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Short Communication

FLOWER CONSTANCY OF BUMBLEBEES – THE CASE OF *ONOBRYCHIS PINDICOLA* (FABACEAE) POLLINATORS

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Abstract

Pollination in high mountain habitats is an important ecosystem service in climate change conditions. The aim of this study was to use pollen load analysis to assess flower constancy and foraging choices of bumblebees foraging on *Onobrychis pindicola,* a high-mountain endemic plant. The flower constancy to the foraging source *O. pindicola* was very high - over half of the bumblebees had pure Onobrychis-type pollen loads. In the mixed pollen loads we found one to seven pollen types other than Onobrychis-type and the functional flower morphology was different from the flag type. Some were gullet while others were dish/bowl functional morphology type. Thus the theory/belief that once discovering the flag blossom as a foraging resource bumblebees tended to visit other plants with such functional morphology was rejected. An abundance of plants did not determine food choice. We could not trace an obvious pattern of the bumblebees' preference to functional blossom morphology but they were attracted to dish-bowl blossoms.

Keywords: blossom functional morphology, flower constancy, pollen loads

INTRODUCTION

Onobrychis pindicola subsp. *urumovii* Degen & Dren. (Fabaceae) is an endemic plant with very restricted distribution in just the Pirin and Slavjanka Mountains on the Balkan Peninsula in south-west Bulgaria (Velchev, 1992; Euro+Med PlantBase, 2011). This *calciphilous* perennial plant is closely related to the common *O. montana* DC (Kozuharov, 1976). This taxon is abundant in its restricted range and according to IUCN criteria categorized as least concern, (Petrova & Vladimirov, 2008) but an element in several Natura 2000 habitats (Roussakova, 2015). We previously studied the breeding systems of *O. pindicola* subsp. *urumovii* growing on Pirin marbles, and the plants were found to be self-incompatible and dependent upon insect vectors for pollen transport (Kozuharova, 1999; Kozuharova & Richards, 2016). The aim of this study was to use pollen load analysis to assess flower constancy and foraging choices of bumblebees foraging on *Onobrychis pindicola.*

MATERIAL AND METHODS

Study sites and field observations

The field observations were conducted in the marbleized karst regions of the North Pirin Mts. Seven study sites of 40 m^2 were chosen. Study sites were grouped in the following Natura 2000 habitats: 6170 - subalpine calcareous grasslands (sites 10, 11, 15), alpine calcareous grasslands (sites 3, 4); 4070 - dominated by *Pinus mugo* (site 20)*;* 95A0 - woods of *Pinus heldreichii* (site 12). Altitudes ranged between 1850 - 2410 m a. s. l. (Fig. 1). The investigations were conducted during the summers of 1995, 1996, 2001 and 2015. Pollinating bumblebees were identified in the field after Edwards and Jenner (2005) and Gogala (2015) and their behaviour was recorded. The plant species flowering in the vicinity of *O. pindicola* were ranked according to their approximate abundance (Figs. 2-4) on a scale of 1 (solitariae) to 6 (sociales). These plants were evaluated in accordance with their functional flower morphology (after Faegri & van der Pijl, 1971). Dish/bowl „blossom" is characterized by radial symmetry and free access to the nectar

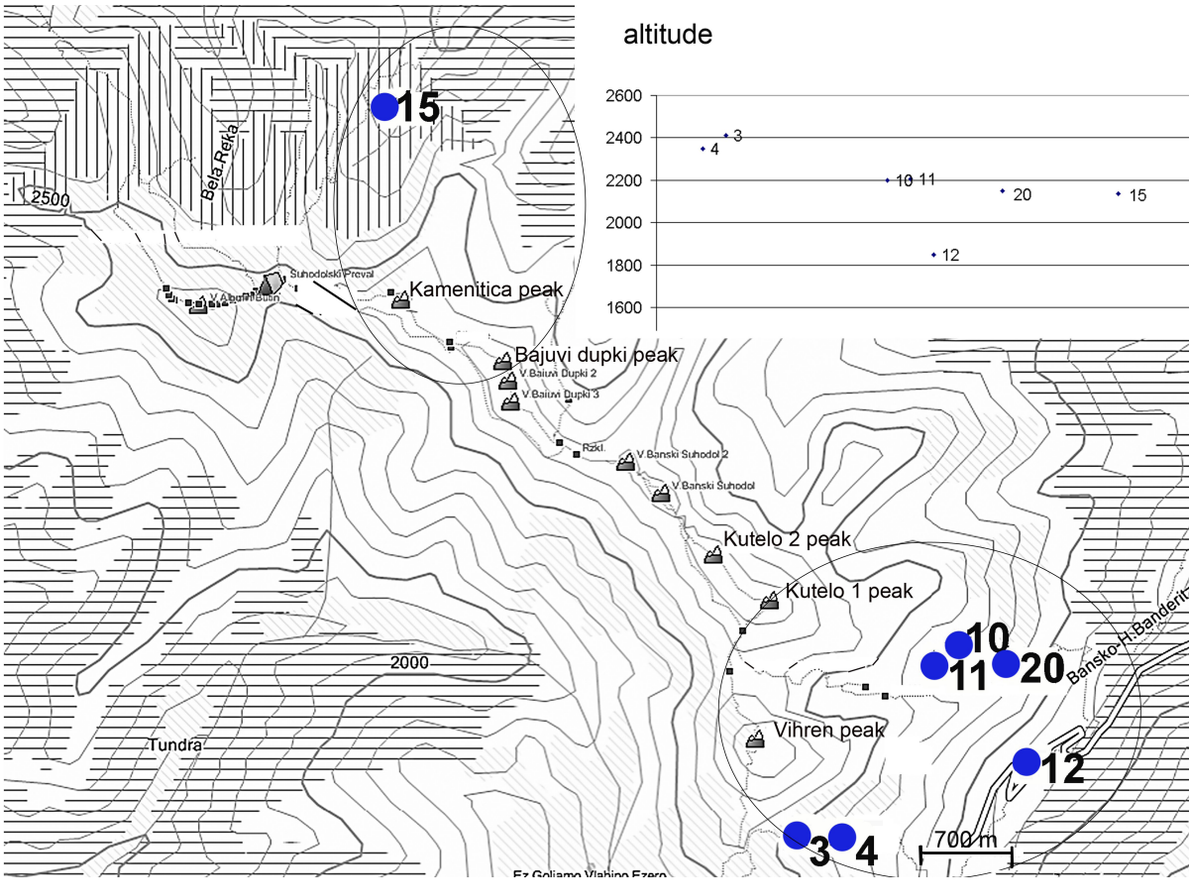

Fig. 1 Study sites

and pollen. Blossoms with bilateral symmetry are flag (sexual organs in the lower part, pollen is deposited on the abdominal side of the insect) and gullet (sexual organs restricted to the functionally upper side, pollen is deposited on the dorsal side of the insect).

Analysis of the pollen loads

Pollen loads (N=71) were collected individually in jelly capsules from bumblebees foraging on the flowers of *Onobrychis pindicola.* The pollen loads from both legs of each bumblebee were homogenized and analyzed. Pollen were identified after Faegri et al., 1989) and counted (at least 1000 pollen grains) under an Amplival Carl Zeiss Jena light microscope.

Data analysis

The composition of pollen loads was evaluated in percentage. Descriptive statistic was used to analyze the data.

RESULTS

Analysis of the pollen loads

Onobrychis pindicola was mostly pollinated by *Bombus pyrenaeus* but it was also by *B. wurflenii /lapidarius, B. lucorum/terrestris* and *B. pratorum*. Field observations revealed high flower constancy; once the bumblebees visited *O. pindicola,* they tended to follow its flowers, rarely switching to other plants such as *Scutellaria alpina, Thymus* sp. or *Hypericum tetrapterum* on the same foraging trip. This high flower constancy observed in the field was confirmed through the pollen analysis. Consequently *Onobrychis pindicola* became their "major" (term invented by Heinrich 1979) source and they kept to it (Figs. 2-4).

In the area of the Vihren peak (study sites 12, 20, 10, 11, 3 and 4, Figs. 2 and 3) the *Onobrychis*-type pollen was presented in all pollen loads of bumble bee workers. The content of it was on average 94.7%. Pure *Onobrychis*-type pollen loads were 52%. Less than 50% *Onobrychis*-type pollen contained only 5% of the pollen loads. *B. pyrenaeus* workers were the most active visitors with on average 94.1% of *Onobrychis*-type pollen in their loads (Fig. 2) and 50% of these loads were pure 100% *Onobrychis*-type

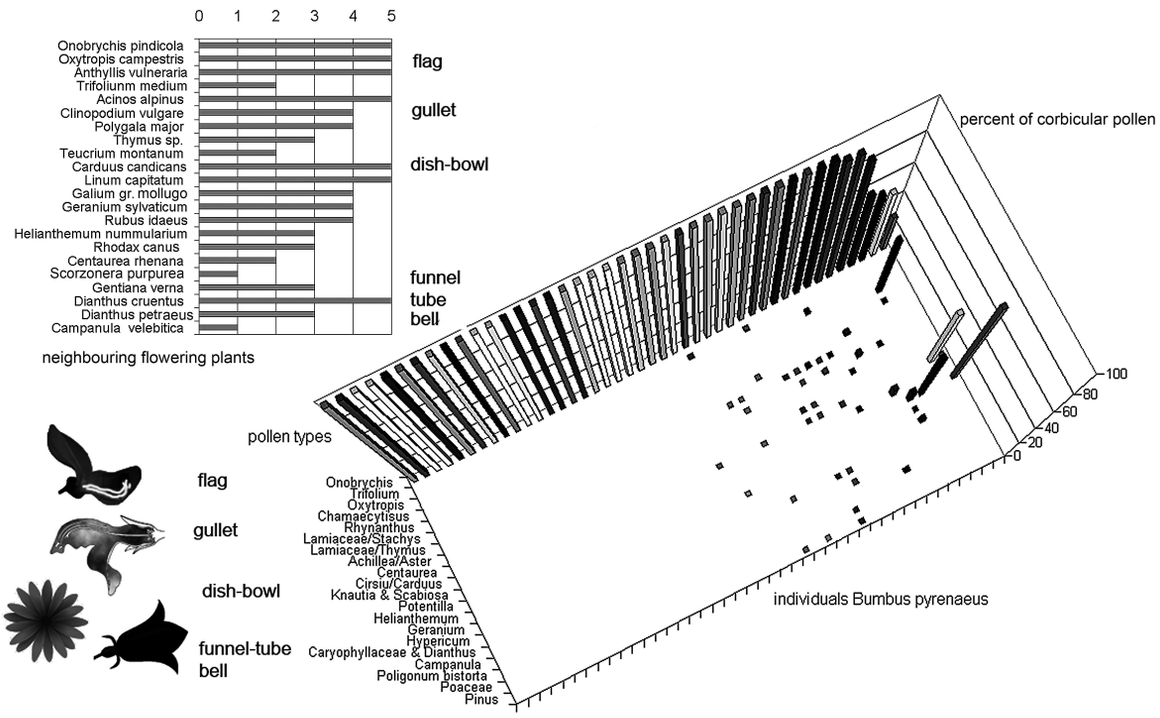

Fig. 2. Percent of corbicular pollen in the pollen loads of 43 individuals *Bombus pyrenaeus* pollinators of *Onobrychis pindicola* in the area of Vihren peak (study sites 12, 20, 10, 11, 3 and 4).

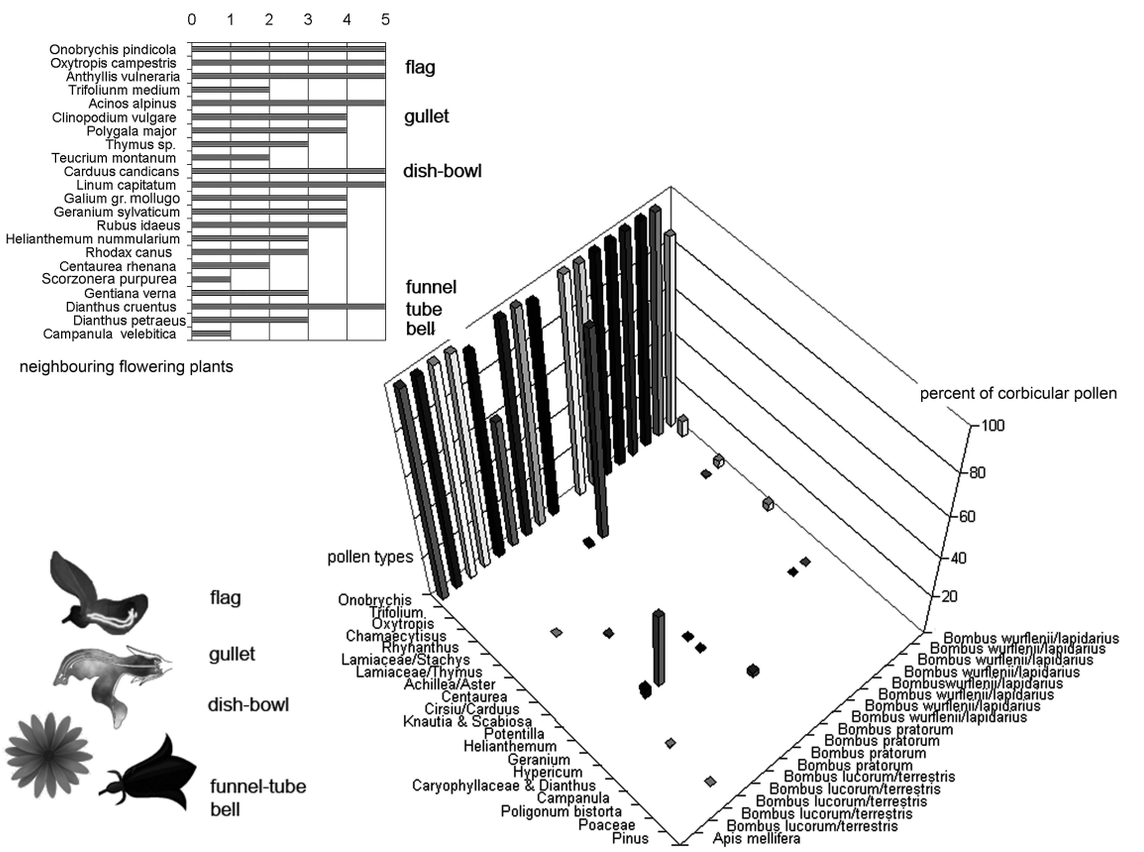

Fig. 3. Percent of corbicular pollen in the pollen loads of 5 individuals *Bombus lucorum/terrestis,* 4 individuals *B. pratorum,* 8 individuals *B. wurflenii/lapidarius,* and one individual *Apis mellifera* pollinators of *Onobrychis pindicola* in the area of Vihren peak (study sites 12, 20, 10, 11, 3 and 4).

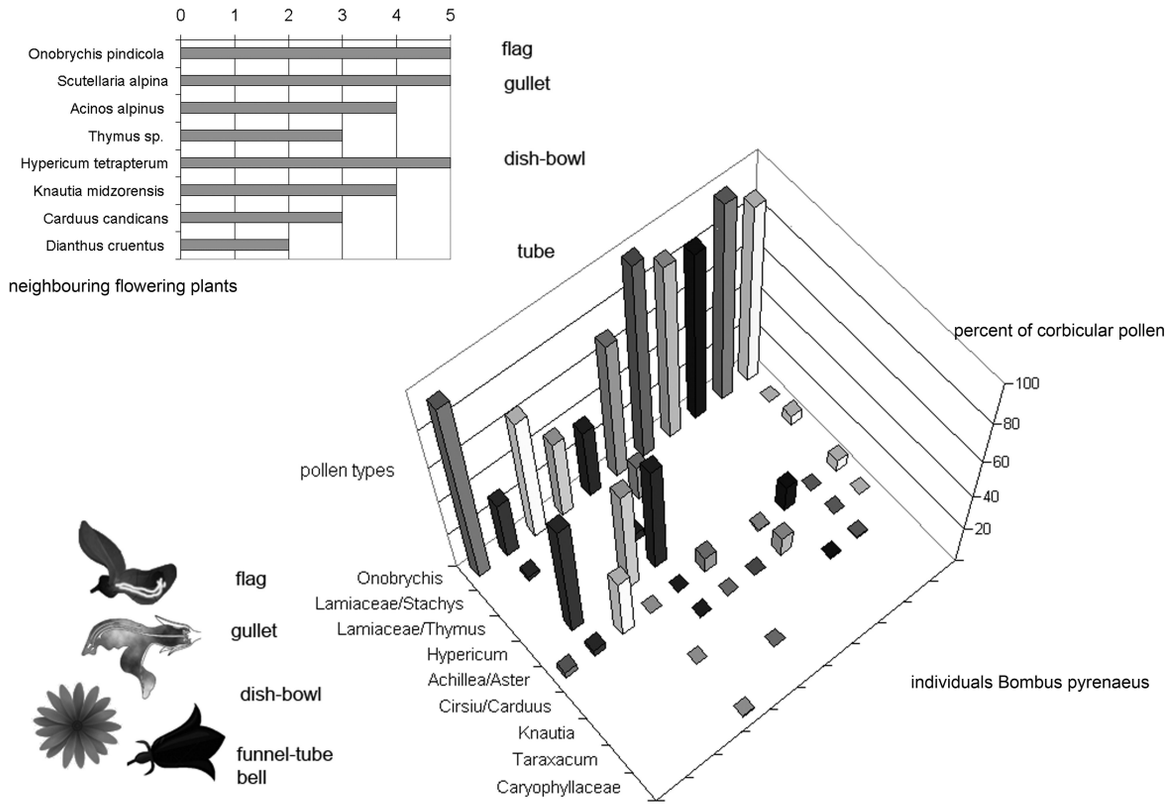

Fig. 4. Percent of corbicular pollen in the pollen loads of 11 individuals Bombus pyrenaeus pollinators of Onobrychis pindicola in the area of Kamenititza peak (study site 15).

pollen. The loads occasionally contained as much pollen of other plants as the *Onobrychis*-type pollen (for instance *Chamaecitysus*-type, Fig. 2). *Onobrychis*-type pollen was less than 50% in only one of the loads (Fig. 2), which was dominated by pollen from *Scabiosa* sp., an uncommon plant. *B. wurflenii /lapidarius* were not the most active visitors of the flowers of *O. pindicola* and preferred *Oxytropis campestris.* However the pollen loads collected from pollinators of sainfoin flowers contained on average 98.2% *Onobrychis*-type pollen and 62% of the pollen loads consisted of pure *Onobrychis*-type pollen (Fig. 3). *B. lucorum/terrestris* workers also demonstrated high flower constancy with on average 74.4% *Onobrychis*-type pollen in their pollen loads (Fig. 3). The same was for *B. pyerenaeus* workers with an average of 98.2% *Onobrychis*-type pollen in their loads (Fig. 3).

In the area of the Kamenititza peak (study sites study site 15, Fig. 4) the pollen loads of bumblebee workers on average consisted of 73.3% *Onobrychis*-type pollen, but none of them of pure, *Onobrychis*-type pollen. In this situation there was high *Hypericum*-type pollen content (Fig. 4). In comparison the pollen loads of *Bombus lapidarius* and *B. pratorum* workers collected from *Stachys alpina* flowers contained predominantly *Hypericum*-type pollen and some grains of *Stachys*-type pollen. This reveals that the bees alternated between both species and used *Hypericum tetrapterum* as a pollen source and *Stachys alpina* as a nectar source. In a few cases the bumble bees behaviour observed in the field was not well reflected in the pollen loads. For instance two individuals, collected in the flowers of *Thymus* sp., had pollen loads dominated by *Onobrychis*-type pollen, while *Thumus*-type pollen was absent.

We could not trace obvious pattern of particular preferences to functional blossom morphology based on the pollen analysis. The dish-bowl blossoms were rather attractive to the bumblebees as pollen from various plants with such a morphology was found in the pollen loads (Fig. 5).

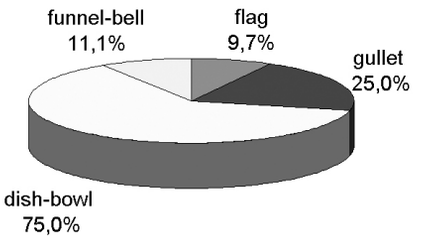

Fig. 5. Occurrence of pollen of foraging plants classified in accordance to their functional blossom morphology detected in the pollen loads of 71 bumblebees.

DISCUSSION

Pollination in high mountain habitats is a particularly important ecosystem service of in climate change conditions. We used *Onobrychis pindicola,* a high mountain endemic plant, as a test object to trace some behavioral specifics of bumblebees. The flower constancy to their foraging source was very high as more than half of the bumblebees had pure *Onobrychis*-type pollen loads. Both our field observations and pollen analysis revealed that bumblebees tended to split and share plant resources, which reduced competition in the mountain habitats of Pirin Mt. marble ridge. We found in the mixed pollen loads from one to seven pollen types (presented usually with just a few pollen grains) other than *Onobrychis*-type. The functional flower morphology of these other foraging plants, either gullet or dish/bowl, differed from the flag type (Figs. 2-4). Bumblebees reduced competition by using many different strategies (Goulson & Sparrow, 2009). This is an adaptation to the efficient pollination of more than one plant species by not blocking the stigma with hetero specific pollen (Thomson, 1982; Galen & Gregory, 1989; Jakobsson et al., 2009). Bumblebee species with tongues of similar length tend to have a higher dietary niche overlap (Goulson et al., 2008) and vice versa (Teper, 2005). Those that foraged primarily on Fabaceae also tended to have long tongues (Goulson et al., 2008). *O. pindicola* received the pollination service mainly from the short tongued bumble bees *B. pyrenaeus*. Abundance of plants was not determining food choice factor because in the pollen loads often were presented pollen types of sporadic plant species (Figs. 2-4).

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