♀	10-11 a.m., 12-2 p.m.
<i>Osmia cornuta</i> (Latreille, 1805)	5	4 ♀ + 1 ♂	12-2 p.m.
<i>Bombus terrestris</i> (Linnaeus, 1758)	4	♀	10 a.m.-2 p.m.
<i>Andrena gravida</i> (Imhoff, 1832)	3	2 ♀ + 1 ♂	12-2 p.m., 3-4 p.m.
<i>Bombus pascuorum</i> (Scopoli, 1763)	2	♀	11-12 a.m., 1-2 p.m.
<i>Lasioglossum laterale</i> (Brullé, 1832)	2	♀	11 a.m.-1 p.m.
<i>Osmia bicolor</i> (Schrank, 1781)	2	♀	12-12 p.m.
<i>Polistes nimpha</i> (Christ, 1791)	2	♀	12-1 p.m.
<i>Andrena bimaculata</i> (Kirby, 1802)	1	♂	2-3 p.m.
<i>Andrena bluethgeni</i> (Stöckert, 1930)	1	♀	11-12 a.m.
<i>Andrena dorsata</i> (Kirby, 1802)	1	♀	11-12 a.m.
<i>Andrena jacobii</i> (Perkins, 1921)	1	♂	4-5 p.m.
<i>Andrena nitida</i> (Müller, 1776)	1	♀	12-1 p.m.
<i>Andrena vaga</i> (Panzer, 1799)	1	♀	1-2 p.m.
<i>Bombus ruderarius</i> (Müller, 1776)	1	♀	12-1 p.m.
<i>Lasioglossum bluethgeni</i> (Ebmer, 1971)	1	♀	2-3 p.m.
<i>Lasioglossum xanthopus</i> (Kirby, 1802)	1	♀	11-12 a.m.
<i>Prionemimimula</i> (Wesmael, 1851)	1	♀	2-3 p.m.
Total:	299		10 a.m -6 p.m.

♀ = female, ♂ = male, a.m. = before noon, p.m. = after noon

Table 5. Aculeata pollinators (genus) of *Pulsatilla grandis* in decreasing frequency (2018)

Genus	Number of individuals	Ratio of all flower visits (%)
<i>Apis</i>	239	79.9
<i>Bombus</i>	31	10.4
<i>Andrena</i>	15	5.0
<i>Osmia</i>	7	2.3
<i>Lasioglossum</i>	4	1.3
<i>Polistes</i>	2	0.7
<i>Priocnemis</i>	1	0.3
Total:	299	100

We observed the most flower-visitation between 10-11 a.m., followed by the period between 12-1 p.m. (Table 6). *Apis* and *Bombus* species visited the flowers between 10 a.m. and 6 p.m., while *Andrena* species between 10 a.m. and 5 p.m.

Table 6. Temporal distribution of *Pulsatilla grandis* pollinators (2018, daylight saving time)

Time	Number/hour	Ratio (%)
10-11 a.m.	66	22.0
11-12 a.m.	55	18.4
12-1 p.m.	60	20.1
1-2 p.m.	40	13.4
2-3 p.m.	25	8.4
3-4 p.m.	16	5.3
4-5 p.m.	26	8.7
5-6 p.m.	11	3.7
Total:	299	100

a.m. = before noon, p.m. = after noon

We collected the most number of insects (34 individuals) on 8 April between 10-11 a.m., while we collected the fewest (1 individual) on 10 April between 10-11 a.m. There was no visitation before 10 a.m. We recorded visitations between 4-6 p.m. only on the first few days. Later no visitation was observed after 4 p.m.

We found 5 males besides the females and workers (*Andrena bicolor*, *A. gravida*, *A. bimaculata*, *A. jacobii*, *Osmia cornuta*).

Three quarters of bee-like species collected from *P. grandis* have a wide range (Palearctic, Western Palearctic and Eurosiberian). One quarter of the species occurs in certain regions of Europe, and only 15% lives in the Mediterranean with a range extending to Central Europe.

The ratio of species preferring warm habitats (eremophil) was only 20%. The species of cool and wet habitats represented 35%. Most of the species (45%) are eurytopic.

The species are polilectic, except *Andrena vaga* which is an oligolectic species. According to the studies of Zs. Józán in the Bakony Hills, Western and Southern Transdanubia 45% of species visited less than 25 plant species, 30% visited 26-50 species, 10% visited 51-75 species, while 15% visited more than 76 plant species for pollen or nectar.

Collected species

Apidae

Apis mellifera Linnaeus, 1758

It was the most dominant pollinator species. Our only social bee species that has perennial colonies. It is a eurytopic species (Móczár, 1957), its subspecies can be found worldwide (Móczár, 1957). The workers visit practically the flowers of every plant species that produce pollen or nectar (unpublished data of the author). In early spring they visit the catkins of *Salix* species (*Salix caprea*, *Salix cinerea*) which produce a high amount of pollen. *P. grandis* seems to be a good source of pollen and nectar as well. The pollen baskets of specimens collected from its flowers were full of pollen. After winter the workers feed the first larvae generation with this protein rich forage. From the end of March they start to visit the flowers of fruit trees, so the visitation of other plants become less significant. It has economic and ecological significance (Rand et al., 2015).

Bombus haematurus Kriechbaumer, 1870

This bumblebee species has Ponto-Mediterranean range (Móczár, 1957). Its number has been increasing rapidly in Hungary in the last decades. The species was first observed in Hungary in 1990 in the Mecsek Hills. Later about 40 occurrences have been recorded in the Bakony Hills, Transdanubian Hills, Mecsek Hills, Villányi Hills, furthermore in Vas County and near Bősárkány (North Hungary) (Józán, 1995, 1996b, 1998, 2000a, 2003, 2006, 2009, 2016, 2017). Sárospataki et al. (2004) classified the species as rare and highly threatened. However, the studies of recent years show it is not so rare. It is also supported by the fact that the species was observed together with other common species on the Csatár Hill. Visited plant species: *Rubus idaeus*, *R. fruticosus*, *Geum urbanum* (Rosaceae), *Vicia grandiflora* (Fabaceae), *Galeobdolon luteum*, *Lamium purpureum*, *L. maculatum* (Labiatae), *Symphytum officinale* (Boraginaceae), *Corydalis cava*, *Lonicera* sp., *Philadelphus coronarius* (other plant families) (Józán, 2009, 2017).

Bombus hypnorum (Linnaeus, 1758)

B. hypnorum is a Eurosiberian bumblebee which prefers habitats with cool and humid microclimate (Móczár, 1953, 1957). It is moderately rare in Hungary (Móczár, 1953; Sárospataki et al., 2004). The species has been observed in 30 localities in Hungary from Bősárkány through the Bakony Hills, the Transdanubian Hills, the Mecsek Hills until the Villányi Hills (Józán, 1990, 1992a, 1995, 1996a, 1998, 2000a, 2000b, 2001, 2006, 2016, 2017). Visited plant species: *Rubus idaeus*, *Rosa* sp. (Rosaceae), *Lamium maculatum* (Labiatae), *Calendula officinalis*, *Cirsium arvense* (Asteraceae), *Anchusa officinalis*, *Echium vulgare* (Boraginaceae), and *Berberis vulgare*, *Epilobium montanum*, *Knautia drymeia*, *Lycium barbarum*, *Salix cinerea*, *Symphoricarpos albus*, *Philadelphus coronarius* (other plant families) (Józán, 2017).

Bombus lapidarius (Linnaeus, 1758)

It is a common eurytopic species living in the Western-Palaearctic range (Móczár, 1953, 1957; Sárospataki et al., 2004). It is common everywhere in Hungary. It has been found in lots of localities during our studies (Józán, 1985, 1992a, 1992b, 1995, 1996a, 1996b, 1998, 2000a, 2000b, 2001, 2002, 2003, 2006, 2009, 2016, 2017). Its pollination

was recorded on 50 plant species of 23 plant families. Most of the species belong to the Fabaceae, Labiatae and Asteraceae families (Józán, 2009, 2016, 2017).

Bombus pascuorum (Scopoli, 1763)

It is a eurytopic Eurosiberian bumblebee species (Móczár, 1953, 1957; Sárospataki et al., 2004). *B. pascuorum* is the most common bumblebee species in Hungary according to our studies (Józán, 1990, 1992a, 1992b, 1995, 1996a, 1998, 2000a, 2000b, 2001, 2006, 2009, 2014, 2016, 2017). It has been collected from 70 plant species of 22 plant families (Józán, 2009, 2016, 2017).

Bombus pratorum (Linnaeus, 1761)

B. pratorum lives in habitats with cool and humid microclimate. Its range is of Eurosiberian type (Móczár, 1953, 1957). *B. pratorum* was classified as moderately rare in Hungary (Sárospataki et al., 2004). During our faunistic studies it has been collected from 40 localities in Transdanubia (Józán, 1990, 1992a, 1996a, 2000b, 2001, 2002, 2006, 2017). Visited plant species: *Rubus idaeus* (Rosaceae), *Astragalus onobrychis*, *Trifolium pratense* (Fabaceae), *Aegopodium podagraria*, *Anthriscus sylvestris* (Apiaceae), *Ajuga reptans*, *Galeobdolon luteum*, *Marrubium peregrinum*, *Salvia nemorosa* (Labiatae), *Centaurea jacea*, *C. micranthos*, *Taraxacum officinale* (Asteraceae), *Stellaria* sp. (Caryophyllaceae), *Echium vulgare*, *Pulmonaria officinalis*, *Symphytum officinale*, *S. tuberosum* (Boraginaceae), other plant families: *Allium ursinum*, *Berberis vulgaris*, *Cerintho minor*, *Geranium* sp., *Knautia arvensis*, *K. drymeia*, *Melampyrum nemorosum*, *Ribes rubrum*, *Solanum nigrum*, *Symphoricarpos albus* (Józán, 2017).

Bombus ruderalis (Müller, 1776)

It is a eurytopic bumble bee species with a Western Palearctic range (Móczár, 1957). It is moderately common in Hungary (Móczár, 1953; Sárospataki et al., 2004). We have found this species in many different localities during our faunistic studies (Józán, 1990, 1992a, 1992b, 1995, 1996a, 1996b, 1998, 2000a, 2000b, 2001, 2002, 2006, 2009, 2016, 2017). It has been collected from more than 40 species of 14 plant families (Józán, 2009, 2017).

Bombus terrestris (Linnaeus, 1758)

It is a eurytopic bumble bee species that occurs in the Palearctic region (Móczár, 1957). It is very common in every part of Hungary (Józán, 2001; Móczár, 1953; Sárospataki et al., 2004). It has been collected from 110 localities (Józán, 1985, 1990, 1992a, 1992b, 1995, 1996a, 1996b, 1998, 2000a, 2000b, 2001, 2002, 2003, 2006, 2009, 2014, 2015, 2016, 2017). Its pollination has been observed on nearly 100 species of 35 plant families. Most significant families were the Fabaceae, Labiatae and Asteraceae (Józán, 2009, 2016, 2017).

Andrenidae

Andrena bicolor Fabricius, 1775

This Eastern Palearctic mining bee species prefers cool and humid habitats (Dylewska, 1987; Móczár, 1957). *A. bicolor* is moderately common in Hungary (Móczár and Warncke, 1972). It has two generations per year, the first being on the wing in spring and the second in summer (Schmid-Egger and Scheuchl, 1977). It has

been found in nearly 100 localities during our faunistic studies (Józán, 1990, 1992a, 1992b, 1996a, 1996b, 1998, 2000a, 2000b, 2001, 2002, 2003, 2006, 2009, 2014, 2016, 2017). It has been collected from 35 species of 15 plant families. The summer generation visits mainly the flowers of *Campanula* species (Schmid-Egger and Scheuchl, 1977; Józán, 2009, 2016, 2017).

Andrena bimaculata (Kirby, 1802)

A. bimaculata is an eurytopic mining bee species that is widespread in the Palearctic region. It has two generations per year (Dylewska, 1987; Schmid-Egger and Scheuchl, 1977). It is common in Hungary (Móczár and Warncke, 1972; Józán, 2011). It has been collected from more than 130 localities (Józán, 1985, 1990, 1992a, 1992b, 1995, 1996a, 1996b, 1998, 2000a, 2000b, 2001, 2003, 2009, 2014, 2016, 2017). It has been observed on 36 species of 13 plant families. The spring generation swarms mainly on the catkins of *Salix* species (Schmid-Egger and Scheuchl, 1977; Józán, 2009, 2016, 2017).

Andrena bluethgeni Stöckhert, 1930

The range of this species is not well-known. It has been found in Central and Northern Europe (Schmid-Egger and Scheuchl, 1977). It is very rare in Hungary. In the last century it was collected only from 4 localities near Budapest and from one locality in the North Hungarian Mountains (Móczár and Warncke, 1972). In the last decades we have observed it in six localities of the Balaton Uplands, near Esztergom, in the Zákány Hills and in Külső-Somogy (Józán, 1992a, 2000b, 2016). Visited plant species: *Amygdalus communis*, *Cerasus vulgare*, *Prunus cerasifera*, *P. spinosa* (Rosaceae), *Capsella bursa-pastoris* (Cruciferae), *Acer platanoides* (Aceraceae) (Józán, 2016).

Andrena dorsata (Kirby, 1802)

A. dorsata is a eurytopic Western Palearctic mining bee species with two generations (Dylewska, 1987). It is common in Hungary. It has been collected from more than 200 localities during our faunistic studies (Józán, 1985, 1990, 1992a, 1992b, 1995, 1996a, 1996b, 1998, 2000a, 2000b, 2002, 2003, 2006, 2009, 2014, 2016, 2017). It has been noted on nearly 100 species of 27 plant families. Most of them belong to the Rosaceae, Cruciferae and Asteraceae families (Józán, 2009, 2016, 2017).

Andrena gravida Imhoff, 1832

A. gravida is a widespread mining bee species in Europe (Dylewska, 1987). Its only generation flies from early spring until May. It is common in Hungary (Móczár and Warncke, 1972). It has been found in nearly 150 localities (Józán, 1990, 1992a, 1992b, 1995, 1996a, 1996b, 1998, 2000a, 2000b, 2001, 2002, 2003, 2006, 2009, 2016, 2017). It has been collected from more than 50 species of 25 plant families. Most of them are members of the Rosaceae and Cruciferae families (Józán, 2009, 2016, 2017).

Andrena jacobi Perkins, 1921

It is a Western Palearctic species with one generation swarming in spring (Dylewska, 1987). It is moderately common in Hungary (Móczár and Warncke, 1972). We have found it in 84 localities during our studies (Józán, 1990, 1992a, 1992b, 1995, 1996a, 1996b, 1998, 2000a, 2000b, 2001, 2017) It has been collected from 24 species of 12 plant families. It has mainly visited the species of Rosaceae family (Józán, 2017).

Andrena nitida (Müller, 1776)

A. nitida has the same range as the former species (Dylewska, 1987). Its only generation flies in the spring months. It is quite common in Hungary (Móczár and Warncke, 1972). It has been found in nearly 130 localities (Józán, 1990, 1992a, 1992b, 1995, 1996a, 1996b, 1998, 2000b, 2001, 2002, 2003, 2009, 2015, 2016, 2017). It has visited more than 50 species of 25 plant families. Most of them are members of Rosaceae and Cruciferae families (Józán, 2016, 2017).

Andrena vaga Panzer, 1799

A. vaga is a mining bee species that lives in Southern Europe (Dylewska, 1987). It flies in early spring. It is not rare in Hungary but not abundant (Móczár and Warncke, 1972). It has been collected from 60 localities in the last decades (Józán, 1985, 1990, 1992a, 1995, 1996a, 1996b, 1998, 2000b, 2001, 2003, 2006, 2015, 2016, 2017). Visited plant species: *Prunus spinosa*, *Sorbus intermedia* (Rosaceae), *Lamium purpureum* (Labiatae), *Salix cinerea*, *S. rosmarinifolia* (Salicaceae) (Józán, 2016, 2017).

Halictidae

Lasioglossum bluethgeni Ebmer, 1971

L. bluethgeni is a Pontomediterranean sweat bee species (Ebmer, 1988; Móczár, 1967). It has two generations. The spring generation contains only of females. It has been observed in many locations in Hungary, but it is not common. It has been collected from 35 Transdanubian localities in the last decades (Józán, 1992b, 1995, 1996a, 1998, 2000b, 2006, 2009, 2015, 2016, 2017). It has visited the flowers of the following species: *Anthriscus cerefolium*, *A. sylvestris*, *Daucus carota*, *Pastinaca sativa* (Apiaceae), *Capsella bursa-pastoris*, *Rorippa amphibia*, *Lepidium draba* (Cruciferae), *Hieratium* sp. (Asteraceae), *Echium vulgare*, *Lithospermum purpureo-coeruleum* (Boraginaceae), other families: *Cotinus coggygia*, *Dictamnus albus*, *Scabiosa ochroleuca* (Józán, 2009).

Lasioglossum fulvicorne Kirby, 1802

It is a eurytopic species with a Palearctic range (Ebmer, 1988; Móczár, 1967). It is rare in Hungary. It has been collected only from Nagybjom (Józán, 1992a), Tótújfalu (Józán, 1995), Kővágószőlős (Józán, 1996a) and Zics (Józán, 2000b). Visited plant species: *Anthemis austriaca*, *Berteroa incana*, *Capsella bursa-pastoris*, *Dorycnium germanicum*, *Ficaria verna*, *Orlaya grandiflora*.

Lasioglossum laterale (Brullé, 1832)

This sweat bee species occurs in the Northern part of the Mediterranean (Ebmer, 1988; Móczár, 1967). It has two generations. The spring generation contains only of females. It is moderately common in Hungary (Móczár, 1967). It has been collected from more than 70 localities in the Bakony Hills and in the southern part of Transdanubia (Józán, 1990, 1992a, 1995, 1996a, 1996b, 1998, 2000a, 2000b, 2003, 2006, 2009, 2017). It has been collected from 20 species of 10 plant families.

Lasioglossum xanthopus (Kirby, 1802)

L. xanthopus is a Western Palearctic species (Ebmer, 1988; Móczár, 1967). It also has two generations. The spring generation contains only of females. It is moderately common in Hungary (Móczár, 1967). We have found it in less localities than the former

species (Józán, 1992a, 1995, 1998, 2000a, 2000b, 2003, 2016, 2017). According to our observations it does not occur abundantly in any type of habitat. It has been recorded on 24 species of 13 plant families. The distribution of species was quite equal between families (Józán, 2016, 2017).

Megachilidae

Osmia bicolor (Schrank, 1781)

It is a widespread species in Europe. It swarms in spring and nests in snail shells (Móczár, 1958). It is moderately common in Hungary (Móczár, 1958). It has been collected from 75 Transdanubian localities (Józán, 1985, 1990, 1992a, 1992b, 1995, 1996a, 1996b, 1998, 2000b, 2003, 2006, 2009, 2015, 2016). It has been recorded on 22 species of 15 plant families. Most of them are member of the Rosaceae family (Józán, 2009, 2014, 2016, 2017).

Osmia cornuta (Latreille, 1805)

It is a widespread species ranging from the northern part of the Mediterranean to Central Europe. It is common in Hungary (Móczár, 1958). It nests in hollow stems or other holes. The cells are separated with mud (Móczár, 1958). The males are often on wings in early March foraging on *Salix* catkins (author's observation). It rarely can be seen in May. It was collected from 16 species of 14 plant families. Most of them are member of the Rosaceae family (Józán, 2009, 2016, 2017).

Pompilidae

Priocnemis mimula Wesmael, 1851

It is a Palearctic pompilid species. It is distributed mainly in the hilly and mountainous regions of Hungary. It is common but not abundant (Móczár, 1956). It is a parasitoid species. It visits the flowers for nectar (Móczár, 1956). It has a limited role in pollination due to the lack of body hairs.

Vespidae

Polistes nimpha (Christ, 1791)

It is a Palearctic Vespidae species. It is very common in Hungary. First it feeds the larvae with nectar and other sweet saps. Later the larvae feed on chopped insects (Móczár, 1995). Its role in pollination is similar to the former species.

Discussion

Apis mellifera was the main pollinator of *P. grandis* in both years of our study. It was followed by *Bombus* species. The high number of *Apis mellifera* can be partly explained by the fact that an apiary can be found 300 meters from the population of *P. grandis*. The fruit trees of the Csatár Hill were not blossoming at the time of early *P. grandis* flowering, so the studied flowers were the only pollen sources for *Apis mellifera*. The significant role of honey bees in successful fructification was observed in the case of later flowering orchid species as well (Biró et al., 2015).

Apis and *Bombus* species had the longest collecting period in both years, which can be explained with the fact that both genus contains eurytopic species.

In 2018 early spring was cold, so a large number of insects visited the flowers of *Pulsatilla* on 4 April even in windy weather.

In 2018 the mornings were quite cool so the flowers were not visited before 10 a.m. In the first days of our study the insects collected pollen until 6 p.m. but later their activity decreased in late afternoon which is the consequence of the long winter as well.

Males visit the flowers only for nectar. They do not have special collecting hairs, so the probability of carrying pollen from a flower to another is lower. In the lack of allogamy *P. grandis* can reproduce with self-pollination as well (Walker and Pinches, 2011; Zimmermann, 1935; Lindell, 1998). As a consequence, male insects can help the pollination of this species too. They can carry the pollen of the same plant to the stigma as they searching for nectar in the flower.

It is normal that the ratio of eremophil bee species was low (20%) in the early spring aspect.

In comparison with the observations of Kratochwil (1988) on *P. vulgaris* we found mainly social bee species (*Apis mellifera*, *Bombus*), while *Andrena* and *Osmia* species were the most frequent among solitary species. *Andrena bicolor* was the most frequent species of the genus in our study as well. We did not collect *Lasioglossum lineare*, but we found *L. fulvicorne*, *L. laterale* and *L. xanthopus*. Two non-Apoidea Hymenoptera species was collected: *Priocnemis mimula* and *Polistes nimpha*.

Our results support the observations of Weryszko-Chmielewska and Sulborska (2011) on *Pulsatilla* species as Bee-like species were the main pollinators of *P. grandis*.

The studies of Sauberer and Panrok (2015) on *Pulsatilla* species showed different results from our observations on *P. grandis*. We collected only 1-2 individuals of 4 Halictidae species (*L. fulvicorne*, *L. laterale* and *L. xanthopus*) and the number of individuals of *Apis* and *Bombus* species was significantly higher than the number of individuals of the *Andrena* genus.

Our results meet the observations of Zimmermann (1935), Walker (2011) and Walker and Pinches (2011): *Apis mellifera* and *Bombus* species were the most frequent pollinators. However, in our case *B. lapidarius* was the most frequent species of the genus instead of *Bombus terrestris* (Zimmermann, 1935).

Compared to the results of Wells and Barling (1971) we collected only 1-2 *Bombus pascuorum* and *Osmia bicolor*.

Pulsatilla species produce pollen in early spring when the activity of Aculeata species is starting as well. They attract the insects with a huge amount of pollen and some nectar.

Pulsatilla species have many strategies to ensure the success of allogamy, especially in the case of early flowering species.

As the seed dispersal distances are short the gene flow ensured by pollination plays an important role in the maintenance of the genetic variability of populations. Therefore, the significance of pollinators is high (Hensen et al., 2005).

In our former studies we found significant differences between the number of fruits in the different *P. grandis* populations in the southern part of Bakony Hills (Mészáros T. in prep.) which can indicate the important role of pollinators as well. According to our studies a large number of pollinators have visited the flowers of *P. grandis*. The success of fructification can depend not only on the number of pollinators but on habitat parameters or weather conditions as well. As a consequence, further studies are needed to determine the role of the studied insects in the success of fructification.

Conclusion

No special plant-pollinator relation was found between the studied species and its pollinators. This result is not surprising as only 1-2% of bee species are specialists. Most of the bee species feed on more plant species and most of the plant species are visited by many insects.

The number of bees has decreased considerably in the last decades which can be caused by the changes in landscape structure and the retreat of visited plant species, so the pollination crisis is becoming more obvious (Potts et al., 2010; Biesmeijer et al., 2007). Besides the retreat of food plants the increasing presence of dangerous chemicals reduces the number of pollinators as well (Dainese et al., 2018).

The early flowering of *P. grandis* is very important for bees appearing in early spring as these flowers are the first food sources for them after the pupa stage. In this period of the year only a few plant species produces pollen. After the flowering of *P. grandis* its pollinators (which have longer life cycle than the flowering period of *P. grandis*) visit other plant species occurring in increasing number, so the bees can choose from more food sources. We can speak about mutual dependency as pollinators are necessary for successful fertilization (and with that for the maintenance of genetic diversity of populations), and the flowers *P. grandis* are food sources for early pollinators. Either the decrease of pollinators or early flowering plant populations influences the relation negatively.

With our study we filled the gap of missing information about the pollinators of *P. grandis* and would like to draw attention to the key role of early flowering plant species in the maintenance of bee diversity. In the lack of these species even the non-specialist pollinators can not maintain their populations. Therefore it is important to conserve and maintain the populations of early flowering plant species.

Acknowledgements. We would like to thank Mrs. Józán, Dénes Péteri and Gábor Barad for their help in insect collection. We would like to thank Judit Bódis and István Galambos for their comments on the manuscript. The publication is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

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