

# CONTRASTING POLLINATION EFFICIENCY AND EFFECTIVENESS AMONG FLOWER VISITORS OF *MALVA SYLVESTRIS*, *BORAGO OFFICINALIS* AND *ONOBRYCHIS VICIIFOLIA*

Anna Gorenflo\*<sup>1</sup>, Tim Diekötter<sup>2</sup>, Mark van Kleunen<sup>3,4</sup>, Volkmar Wolters<sup>5</sup>, Frank Jauker<sup>5</sup>

<sup>1</sup>Applied Entomology, Department of Insect Biotechnology, Justus Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany

<sup>2</sup>Department of Landscape Ecology, Institute for Natural Resource Conservation, Kiel University, Olshausenstrasse 75, 24118 Kiel, Germany

<sup>3</sup>Ecology, Department of Biology, University of Konstanz, Universitätsstrasse 10, 78457 Konstanz, Germany

<sup>4</sup>Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou 318000, China

<sup>5</sup>Department of Animal Ecology, Justus Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany

**Abstract**—Biotic pollination is an important factor for ecosystem functioning and provides a substantial ecosystem service to human food security. Not all flower visitors are pollinators, however, and pollinators differ in their pollination performances. In this study, we determined the efficiencies of flower visitors to the plant species *Malva sylvestris*, *Borago officinalis* and *Onobrychis viciifolia* by analysing stigmatic pollen deposition. We further calculated pollinator effectiveness by scaling up single-visit pollen deposition using visitation frequency. Flower-visitor groups differed in their efficiencies at the single-visit level and not all of them deposited more pollen compared to unvisited stigmas. Bumblebees tended to be most efficient in depositing pollen per single visit across the three plant species. Due to the by far highest visitation frequencies, *Apis mellifera* showed the highest effectiveness in depositing pollen per hour for *M. sylvestris* and *B. officinalis*, but not for *O. viciifolia*, for which the *Bombus lapidarius* complex was both the most frequent and the most effective pollinator group. Hence, the most frequent flower visitors were most effective in our study. For non-dominant pollinator groups, however, visitation frequencies contributed disproportionately to pollinator effectiveness. Thus, combining pollen deposition per single-visit with visitation frequency is necessary to reveal true pollinator performance and to better understand flower-visitor interactions.

**Keywords:** Apoidae, pollen analysis, pollination service, pollinator importance, solitary bees

## INTRODUCTION

Pollen transfer is an essential process for seed and fruit production in sexually reproducing plants and around 90% of all angiosperm species are animal pollinated (Ollerton et al. 2011). Biotic pollination is therefore an important factor for wild plant reproduction (Ollerton et al. 2011) and an important ecosystem service to human food security (Gallai et al. 2009; Calderone et al. 2012). Despite the importance of biotic pollination, many obscurities about plant-pollinator interactions still need to be solved (Mayer et al. 2011). For example, the terms “flower visitor” and “pollinator” are often used synonymously, without actual proof of pollen transfer (Ne’eman et al. 2010). In addition, performances as pollinators have been shown to differ among flower visitors (Ne’eman et al. 2010; Jędrzejewska-Szmek & Zych 2013; Popic et al. 2013; Ballantyne et al. 2015). This general gap in knowledge complicates finding appropriate pollinators for agricultural systems (Slaa et al. 2006), interpreting specialisation and generalisation (Jędrzejewska-Szmek &

Zych 2013; Popic et al. 2013; Ballantyne et al. 2015) and predicting ecological and economic consequences of pollinator loss or invasions (Goulson 2003).

Insects, and above all bees, are considered the most important pollinators (Buchmann & Nabhan 1996). Pollen is transferred passively while insects utilise flowers for foraging, as shelter or mating site, among other reasons (Inouye et al. 1994). Not all flower visitors, however, are involved in pollination. Reasons are mismatches in morphology or behaviour or illegitimate exploitations of floral resources (i.e. nectar and pollen thieves or robbers) (Inouye 1980). Therefore, pollination services of a given flower visitor may differ among plant species and pollination success for a given plant species differs among pollinators (Fenster et al. 2004; King et al. 2013; Popic et al. 2013; Ballantyne et al. 2015).

Both indirect (e.g. visit duration, visitor frequency, pollen removal, stigma receptivity) and direct measurements (e.g. stigmatic pollen deposition, seed set) have been used to analyse pollination performances of flower visitors (summarised by Ne’eman et al. 2010), but previous research suggests that direct measurements are more reliable (Johnson & Steiner 2000; Adler & Irwin 2006; King et al. 2013;

Received 24 November 2016, accepted 04 May 2017

\*Corresponding author: annagorenflo@googlemail.com

Ballantyne et al. 2015). Therefore, to analyse pollination performances, distinguish flower visitors from true pollinators and determine the strength of plant-visitor interactions, it seems crucial to investigate the transfer of pollen by flower visitors more closely for multiple plant species.

In this study, we analysed the performance of flower visitors to the three plant species *Malva sylvestris*, *Borago officinalis* and *Onobrychis viciifolia* directly by determining stigmatic pollen deposition. The three plant species are visited by a variety of insect species. Yet, it is not known, whether all flower visitors are pollinators and whether pollinators differ in their pollination performances when direct measurements are used. We first compared pollen loads on unvisited and visited stigmas to evaluate pollination performance of abiotic factors versus flower visitors overall. Then, pollination performances of flower visitors to *M. sylvestris*, *B. officinalis* and *O. viciifolia* were analysed in a hierarchical approach: (1) A flower-visitor group was identified as efficient pollinator when more pollen grains were deposited on stigmas per single visit compared to pollen loads on unvisited stigmas. (2) Pollinators were ranked regarding their pollination efficiencies by comparing single-visit pollen depositions. (3) Pollinators were ranked regarding their pollination effectiveness by comparing their pollen depositions per hour, including visitation frequencies (Fumero-Cabán & Meléndez-Ackerman 2007; Madjidian et al. 2008; Rader et al. 2009).

## MATERIALS AND METHODS

### Study site and plant species

The study was conducted at the research farm “Oberer Hardthof” of the University of Giessen, Germany, which is surrounded by farmland and a small area of woodland. Four flower mixtures (“Lebensraum1”, “Odin1”, “Odin2”, and “Veitshöchheimer Bienenweide”) were sown on former arable land on 28 May 2013 in a block design, consisting of a row of eight blocks with four plots (4 m × 4 m) per block. Within each block, plots of the four flower mixtures were randomised and separated from each other by 3.5 m-wide strips of grassland. For determining pollination performances of flower visitors, we chose the three plant species *M. sylvestris*, *B. officinalis* and *O. viciifolia*, which were present in all (*M. sylvestris* and *O. viciifolia*) or in three (*B. officinalis*) of the four flower mixtures sown at the study site. Differences in pollinator performance between seed mixtures were not tested, because plant-community composition data did not allow for meaningful hypotheses.

*Malva sylvestris*, *B. officinalis* and *O. viciifolia* are frequently applied in flower mixtures, sown as flower strips in agricultural landscapes, and are cultivated as crops or for pharmaceutical purposes (Janick et al. 1989; Gasparetto et al. 2012; Hayot Carbonero et al. 2011). *Malva sylvestris* is native to Germany and the other two species are considered to be neophytes. The common mallow *M. sylvestris* (Malvaceae) generates actinomorphic, dish-shaped and upturned pinkish-purple flowers with at least 11 ovules per flower (Kumar et al. 2014). The central style divides into

numerous filamentous stigmas and is surrounded by several anthers at its base (Kumar et al. 2014; Appendix I). Flowers of borage *B. officinalis* (Boraginaceae) are actinomorphic, dish-shaped and downward directed and change from pink to blue as they age. There are four ovules per flower (De Haro-Bailón & Del Rio 1998). The style ends in a terminal stigma and is surrounded by cone-like anthers at its base (Ghorbel & Nabli 1998; Appendix I). The common sainfoin *O. viciifolia* (Fabaceae) produces inflorescences with up to 120 white to pink, papilionaceous flowers (Goplen et al. 1991) with a single ovule per flower (Galloni et al. 2007). The stigma and the anthers are enclosed within the two fused and boat-shaped keel petals (Appendix I).

The three plant species produce pollen and large amounts of nectar. Nectaries are located at the base of the corollas and are indicated by prominent nectar guides in *M. sylvestris* and *O. viciifolia* (Westrich 1989). The plant species are considered self-compatible, but flowers show features to avoid self-pollination or self-fertilisation, i.e. spatial and temporal separation of mature stigmas and anthers (*M. sylvestris*, *B. officinalis*) (Montaner et al. 2000; Kumar et al. 2014) or presence of a stigmatic cuticle (*O. viciifolia*) (Galloni et al. 2007). Given these floral traits and observational data on flower visitors, the three plant species are assumed to rely mainly on insects such as bees (*M. sylvestris*, *B. officinalis*, *O. viciifolia*), hoverflies and butterflies (*M. sylvestris*) for pollen transfer between flowers of the same (geitonogamy) or other conspecific plants (allogamy) (Richards & Edwards 1988; Corbet et al. 1991; Goplen et al. 1991; Comba et al. 1999; Kumar et al. 2014).

### Pollination efficiencies of flower visitors

We analysed the pollination efficiencies of flower visitors to *M. sylvestris*, *B. officinalis* and *O. viciifolia* directly by determining stigmatic pollen deposition per single visit. Sampling took place between 7:30 a.m. and 6:30 p.m. on warm and sunny days with low wind speeds from 3 June to 4 July 2014 (on 19 days for *M. sylvestris*, 15 days for *B. officinalis* and 13 days for *O. viciifolia*). Stigmas were sampled from all 32 plots (*M. sylvestris*, 1055 stigmas), from 16 plots representing all flower mixtures (*O. viciifolia*, 411 stigmas) and from 11 plots representing two flower mixtures (*B. officinalis*, 252 stigmas). Flowers of the three plant species were bagged before blooming (perforated polypropylene bags, holes 2 mm in diameter) and uncovered after flowers had fully opened and stigmas had turned receptive. We photographed the first flower visitor from an appropriate distance to prevent disturbances and removed the stigmas after the first visitor had left. Only flower-visitor groups with at least eight visited stigmas were further analysed. At the same time, receptive stigmas from bagged flowers were sampled as controls before any visit took place to account for pollen transfer due to self- or wind-pollination, animals smaller than 2 mm, which could enter the bags, or the experimental handling of the flowers. Sampled stigmas were stored in individual wells of plastic 96 cell-culture arrays in a freezer (-20 °C) until pollen analysis.

For counting attached pollen grains, visited and unvisited stigmas were unfrozen and mounted in glycerin-jelly slides stained with basic fuchsin following the protocol of Kearns

& Inouye (1993) to increase the contrast (Appendix I). Morphologically conspecific pollen grains attached to stigmas were counted by light microscopy (between  $\times 40$  and  $\times 400$ ). We identified the photographed flower visitors and grouped them according to size, coloration and/or taxonomy. Flower-visitor groups were considered to be efficient pollinators when pollen loads on visited stigmas exceeded those on unvisited stigmas. Pollinator efficiencies (i.e. mean numbers of pollen grains deposited per single visit) were calculated by subtracting the mean numbers of pollen grains found on unvisited stigmas, serving as controls, from mean numbers of pollen grains on stigmas visited by the flower-visitor groups for each plant species.

#### Visitation frequencies and pollinator effectiveness

For estimating pollinator effectiveness in terms of pollen deposition per hour, visitation frequencies of flower visitors to the three plant species were recorded during separate observations. Observations took place between 7:30 a.m. and 6:30 p.m. from 17 June to 6 July 2014 (on six days for *M. sylvestris*, 10 days for *B. officinalis* and eight days for *O. viciifolia*). *Malva sylvestris* was observed in 20 plots (representing all flower mixtures), *B. officinalis* was observed in seven plots (representing two flower mixtures) and *O. viciifolia* was observed in three plots (representing three flower mixtures). Per plot, six flowers of *M. sylvestris* and *B. officinalis* and one to four inflorescences of *O. viciifolia* (around 25 flowers in total) were observed for 15-minute periods (*M. sylvestris*) or 20-minute periods (*B. officinalis* and *O. viciifolia*) over the day. In total, receptive and unbagged flowers were observed for 201 15-minute periods (50.25 hours, *M. sylvestris*), 168 20-minute periods (56 hours, *B. officinalis*) and 113 20-minute periods (37.67 hours, *O. viciifolia*).

Per-hour visitation frequencies were calculated by dividing the total number of visits recorded for a flower-visitor group by the total number of observational hours and the number of flowers simultaneously observed. Pollinator effectiveness (i.e. mean numbers of pollen grains deposited per hour) was calculated only for flower-visitor groups being efficient pollinators per single visit. For this, the mean number of pollen grains deposited per single visit by each pollinator group was multiplied with its corresponding visitation frequency.

TABLE 1. The total numbers of visited and unvisited stigmas sampled from *Malva sylvestris*, *Borago officinalis* and *Onobrychis viciifolia* and the mean numbers of pollen grains on stigmas ( $\pm$  SE).

| Plant species         | Visitation type | No. of stigmas | Mean no. of pollen grains |
|-----------------------|-----------------|----------------|---------------------------|
| <i>M. sylvestris</i>  | Unvisited       | 304            | 57.4 $\pm$ 3.5            |
|                       | Visited         | 751            | 246.3 $\pm$ 8.1****       |
| <i>B. officinalis</i> | Unvisited       | 79             | 6.1 $\pm$ 0.9             |
|                       | Visited         | 173            | 14.2 $\pm$ 1.6**          |
| <i>O. viciifolia</i>  | Unvisited       | 130            | 0.8 $\pm$ 0.1             |
|                       | Visited         | 281            | 2.7 $\pm$ 0.2***          |

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

P-values were calculated using Wilcoxon rank-sum tests and indicate differences between the mean numbers of pollen grains on visited compared to unvisited stigmas.

#### Statistical analysis

Wilcoxon rank-sum tests were used to test for differences in the numbers of pollen grains on visited and unvisited stigmas for each plant species (function "wilcox.test" in the stats package of R version 3.2.3; R Core Team 2015). To differentiate flower visitors from pollinators and rank flower-visitor groups according to their pollination efficiency, numbers of pollen grains on unvisited stigmas and on stigmas visited by the different flower-visitor groups were compared by Poisson generalized linear models (GLMs) with a correction for overdispersion (i.e. family was set to quasipoisson) for each plant species (function "glm" in the stats package of R version 3.2.3). To provide levels of uncertainty in pollinator effectiveness (a product of the mean pollen deposition and a constant visitation frequency), we calculated standard errors by multiplying the error of pollen deposition efficiencies with the corresponding visitation frequency.

## RESULTS

#### Pollination efficiencies of flower visitors

Visited stigmas had significantly increased pollen grain numbers compared to unvisited stigmas for each plant species, the mean increase ranging from over two-fold (*B. officinalis*) to over three-fold (*O. viciifolia*) and over four-fold (*M. sylvestris*) (Tab. 1). Stigmas sampled from the three plant species were visited mostly by bees, with other insects being less common.

For analysing pollen deposition, enough (eight or more) visited stigmas from *M. sylvestris* were obtained for four flower-visitor groups: *Apis mellifera*, the *Bombus lapidarius* complex (mainly *B. lapidarius*, but possibly also including the similarly coloured species *B. ruderarius*, *B. pratorum*, *B. soroeensis*), the *B. terrestris* complex (mainly *B. terrestris* or *B. lucorum*, but possibly also including the similarly coloured species *B. magnus*, *B. cryptarum*) and Halictidae (including only species of the genera *Halictus* and *Lasioglossum* smaller than 10 mm) (Tab. 2; Appendix II). Pollen deposition for each group of flower visitors was always significantly larger than pollen loads on unvisited stigmas (Tab. 2). Mean

TABLE 2. The numbers of stigmas sampled, mean numbers of pollen grains on stigmas after a single visit ( $\pm$  SE), pollinator efficiencies (i.e. mean numbers of pollen grains deposited per single visit, corrected for pollen loads on unvisited stigmas), visitation frequencies (visits per flower and hour and percentage of total visits in parenthesis) and pollinator effectiveness (i.e. mean numbers of pollen grains deposited per hour) of the analysed flower-visitor groups of *Malva sylvestris*, *Borago officinalis* and *Onobrychis viciifolia*. Please note that pollinator efficiencies and visitation frequencies are rounded, but pollinator effectiveness is calculated from exact values.

| Plant species         | Flower-visitor group         | Stigmas sampled | Pollen per stigma (mean $\pm$ SE) | Pollinator efficiency | Visitation frequency | Pollinator effectiveness |
|-----------------------|------------------------------|-----------------|-----------------------------------|-----------------------|----------------------|--------------------------|
| <i>M. sylvestris</i>  | <i>A. mellifera</i>          | 683             | 227.9 $\pm$ 7.1***                | 170.5                 | 9.0 (88.4)           | 1,535.1 $\pm$ 63.9       |
|                       | <i>B. lapidarius</i> complex | 27              | 625.1 $\pm$ 76.7***               | 567.7                 | 0.6 (5.9)            | 342.7 $\pm$ 46.3         |
|                       | <i>B. terrestris</i> complex | 11              | 669.8 $\pm$ 127.0***              | 612.4                 | 0.2 (1.8)            | 113.8 $\pm$ 23.6         |
|                       | Halictidae                   | 13              | 169.7 $\pm$ 37.2***               | 112.3                 | 0.1 (1.1)            | 7.8 $\pm$ 4.2            |
| <i>B. officinalis</i> | <i>A. mellifera</i>          | 106             | 13.3 $\pm$ 1.7**                  | 7.2                   | 8.4 (87.3)           | 60.3 $\pm$ 14.3          |
|                       | <i>B. terrestris</i> complex | 22              | 19.6 $\pm$ 7.2***                 | 13.4                  | 0.7 (7.3)            | 9.4 $\pm$ 5.0            |
|                       | <i>B. lapidarius</i> complex | 9               | 26.3 $\pm$ 10.4***                | 20.2                  | 0.1 (0.8)            | 1.5 $\pm$ 0.8            |
|                       | <i>B. sylvarum</i>           | 9               | 23.7 $\pm$ 9.6***                 | 17.6                  | 0.1 (0.8)            | 1.3 $\pm$ 0.7            |
|                       | <i>Megachile</i>             | 9               | 8.7 $\pm$ 2.9                     | -                     | 0.1 (1.0)            | -                        |
|                       | Halictidae                   | 8               | 4.6 $\pm$ 1.4                     | -                     | 0.1 (0.7)            | -                        |
| <i>O. viciifolia</i>  | <i>B. lapidarius</i> complex | 187             | 3.0 $\pm$ 0.3***                  | 2.2                   | 1.4 (60.2)           | 3.1 $\pm$ 0.4            |
|                       | <i>B. sylvarum</i>           | 11              | 4.3 $\pm$ 1.4***                  | 3.5                   | 0.2 (8.9)            | 0.7 $\pm$ 0.3            |
|                       | <i>Megachile</i>             | 39              | 2.7 $\pm$ 0.8***                  | 1.9                   | 0.1 (5.4)            | 0.3 $\pm$ 0.1            |
|                       | <i>A. mellifera</i>          | 9               | 2.0 $\pm$ 0.7                     | -                     | 0.3 (11.4)           | -                        |
|                       | Halictidae                   | 21              | 0.9 $\pm$ 0.3                     | -                     | 0.1 (2.2)            | -                        |

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

P-values were calculated using Poisson generalized linear models (GLMs) with a correction for overdispersion (i.e. family was set to quasipoisson) and indicate differences between the mean numbers of pollen grains on stigmas visited by the analysed flower-visitor groups compared to unvisited stigmas.

Standard errors of pollinator effectiveness were calculated by multiplying the error of pollen deposition efficiencies with the corresponding visitation frequency.

pollen deposition ranged from 112.3 to 612.4 pollen grains per single visit after subtracting the mean number of pollen grains found on unvisited stigmas (Tab. 2, Fig. 1A). Both bumblebee groups showed significantly higher pollination efficiencies than *A. mellifera* and Halictidae (GLM: all  $P < 0.001$ ).

For *B. officinalis*, enough visited stigmas were obtained for six flower-visitor groups: *A. mellifera*, *B. lapidarius* complex, *B. sylvarum*, *B. terrestris* complex, *Megachile* and Halictidae (Tab. 2; Appendix II). Pollen deposition was always significantly larger than pollen loads on unvisited stigmas, except for bees of the genus *Megachile* and the family Halictidae (Tab. 2). Mean pollen deposition ranged from 7.2 to 20.2 pollen grains per visit after subtracting the mean number of pollen grains found on unvisited stigmas (Tab. 2, Fig. 1B). Pollination efficiency of the *B. lapidarius* complex was significantly higher than the efficiency of *A. mellifera* (GLM:  $P < 0.05$ ).

For *O. viciifolia*, enough visited stigmas were obtained for five flower-visitor groups: *A. mellifera*, *B. lapidarius* complex, *B. sylvarum*, *Megachile* and Halictidae (Tab. 2; Appendix II). Pollen deposition was always significantly

larger than pollen loads on unvisited stigmas, except for *A. mellifera* and Halictidae (Tab. 2). Mean pollen deposition ranged from 1.9 to 3.5 pollen grains per visit after subtracting the mean number of pollen grains found on unvisited stigmas of *O. viciifolia*, (Tab. 2, Fig. 1C). There was no significant difference in pollination efficiency between the *B. lapidarius* complex, *B. sylvarum* and *Megachile*.

#### Visitation frequencies and pollinator effectiveness

Virtually all insects visiting the three plant species during visitation surveys were honeybees, bumblebees or solitary bees (*M. sylvestris*: 97.6%, *B. officinalis*: 99.0%, *O. viciifolia*: 97.9%; Appendix III).

The four flower-visitor groups of *M. sylvestris* analysed for pollination efficiency were the most frequent flower visitors, with *A. mellifera* being by far the most frequent visitor overall (Tab. 2). Accordingly, *A. mellifera* turned out to be the most effective flower-visitor group per time unit (1,535.1 pollen grains/hour compared to the *B. lapidarius* complex: 342.7 grains/hour, the *B. terrestris* complex: 113.8

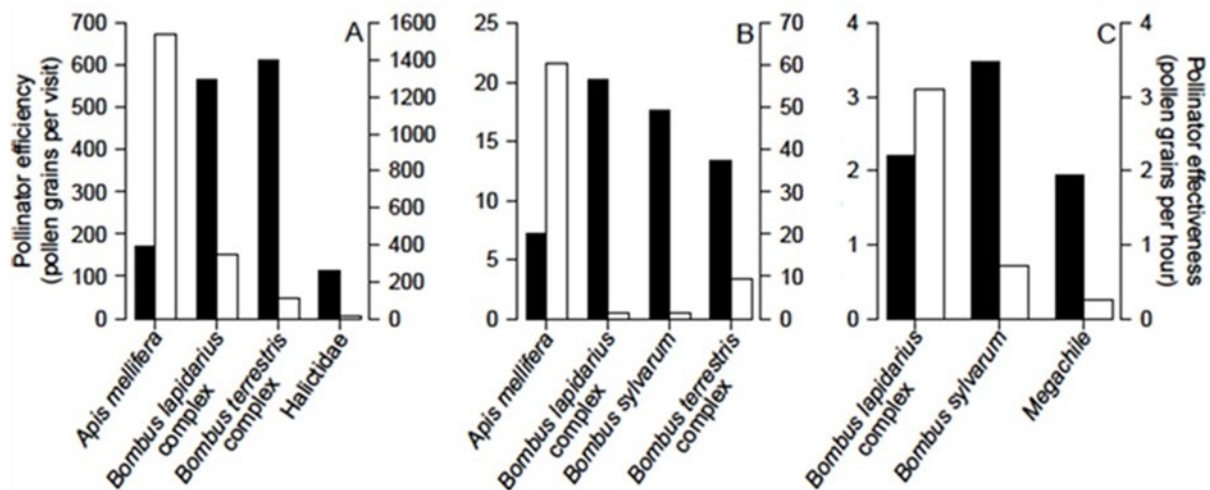


FIGURE 1. Pollinator efficiencies (black bars, i.e. mean numbers of pollen grains deposited per single visit) and pollinator effectiveness (white bars, i.e. mean numbers of pollen grains deposited per hour) of the flower-visitor groups exceeding mean number of pollen grains on unvisited stigmas of (A) *Malva sylvestris*, (B) *Borago officinalis* and (C) *Onobrychis viciifolia*.

grains/hour and Halictidae: 7.8 pollen grains/hour; Tab. 2, Fig. 1A).

The six flower-visitor groups of *B. officinalis* analysed for pollination efficiency were among the seven most frequent flower visitors, again *A. mellifera* being the most frequent visitor (Tab. 2). *Apis mellifera* was also again the most effective flower-visitor group per time unit (60.3 pollen grains/hour compared to the *B. terrestris* complex: 9.4 pollen grains/hour, the *B. lapidarius* complex: 1.5 pollen grains/hour and the *B. sylvarum*: 1.3 pollen grains/hour; Tab. 2, Fig. 1B).

The five flower-visitor groups of *O. viciifolia* analysed for pollination efficiency were also the most frequent flower visitors. The *B. lapidarius* complex was the most frequent flower-visitor group (Tab. 2) and was also most effective per time unit (3.1 pollen grains/hour compared to *B. sylvarum*: 0.7 pollen grains/hour and *Megachile*: 0.3 pollen grains/hour; Tab. 2, Fig. 1C).

## DISCUSSION

### Pollination efficiencies of flower visitors

Overall, flower visitors deposited more pollen grains than wind or other vectors, supporting the pollinator dependencies of *M. sylvestris*, *B. officinalis* and *O. viciifolia* described in the literature (Richards & Edwards 1988; Corbet et al. 1991; Goplen et al. 1991; Comba et al. 1999; Kumar et al. 2014). We were able to identify four efficient pollinators of *M. sylvestris* (*A. mellifera*, *B. lapidarius* complex, *B. terrestris* complex and Halictidae), four of *B. officinalis* (*A. mellifera*, *B. lapidarius* complex, *B. terrestris* complex and *B. sylvarum*) and three of *O. viciifolia* (*B. lapidarius* complex, *B. sylvarum* and *Megachile*). Hence, assuming that within-flower autogamous pollination is minimised by floral traits of the three plant species, these bees were visiting other conspecific flowers before, thereby taking up pollen that stayed available for the pollination of subsequent flowers (Inouye et al. 1994; Ne'eman et al.

2010). On the other hand, two of the analysed flower-visitor groups of *B. officinalis* (*Megachile* and Halictidae) and two of *O. viciifolia* (*A. mellifera* and Halictidae) were not efficient pollinators. Thus, not all flower visitors can be classified as efficient pollinators, even when belonging to a group of potential pollinators (i.e. all flower-visitor groups were bees), or being efficient pollinators to other plant species (i.e. honeybees were efficient pollinators of both other plant species, Halictidae and *Megachile* of one other plant species each).

Variation in pollen deposition efficiency occurs due to differences in the degree to which flower and visitor traits match, defining a visitor's ability to take up and deposit pollen grains. Because possible factors determining pollen transfer, such as body size (Willmer & Finlayson 2014), hairiness, tongue length (Hobbs et al. 1961), floral constancy or preference (Waser 1986), pollen-transporting structures and nectar- and pollen-collecting behaviours (Inouye 1980; Thorp 1999; Michener 2007) are highly diverse in bees, generalisation about pollination performances is difficult (Fenster et al. 2004). Nevertheless, generalist bumblebee groups (Goulson & Darvill 2004) were the most efficient (for *M. sylvestris* and *B. officinalis*) or among the most efficient pollinators (for *O. viciifolia*) across the three plant species in our study. This supports that bumblebees can often be more efficient per single visit across plant species of different families than honeybees (Wilson & Thomson 1991; Willmer et al. 1994; Javorek et al. 2002; Ballantyne et al. 2015; Zhang et al. 2015).

Bumblebees are characterised by a large surface due to their large and hairy bodies, which probably increases the probability to touch anthers and stigmas and the amount of pollen transferred. Bumblebees' long tongues (Goulson et al. 2005) make them better pollinators of flowers with deep corollas (Hobbs et al. 1961) and probably induced positioning on the studied flowers favourable for contact with anthers and stigmas. Furthermore, like for other Fabaceae (Córdoba & Cocucci 2011), bumblebees were

strong enough to open flowers of *O. viciifolia* and thus could reach the nectar hidden between the base of the banner and keel petals. By pushing their heads and tongues towards the nectar, keel petals folded down while stigma and anthers were released and touched the bumblebees' ventral side, enabling pollination. In *B. officinalis* dehiscence of anthers is introrse and pollen grains are held within the cone-like anthers (Corbet et al. 1988). This controls pollen removal by flower visitors, since large amounts of pollen are released only in response to sonication (Buchmann 1983; De Luca & Vallejo-Marín 2013). Bumblebees are well known to perform buzz pollination (Buchmann 1983; King & Buchmann 2003). They were observed to sonicate on *B. officinalis* in this study (see also Corbet et al. 1988), which probably increased the amount of pollen released and transferred to subsequent flowers in *B. officinalis*.

Other bees analysed in this study were generally smaller, less hairy and had shorter tongues, probably causing morphological and behavioural mismatches, which restricted pollen transfer compared to bumblebees in most cases. Bees of the family Halictidae seemed of inappropriate size to flowers of all three plant species, not touching stigmas while drinking nectar, but rather occasionally on their way to the nectaries or when foraging on pollen. Honeybees had to crawl underneath stigmas of *M. sylvestris* to reach the nectar or inserted the tongue laterally into flowers of *M. sylvestris* and *O. viciifolia*, reducing the contact with anthers and stigmas (see also Comba et al. 1999). Furthermore, honeybees were observed to intensively groom pollen from their bodies and discard it after visiting flowers of *M. sylvestris*, making the pollen unavailable for transfer (Inouye et al. 1994). In addition, sonication is not performed by bees of the genus *Apis* (Buchmann 1983; King & Buchmann 2003) and only very rarely within the Megachilidae (Neff & Simpson 1988), probably reducing their pollination efficiency on *B. officinalis*. In contrast to the literature (Richards & Edwards 1988; Goplen et al. 1991), *A. mellifera* was not a successful pollinator of *O. viciifolia* in this study, probably because closed flowers of *O. viciifolia* could not be opened easily, as has been shown for other Fabaceae (Córdoba & Cocucci 2011). Bees of the genus *Megachile*, however, have relatively long tongues and were strong enough to trigger the lever mechanism of *O. viciifolia*, as shown for other papilionate flowers (Córdoba & Cocucci 2011), explaining a pollen deposition efficiency similar to the ones of the bumblebee groups.

In summary, *A. mellifera* and Halictidae on *M. sylvestris* and *A. mellifera* on *B. officinalis* were pollinators of the respective plant species, although much less efficient than the bumblebee groups. According to the observed behaviours, the inefficient pollinators can be classified as (pollen or nectar) thieves or base workers instead (*sensu* Inouye 1980), feeding on floral resources without pollinating. All analysed flower-visitor groups of *M. sylvestris* were efficient pollinators, whereas two groups of *B. officinalis* and *O. viciifolia* were inefficient pollinators. This may reflect an increasing specialisation of the floral morphology from *M. sylvestris* to *B. officinalis* to *O. viciifolia*, which hinders pollination by less suitable flower visitors.

The efficient pollinators deposited all at least as many pollen grains on stigmas as ovules present per flower (see Material and Methods). Hence, all efficient pollinators can potentially provide pollination services for maximum seed set of the plant species studied in our system. Differences in foraging behaviour within a plant or within single flowers may still affect conspecific pollen grain quality with regard to viability or the degree of kinship, both affecting germination success (Snow et al. 1996). Similarly, low flower constancy increases transfer of heterospecific pollen (Waser 1986), which could lead to stigma clogging and prevent conspecific pollen to germinate. Thus, including fertilisation, e.g. seed set and germination rates, is an important next step to determine the required quantity and quality of conspecific pollen, and the effect of heterospecific pollen deposited by efficient pollinators (Garibaldi et al. 2014; Zhang et al. 2015).

Variation in the efficiency within flower-visitor groups may be explained by differences between species within groups (King et al. 2013) or between individuals within species (Jauker et al. 2016). This seems especially important for bumblebees, showing a wide intraspecific range of body sizes and tongue lengths (Goulson et al. 2002; Willmer & Finlayson 2014). Since only female bees collect pollen, morphological and behavioural differences between sexes may also lead to intraspecific differences in bees (Ne'eman et al. 2006). Future studies including analyses of pollinator performances at the lowest taxonomic rank possible and even within species will be necessary for further insights into pollination variability.

#### Visitation frequencies and pollinator effectiveness

Most visitors to flowers of *M. sylvestris*, *B. officinalis* and *O. viciifolia* were bees. The flower-visitor groups analysed for pollen deposition were among the most frequent visitors. *Apis mellifera* dominated the number of flower visits to *M. sylvestris* and *B. officinalis*, whereas bumblebees of the *B. lapidarius* complex were the most frequent visitors to flowers of *O. viciifolia*. Differences in dominant visitors between plant species suggest differing flower preferences and high flower constancy of *A. mellifera* (Hill et al. 1997) and bumblebees of the *B. lapidarius* complex (Chittka et al. 1997; Raine & Chittka 2005; Zych & Stpiczyńska 2012). It also exemplifies, that the overall most abundant pollinator species, the generalist honeybee, is not necessarily the most frequent visitor to all present plant species.

For *M. sylvestris* and *B. officinalis*, *A. mellifera* was the most effective pollinator, its high abundance compensating for the relatively low pollinator efficiency per single visit. For *O. viciifolia*, bumblebees of the *B. lapidarius* complex were the most effective pollinators, again based on higher abundances than the similarly efficient *B. sylvarum* and bees of the genus *Megachile* at the single-visit level. Hence, in this study the most frequent flower-visitor group of each plant species was most effective in depositing conspecific pollen per time unit, even though not showing the highest pollinator efficiency per single visit. In combination with previous studies, this suggests that the most frequent visitors are often the most important pollinators overall, even when being poor or equally efficient pollinators per single visit

(Olsen 1997; Vázquez et al. 2005; Madjidian et al. 2008; Rader et al. 2009; Zych et al. 2013). Even though pollen limitation was of minor importance in our study and several pollinator species can potentially cause maximum seed set, the results suggest that visitation rate could play a more important role in plant reproduction than pollination success on a per-interaction basis (Vázquez et al. 2005; Sahli & Conner 2006). Thus, pollinator abundance seems a similar important conservation measure for ecosystem functioning as species richness for biodiversity (Kleijn et al. 2015).

Visitation frequencies, however, did not explain pollinator effectiveness entirely. Flower visitors not contributing to pollination at all were similarly frequent as legitimate pollinators in both *B. officinalis* (*Megachile* and *Halictidae*) and *O. viciifolia* (*A. mellifera*). Furthermore, differences in visitation frequencies between flower-visitor groups are not proportional to the differences in pollinator effectiveness. For example, honeybees were 15 times more frequent on *M. sylvestris* than bumblebees of the *B. lapidarius* complex, but only five times more effective. Therefore, it seems more reliable and informative to use direct measurements when determining pollinator performances, instead of deducing effectiveness from visitation frequencies alone (Mayfield et al. 2001; Javorek et al. 2002; Ballantyne et al. 2015). Such information reveals differences in the density dependence of pollination success between pollinators and is ultimately necessary for estimating consequences of pollinator decline for plant population persistence. Revealing true pollinator performances will allow to construct more informative plant-pollinator networks (Ballantyne et al. 2015), find appropriate species for crop pollination (Westerkamp & Gottsberger 2000) and thus conserve and apply pollination service best (Garratt et al. 2014).

### Conclusion

In conclusion, considerable differences in pollinator performance occurred even in closely related taxa. Not all bees were pollinators of the studied plants and pollinators differed in their pollen deposition efficiency per single visit. Although the most frequent flower visitors were most effective, pollinator effectiveness could not be explained by visitation frequencies alone. These findings emphasise the need to connect visitation frequencies to stigmatic pollen deposition to reveal true pollination performances of flower visitors.

### ACKNOWLEDGEMENTS

We thank Daniela Warzecha for helping identify flower visitors and Verena Grob for advice on pollen identification. We further thank Verena Grob, Robert Garling and Daniela Warzecha for their assistance during the flower-visitor surveys.

### APPENDICES

Additional supporting information may be found in the online version of this article:

APPENDIX I. Flowers and stigmas with pollen of (A) *M. sylvestris*, (B) *B. officinalis* and (C) *O. viciifolia*.

APPENDIX II. Analysed flower-visitor groups of (A) *M. sylvestris*, (B) *B. officinalis* and (C) *O. viciifolia*.

APPENDIX III. All flower-visitor groups observed on (A) *M. sylvestris*, (B) *B. officinalis* and (C) *O. viciifolia*.

### REFERENCES

- Adler LS, Irwin RE (2006) Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. *Annals of Botany* 97:141-150.
- Ballantyne G, Baldock KCR, Willmer PG (2015) Constructing more informative plant-pollinator networks: visitation and pollen deposition networks in a heathland plant community. *Proceedings of the Royal Society of London B: Biological Sciences* 282:20151130.
- Buchmann SL (1983) Buzz pollination in angiosperms. In: Jones CE, Little RJ (eds) *Handbook of experimental pollination biology*. Van Nostrand Reinhold Company Inc., New York, pp 73-113.
- Buchmann SL, Nabhan GP (1996) *The forgotten pollinators*. Island Press/Shearwater Books, Washington, DC.
- Calderone SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL (2012) Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992-2009. *PLoS ONE* 7:e37235.
- Chittka L, Gumbert A, Kunze J (1997) Foraging dynamics of bumble bees: correlates of movements within and between plant species. *Behavioral Ecology* 8:239-249.
- Comba L, Corbet SA, Hunt L, Warren B (1999) Flowers, nectar and insect visits: evaluating British plant species for pollinator-friendly gardens. *Annals of Botany* 83:369-383.
- Corbet SA, Chapman H, Saville N (1988) Vibratory pollen collection and flower form: bumble-bees on *Actinidia*, *Symphytum*, *Borago* and *Polygonatum*. *Functional Ecology* 2:147-155.
- Corbet SA, Williams IH, Osborne JL (1991) Bees and the pollination of crops and wild flowers in the European Community. *Bee World* 72:47-59.
- Córdoba SA, Cocucci AA (2011) Flower power: its association with bee power and floral functional morphology in papilionate legumes. *Annals of Botany* 108:919-931.
- De Haro-Bailón A, Del Rio M (1998) Isolation of chemically induced mutants in borage (*Borago officinalis* L.). *Journal of the American Oil Chemists' Society* 75:281-283.
- De Luca PA, Vallejo-Marín M (2013) What's the "buzz" about? The ecology and evolutionary significance of buzz-pollination. *Current Opinion in Plant Biology* 16:429-435.
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35:375-403.
- Fumero-Cabán JJ, Meléndez-Ackerman EJ (2007) Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). *American Journal of Botany* 94:419-424.
- Gallai N, Salles J-M, Settele J, Vaissière BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics* 68:810-821.
- Galloni M, Podda L, Vivarelli D, Cristofolini G (2007) Pollen presentation, pollen-ovule ratios, and other reproductive traits in Mediterranean Legumes (Fam. Fabaceae - Subfam. Faboideae). *Plant Systematics and Evolution* 266:147-164.
- Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O, Bartomeus I, Benjamin F, et al. (2014) Wild

- pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339:1608-1611.
- Garratt MPD, Coston DJ, Truslove CL, Lappage MG, Polce C, Dean R, Biesmeijer JC, Potts SG (2014) The identity of crop pollinators helps target conservation for improved ecosystem services. *Biological Conservation* 169:128-135.
- Gasparetto JC, Martins CAF, Hayashi SS, Otuky MF, Pontarolo R (2012) Ethnobotanical and scientific aspects of *Malva sylvestris* L.: a millennial herbal medicine. *Journal of Pharmacy and Pharmacology* 64:172-189.
- Ghorbel S, Nabli MA (1998) Pollen, pistil and their interrelations in *Borago officinalis* and *Heliotropium europaeum* (Boraginaceae). *Grana* 37:203-214.
- Goplen BP, Richards KW and Moyer JR (1991) Sainfoin for western Canada. Agriculture Canada Publication 1470E:24.
- Goulson D, Peat J, Stout JC, Tucker J, Darvill B, Derwent LC, Hughes WOH (2002) Can alloethism in workers of the bumblebee *Bombus terrestris* be explained in terms of foraging efficiency? *Animal Behaviour* 64:123-130.
- Goulson D (2003) Effects of introduced bees on native ecosystems. *Annual Review of Ecology, Evolution, and Systematics* 34:1-26.
- Goulson D, Darvill B (2004) Niche overlap and diet breadth in bumblebees; are rare species more specialized in their choice of flowers? *Oecologia* 35:55-63.
- Goulson D, Hanley ME, Darvill B, Ellis JS, Knight ME (2005) Causes of rarity in bumblebees. *Biological Conservation* 122:1-8.
- Hayot Carbonero C, Mueller-Harvey I, Brown TA, Smith L (2011) Sainfoin (*Onobrychis viciifolia*): a beneficial forage legume. *Plant Genetic Resources* 9:70-85.
- Hill PSM, Wells PH, Wells H (1997) Spontaneous flower constancy and learning in honey bees as a function of colour. *Animal Behavior* 54:615-627.
- Hobbs GA, Nummi WO, Virotek JF (1961) Food-gathering behaviour of honey, bumble, and leaf-cutter bees (Hymenoptera: Apoidea) in Alberta. *The Canadian Entomologist* 93:409-419.
- Inouye DW (1980) The terminology of floral larceny. *Ecology* 61:1251-1253.
- Inouye DW, Gill DE, Dudash MR, Fenster CB (1994) A model and lexicon for pollen fate. *American Journal of Botany* 81:1517-1530.
- Janick J, Simon JE, Quinn J, Beaubaire N (1989) Borage: a source of gamma linolenic acid. In: Craker LE, Simon JE (eds) Herbs, spices, and medicinal plants: recent advances in botany, horticulture and pharmacology. Vol 4. Oryx Press, Phoenix, AZ, pp 145-168.
- Jauker F, Speckmann M, Wolters V (2016) Intra-specific body size determines pollination effectiveness. *Basic and Applied Ecology* 17:714-719.
- Javorek KE, Mackenzie SP, Vander Kloet SK (2002) Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on Lowbush Blueberry (Ericaceae: *Vaccinium angustifolium*). *Annals of the Entomological Society of America* 95:345-351.
- Jędrzejewska-Szmek K, Zych M (2013) Flower-visitor and pollen transport networks in a large city: structure and properties. *Arthropod-Plant Interactions* 7:503-516.
- Johnson SD, Steiner KE (2000) Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution* 15:140-143.
- Kearns CA, Inouye DW (1993) Techniques for pollination biologists. University Press of Colorado, Boulder.
- King MJ, Buchmann SL (2003) Floral sonication by bees: mesosomal vibration by *Bombus* and *Xylocopa*, but not *Apis* (Hymenoptera: Apidae), ejects pollen from poricidal anthers. *Journal of the Kansas Entomological Society* 76:295-305.
- King C, Ballantyne G, Willmer PG (2013) Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution* 4:811-818.
- Kleijn D, Winfree R, Bartomeus I, Carvalheiro LG, Henry M, Isaacs R, Klein A-M, Kremen C, M'Gonigle LK, Rader R, Ricketts TH, Williams NM, et al. (2015) Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nature Communications* 6:7414.
- Kumar G, Kadam GB, Saha TN, Girish KS, Tiwari AK, Kumar R (2014) Studies on floral biology of *Malva sylvestris* L. *Indian Journal of Horticulture* 71:295-297.
- Madjidian JA, Morales CL, Smith HG (2008) Displacement of a native by an alien bumblebee: lower pollinator efficiency overcome by overwhelmingly higher visitation frequency. *Oecologia* 156:835-45.
- Mayer C, Adler L, Armbruster WS, Dafni A, Eardley C, Huang SQ, Kevan PG, Ollerton J, Packer L, Ssymank A, Stout JC, Potts SG (2011) Pollination ecology in the 21st century: key questions for future research. *Journal of Pollination Ecology* 3:8-23.
- Mayfield M, Waser NM, Price M (2001) Exploring the 'Most effective pollinator principle' with complex flowers: bumblebees and *Ipomopsis aggregata*. *Annals of Botany* 88:591-96.
- Michener CD (2007) The bees of the world. Johns Hopkins University Press, Baltimore.
- Montaner C, Floris E, Alvarez JM (2000) Is self-compatibility the main breeding system in borage (*Borago officinalis* L.)? *Theoretical and Applied Genetics* 101:185-189.
- Ne'eman G, Shavit O, Shaltiel L, Shmida A (2006) Foraging by male and female solitary bees with implications for pollination. *Journal of Insect Behavior* 19:383-401.
- Ne'eman G, Jürgens A, Newstrom-Lloyd LE, Potts SG, Dafni A (2010) A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Reviews* 85:435-451.
- Neff JL, Simpson BB (1988) Vibratile pollen-harvesting by *Megachile mendica* Cresson (Hymenoptera, Megachilidae). *Journal of the Kansas Entomological Society* 61:242-244.
- Ollerton J, Tarrant S, Winfree R (2011) How many flowering plants are pollinated by animals? *Oikos* 120:321-326.
- Olsen KM (1997) Pollination effectiveness and pollinator importance in a population of *Heterotheca subaxillaris* (Asteraceae). *Oecologia* 109:114-121.
- Popic TJ, Wardle GM, Davila YC (2013) Flower-visitor networks only partially predict the function of pollen transport by bees. *Austral Ecology* 38:76-86.
- R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rader R, Howlett BG, Cunningham SA, Westcott DA, Newstrom-Lloyd LE, Walker MK, Teulon DAJ, 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* 46:1080-1087.
- Raine NE, Chittka L (2005) Comparison of flower constancy and foraging performance in three bumblebee species (Hymenoptera: Apidae: *Bombus*). *Entomologia Generalis* 28:81-89.
- Richards KW, Edwards PD (1988) Density, diversity, and efficiency of pollinators of sainfoin, *Onobrychis viciaefolia* Scop. *The Canadian Entomologist* 120:1085-1100.



- Sahli HF, Conner JK (2006) Characterizing ecological generalization in plant-pollination systems. *Oecologia* 148:365-72.
- Slaa EJ, Sánchez CLA, Malagodi-Braga KS, Hofstede FE (2006) Stingless bees in applied pollination. Practice and perspectives. *Apidologie* 37:293-315.
- Snow AA, Spira TP, Simpson R, Klips RA (1996) The ecology of geitonogamous pollination. In: Lloyd DG, Barrett SCH (eds) *Floral biology: studies on floral evolution in animal-pollinated plants*. Chapman and Hall, New York, pp 191-216.
- Thorp RW (2000) The collection of pollen by bees. *Plant Systematics and Evolution* 222:211-223.
- Vázquez DP, Morris WF, Jordano P (2005) Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecology Letters* 8:1088-1094.
- Waser NM (1986) Flower constancy: definition, cause and measurement. *The American Naturalist* 127: 96-603.
- Westerkamp C, Gottsberger G (2000) Diversity pays in crop pollination. *Crop Science* 40:1209-1222.
- Westrich P (1989) *Die Wildbienen Baden-Württembergs. Allgemeiner Teil*. E Ulmer, Stuttgart.
- Willmer PG, Bataw AAM, Hughes JP (1994) The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. *Ecological Entomology* 19:271-284.
- Willmer PG, Finlayson K (2014) Big bees do a better job: intraspecific size variation influences pollination effectiveness. *Journal of Pollination Ecology* 14:244-254.
- Wilson P, Thomson JD (1991) Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* 72:1503-1507.
- Zhang H, Huang J, Williams PH, Vaissière BE, Zhou Z, Gai Q, Dong J, An J (2015) Managed bumblebees outperform honeybees in increasing peach fruit set in China: different limiting processes with different pollinators. *PLoS ONE* 10:e0121143.
- Zych M, Goldstein J, Roguz K, Stpiczynska M (2013) The most effective pollinator revisited: pollen dynamics in a spring-flowering herb. *Arthropod-Plant Interactions* 7:315-322.
- Zych M, Stpiczynska M (2012) Neither protogynous nor obligatory out-crossed: pollination biology and breeding system of the European Red List *Fritillaria meleagris* L. (Liliaceae). *Plant Biology* 14:285-294.



This work is licensed under a [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/).