

The most effective pollinator revisited: pollen dynamics in a spring-flowering herb

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Abstract Most flowers are visited by a wide range of potential pollinators. However, their efficiency in pollen removal and deposition, and other behavioural factors affecting pollination effectiveness may greatly differ among taxa, and even individuals. Fritillary (*Fritillaria meleagris* L., Liliaceae) is a spring-flowering, critically endangered plant in the Polish flora, red-listed in most of the European countries of its range. Based on indirect evidence, that is, body pollen loads, visitation frequency and seasonal abundance, it is estimated that its key pollinators are queen bumblebees, but, as shown in the literature, the largest *Fritillaria* pollen loads are carried by solitary bees. To study pollinator effectiveness for floral visitors to *F. meleagris*, we performed a garden experiment, where we analysed pollen deposition and assessed pollen removal per single flower-visit in the plant. Similarly to field conditions reported in the literature, our experimental plants were serviced by nectar-seeking bumblebee queens and two taxa of solitary bees, small pollen-collecting *Andrena* and large, nectar-seeking *Anthophora* males. When “quality” component was addressed, despite the character of visits, insects from all groups deposited more pollen than was found on unvisited flowers, but they did not differ significantly from each other in pollen deposition on virgin stigmas. We also found some differences in pollen removal both within- and among-visitor species and control flowers, unfortunately due to extremely high variation of the results they were all statistically insignificant. However, when “quantity” component of insect

performance was concerned, we observed that over 81 % of visits were by bumblebees. *Bombus* queens stayed on flowers significantly less time than small *Andrena* individuals (13 % of recorded visits) and equally long as *Anthophora* males (only 6 % of visits). We conclude that although all the visitor groups can pollinate the flowers of *F. meleagris*, bumblebee queens indeed proved to be the most effective pollinators of the plant, when both quality and quantity components of pollination are concerned.

Keywords Bumblebees · Effectiveness · *Fritillaria meleagris* · Pollen deposition · Pollen removal · Pollination

Introduction

Different floral visitors affect pollination of flowers in different ways depending on their relative size, visit frequency and behaviour. “The most effective pollinator” principle formulated by Stebbins (1970; p. 318) implies that floral characters should evolve to match “the most frequent and effective” visitor. When we look at various metrics used to quantify pollinator performance (presented and/or reviewed in, e.g., Kearns and Inouye 1993; Dafni et al. 2005; Fenster et al. 2004; Ne’eman et al. 2010), we may distinguish between a “quantitative” and “qualitative” component of animal activity, where quality determines animal’s ability to deliver pollen grains to conspecific stigmas, and quantity usually refers to the frequency of visits to a particular plant species (Herrera 1987; Olsen 1997). These two components of insect effectiveness (sensu Willmer 2011) are independent because rare visitors may carry more pollen than the common ones or vice versa (e.g. Mayfield et al. 2001; Fumero-Cabán and Meléndez-Ackerman 2007; Zych 2007; Niemirski and Zych 2011). In some cases, the most frequent visitor

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is indeed the most important pollinator (Motten et al. 1981; Fishbein and Venable 1996; Olsen 1997; Sahli and Conner 2007; Niemirski and Zych 2011), but in other cases, common floral visitors do not contribute significantly to pollination because, for example, they carry no or little pollen on their bodies (Schemske and Horvitz 1984; Zych 2002; Fumero-Cabán and Meléndez-Ackerman 2007; Watts et al. 2012), their efficiency in pollen transfer is low when compared to other animals (Wilson and Thomson 1991; Cane et al. 1993; Mayfield et al. 2001; Thomson and Goodell 2001; Javorek et al. 2002; Adler and Irwin 2006), and they preferentially visit one flower sexual phase in dioecious or dichogamous plants (Bierzychudek 1987; Schlessman et al. 2004; Larsson 2005; Davila and Wardle 2007; Zych 2007). Honey bees (*Apis mellifera* L.), for instance, being the most common floral visitors in many anthropogenic habitats, may be efficient in pollen removal, but inefficient in pollen deposition, and thus are quoted in some cases as parasites rather than actual pollinators (Westerkamp 1991; Wilson and Thomson 1991; Buchmann and Nabhan 1996; Willmer 2011; Ollerton et al. 2012). Also, the particular components of effectiveness of main floral visitors may be highly variable among years and/or sites (Ollerton 1996; Gómez and Zamora 1999; Lamborn and Ollerton 2000; Ivey et al. 2003; Gibson et al. 2006; Ollerton et al. 2007; Davila and Wardle 2008; Lay et al. 2011; Robertson and Leavitt 2011).

In many cases, precise determination of insects' effectiveness components seems difficult or unfeasible. This, for instance, may be related to, so-called, generalist pollination systems, where flowers are visited by dozens or even hundreds of insect species from many taxonomic orders and the evaluation of each taxon is a daunting task (Lindsey 1984; Herrera 1987; Olsen 1997; Gómez and Zamora 1999; Zych 2002, 2007; Gibson et al. 2006; Pérez-Bañón et al. 2007; Niemirski and Zych 2011; Tepedino et al. 2011), or to cases where visitation frequency is low so that adequate sampling in field conditions is logistically impossible (e.g. Ollerton et al. 2009; Zych and Stpiczyńska 2012). Frequently in such cases, indirect methods are used to give an overview of the plant's pollination system (e.g. Lindsey 1984; Lamborn and Ollerton 2000; Zych 2002, 2007; Gibson et al. 2006; Ferrero et al. 2011; Horsburgh et al. 2011; Niemirski and Zych 2011; Tepedino et al. 2011). Although such methods usually include a combination of quality and quantity measures of animal performance on flowers (e.g. number/frequency of visits, insect relative abundance, body pollen load, foraging behaviour), they have some restrictions as, for instance, body pollen loads are not necessarily good estimates of pollen deposition (Adler and Irwin 2006). Also, they do not allow for the estimation of costs of pollination, for instance, in terms of pollen picked up by floral visitors, but not deposited on stigmas. At best, results based on such estimates should be

treated as provisional until supported by more direct methods, for example, measuring pollen loads deposited on a stigma by a given pollinator species, or seed set after visits by certain pollen-carrying vectors or after exclusion of a given insect visitor (Johnson and Steiner 2000; Pellmyr 2002; Willmer 2011). The knowledge on the actual pollen dynamics and pollinator effectiveness may be of great importance especially in the case of threatened plant species where pollination and seed production often represent most vulnerable stages of the species' life history (Schemske et al. 1994; Kwak and Bekker 2006). Therefore, in the present study, we focused on red-listed *Fritillaria meleagris* (Liliaceae), reputedly classic bumblebee flower (Knuth 1899). In a 4-year study, Zych and Stpiczyńska (2012) reported bumblebees as the key pollinators of the plant based on indirect methods of assessing insect efficiency (insect body pollen loads and visit frequency). *Bombus* queens do carry Fritillary pollen on their bodies, are much more frequent visitors than other bees and visit flowers in poor weather conditions. However, small solitary bees of the genera *Andrena* and *Lasioglossum* carried almost three times larger pollen loads than bumblebees or honeybees, suggesting they could be more effective pollinators, at least in terms of quality. Therefore, to assess the quality component of pollinator effectiveness directly for floral visitors to *F. meleagris* and their pollen transfer efficiency, we performed a garden experiment, where we quantified pollen deposition and removal during single flower visits to the plant.

Materials and methods

The plant

In natural conditions, flowers of *Fritillaria meleagris* L. (Liliaceae) last 6–7 days, and they are produced singly (very rarely 2–3 per plant; Fig. 1) and offer both pollen and abundant, highly concentrated nectar produced throughout the life of a flower (Stpiczyńska et al. 2012). The flowers are from purplish pink to pure white with characteristic checkerboard pattern (Knuth 1899). The flowers contain six anthers and one three-carpellate pistil producing, on average, 148 ± 26 ovules (mean \pm SD; Stpiczyńska and Zych, unpublished). The plant is self-compatible, but seeds in natural conditions are mostly xenogamous (Zych and Stpiczyńska 2012). Flower visitors include bumblebees, honeybees, solitary bees and flies (Hedström 1983; Zych and Stpiczyńska 2012). Published estimates of the plant pollination system based on visitation data (Knuth 1899) or indirect measures of insects' effectiveness (Hedström 1983; Zych and Stpiczyńska 2012) report bumblebees as the main pollinators.

The experiment

Our experimental work was completed during April 2010. We created a small artificial population of *F. meleagris* L. (Liliaceae) in the Botanic Garden, University of Warsaw, located in Warsaw, Central Poland, on the western bank of the Vistula river valley, E21°1'49' N52°13'15', 110 m a.s.l. (Werblan-Jakubiec 1991). In the fall of 2009, we planted approximately 500 plants in a 500-m² grassy area in the garden. The plants were grown from commercially available bulbs (Domena, Łomianki, Poland). These served as a source of flowers for the study.

Our experiment was conducted for 5 days in a small grassy garden compartment (approximately 100 m²; hereafter *experimental garden*), isolated from other parts of the garden. It was situated approximately 300 m from the source artificial population, in which prior to experimental work we bagged flower buds. To saturate pollinator community, and to find out whether *Apis mellifera* is indeed an effective pollinator, we placed there a small beehive and a commercially available colony of *Bombus terrestris* (Polski Trzmiel-Marcin Matuszak, Tymieniec-Dwór 8; 62-865 Szczytniki,



Fig. 1 *Fritillaria meleagris* L. (Liliaceae). Photograph by M. Zych

Poland). Before the experiment took place, we had mowed the area trying to remove all the flowers from the neighbour herb plants (mostly of *Corydalis solida*, *Ficaria verna*, *Gagea lutea* and *Taraxacum* sp.) and established there a regular 10 × 5 grid composed of glass test tubes with water, fastened to thin wooden sticks, where each time we presented 50 cut stems with open, unbagged flowers in the stage of pollen presentation. Most of the stems used in the experiment bore a single flower, and no more than 5 % of stems had two flowers. Half of the stems (25) were intended as “adaptation plants”. Insects were allowed to freely forage on adaptation plants for approximately 2 days before the experiment to gain experience with a novel flower type. The remaining 25 stems were treated as experimental. Virgin flowers were unbagged each day at approximately 09.00 h and, if not visited, again bagged at approximately 18.00 h, and adaptation plants remained available for insects throughout the whole period of the study. After a single visit to an experimental flower (i.e. an insect contacted floral reproductive parts), it was collected, its stigmas and anthers removed, and stored separately in Eppendorf tubes in 70 % EtOH. We noted the identity of insect visitors, and in order to check for possible relationship between visit duration and pollen deposition or removal, for 3 days of the experiment also duration of the visits (measured from the moment of entering the flower until departure). In order to distinguish among pollen- and nectar visits, we observed insect’s behaviour in a flower. Whenever an insect clung to a sepal and, facing the nectary, moved upwards, we treated that as a nectar visit, and other behaviour was regarded as seeking pollen. Collected flowers were immediately replaced with new virgin ones. Each day, as the control for both pollen removal and deposition, we would also collect in the same way stigmas and anthers from 3 to 5 random flowers treated in the same way as those used in the experiment (i.e. bagged and unbagged).

To measure pollen deposition on stigmas, each stigma exposed to a single insect visit was softened with 40 % NaOH in a heated bath for 10 min (temperature approximately 60 °C), rinsed in distilled water, placed on a microscopic slide in a drop of glycerin, covered with cover slips and gently pressed to distribute the macerated tissue over the whole slide. The number of *Fritillaria* pollen grains was counted under a light microscope (magnification ×40). The initial tube where the stigma was deposited in ethanol was shaken and its content was poured onto one or more microscopic slides covered with cover slip, and again all *Fritillaria* pollen grains counted. The results of the two procedures were summed and used as estimate of pollen deposition.

To estimate pollen production per flower (in control flowers) or pollen remaining after the single visit, the anthers from an individual flower were placed in a watch glass and pollen extracted from the anthers with a dissecting needle and flushed into another watch glass with

50 % EtOH with a drop of tensioactive liquid (Dafni et al. 2005). After drying, the pollen was suspended in 1,500 μ l of 66 % glycerine; 10 μ l subsamples of the suspended pollen were counted under a light microscope. Two subsamples per flower were counted, and the results were extrapolated to obtain the number of pollen grains in the entire sample.

To estimate the pollinator efficiency of floral visitors (sensu Ivey et al. 2003), for each taxon, we compared mean pollen deposited relative to pollen removed.

We compared pollen deposition and removal among visitor taxa using ANOVA followed by post hoc pairwise tests (Statistica 7.1; Stat Soft Inc. 2005, Tulsa, OK, USA). Data were log-transformed to achieve normality. Visit duration could not be normalized by transformation and was tested using Kruskal–Wallis nonparametric ANOVA.

Results

During 5 days of experiment, we observed 96 individual visits to *F. meleagris* flowers by five insect species from two hymenopteran families, differing in size and behaviour [Andrenidae: *Andrena* sp.; Apidae: *Anthophora plumipes* (Pall.), *Bombus lapidarius* (L.), *B. ruderarius* (Müller), *B. terrestris* (L.)]. *Bombus* species recorded during the experiment represented two tongue-length classes (short-tongued *B. terrestris* and *B. lapidarius* and medium-tongued *B. ruderarius*; Goulson et al. 2008b), and we, however, observed no significant differences in performance among these species and therefore in subsequent analyses treat them together as one functional group. Bumblebees made over 81 % of the visits, *Andrena* made 13 % and *A. plumipes* 6 % (the latter, for simplicity, hereafter referred to as *Anthophora*). Honeybees never visited the flowers, although occasionally they approached flowers and even rested on outer part of the perianth. Bumblebee visits were by large wild queens (body length approximately 20 mm) and not by small workers from our introduced colony (body length approximately 15 mm), and *Anthophora* were only by males (body length approximately 15 mm). Bumblebees and large-bodied solitary bees (*Anthophora*) visited flowers for nectar, and small *Andrena* bees (body length less than 10 mm) visited for pollen. The former two clung to sepals and departed from the flowers with visible pollen loads located on the thorax, while the latter wandered over the androecium and usually left flowers with pollen grains completely covering all body surfaces.

Insects from all groups deposited significantly more pollen than was found on unvisited control flowers (ANOVA on log-transformed data $F_{3,111} = 14.49$, $P \ll 0.001$), but they did not differ significantly from each other in pollen deposition on virgin stigmas (Fig. 2).

Unvisited flowers contained 340000 ± 40300 pollen grains (mean and SD), and a single visit resulted, on average, in removal of 18 ± 139 %, 29 ± 130 % and 37 ± 127 % pollen grains from the flower, respectively, for *Andrena*, *Bombus* and *Anthophora* (Fig. 3). Extremely large variation in the pollen removal data did not allow us to detect any significant differences among taxa or between each taxon and control flowers in the amounts of pollen grains remaining in a flower after a single visit (ANOVA on log-transformed data $F_{3,110} = 0.46$, $P > 0.7$).

Mean efficiency of pollen transfer was slightly but insignificantly higher for pollen-collecting *Andrena* (7.6 ± 60.0 %) than for nectar-collecting *Bombus* (5.8 ± 25.6 %) and *Anthophora* (5.9 ± 20.0 %).

Time spent on flowers varied greatly among the insect groups (Kruskal–Wallis ANOVA: $H_{(2,N=46)} = 7.87$; $P = 0.0195$), and bumblebees performed, on average, significantly shorter visits than *Andrena* individuals (mean and SD 43 ± 51 /median 31 s vs. mean and SD 308 ± 245 /median 195 s; $P = 0.0175$, Kruskal–Wallis test for pairwise comparisons, Fig. 4), and equally long as *Anthophora* (mean and SD 53 ± 41 /median 49 s).

Discussion

Fritillary in our experimental array at the botanic gardens was pollinated by overwintered bumblebee queens and solitary bees, similarly to those seen in field conditions (Hedström 1983; Zych and Stpiczyńska 2012).

In our study, all floral visitors deposited similar amounts of pollen in *F. meleagris* flowers. Therefore, despite the

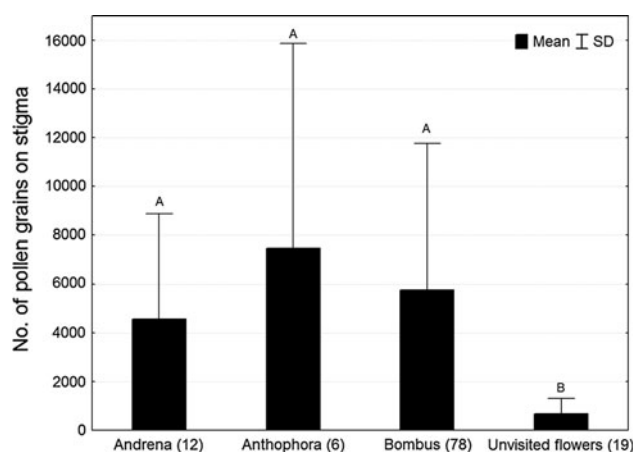


Fig. 2 Average number of *Fritillaria meleagris* pollen grains (and SD) deposited on the virgin stigma after a single visit by *Andrena*, *Anthophora* or *Bombus*, as compared to stigmatic loads in unvisited flowers (control). Numbers in brackets indicate sample size for each group. Means with different letter are different at $P < 0.05$ (post hoc Tukey HSD test for uneven N)

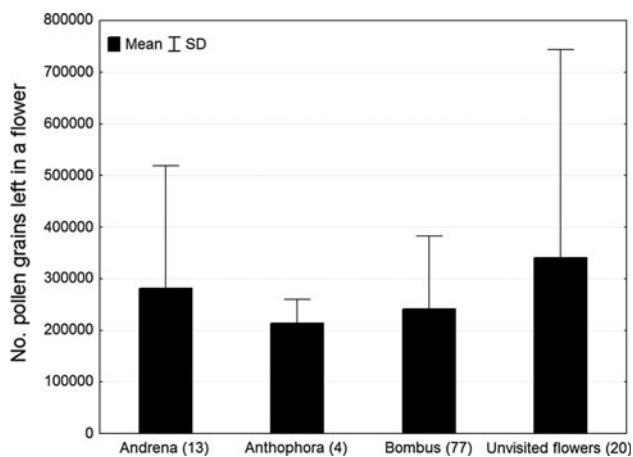


Fig. 3 Number of pollen grains (and SD) left in a *Fritillaria meleagris* flower after a single visit by *Andrena*, *Anthophora* or *Bombus*. The results for insect-visited flowers are not significantly different from those for unvisited flowers (control; ANOVA on log-transformed data, $F_{3,110} = 0.46$, $P > 0.7$). Numbers in brackets indicate sample size for each group

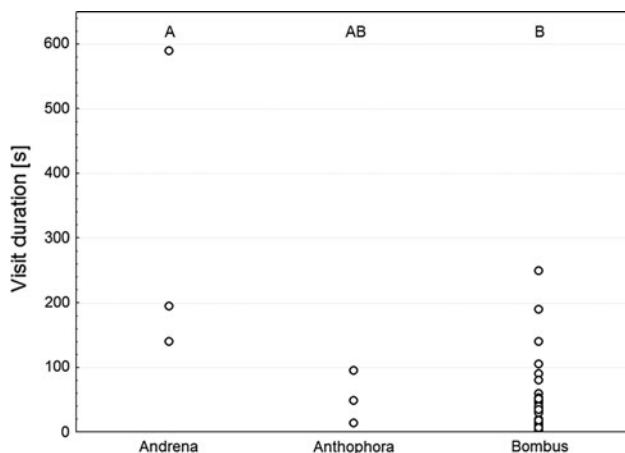


Fig. 4 Visit duration of the main pollinators in *Fritillaria meleagris* flowers. Median values are as follows: *Andrena* 195, *Anthophora* 49, *Bombus* 31, and differ significantly among the taxa (Kruskal–Wallis ANOVA: $H_{2,N=4} = 7.87$; $P < 0.05$). The same letters over the data points indicate that medians are not significantly different at $P < 0.05$ (Kruskal–Wallis test for pairwise comparisons)

differences in body size and flower-visitor behaviour, all floral visitors were similar in the quality component of effectiveness (sensu Olsen 1997). We also observed substantial differences between flower visitors in pollen removal but due to high variation of the results we found them to be statistically insignificant. Interestingly, Sahli and Conner (2007) found a similar lack of effect of body size on removal, but significant differences between nectar and pollen foragers in a study on wild radish. Such differences were also reported by Wilson and Thomson (1991) in a study of *Impatiens capensis* (Balsaminaceae), where nectar foraging *Bombus* removed relatively small

amounts of pollen and deposited much of it compared to pollen foraging *A. mellifera* and solitary bees. When, for the same plant species, the foraging mode was changed and both key visitors (*Bombus* and *A. mellifera*) sought nectar, the difference between taxa disappeared (Young et al. 2007). In the present experiment, we also observed differences in foraging behaviour of pollinators. Bumblebee queens and *Anthophora* males foraged for nectar and *Andrena* for pollen in Fritillary flowers. This, however, did not alter their pollen removal and deposition. If all visitors to Fritillary flowers sought nectar, most likely it would result in inferior performance of *Andrena*, which were the smallest, in terms of body size, of the three observed visitor groups. Fritillary produces rather large, pendant flowers, and when seeking nectar, insects cling to sepals and move upwards, where the nectaries, running along the middle nerve, are situated. In doing so, they receive pollen, which is deposited mostly on the upper side of thorax and wings (Knuth 1899; Hedström 1983). Both *Anthophora* and *Bombus* are large enough to touch anthers and stigma while using nectar, whereas *Andrena* individuals are markedly smaller and could probably collect nectar without touching floral sexual parts. Our results show, however, that regardless of the character of a visit, any floral visitor entering the flower, in search for either pollen or nectar, can successfully pollinate the stigma, and a single visit deposits sufficient pollen to fertilize all ovules. The large variation in estimated pollen removal (which probably may be attributed both to variation in pollen production per flower and crude sampling procedure with too few subsamples analysed) did not allow for reliable estimation of pollinator efficiency, but the fraction of pollen produced in a single Fritillary flower reaching conspecific stigma during a single visit seems similar for all insect visitors (1.3–2.2 %, depending on the insect taxon).

Insect visitors to Fritillary differed more dramatically in the quantity component of pollination. The dominance of *Bombus* pollinators (over 81 % of visits) is consistent with data from the natural populations, where in some years, *Bombus* constitute 100 % of floral visitors (Zych and Stpiczyńska 2012). Some researchers (e.g. Gómez and Zamora 1992; Morris 2003; Vázquez et al. 2005) argue that visitation rate could be a suitable surrogate of pollinator performance. Although attractive in terms of research methodology and concurrent with our present results, this suggestion seems true only provided the visitation is performed by animals that are equivalent in terms of the quality component of pollination. This equivalency is indeed the case for *F. meleagris*, but in other cases, such simplification could be misleading (see, e.g., Zych 2002, 2007; Fumero-Cabán and Meléndez-Ackerman 2007; Watts et al. 2012; Sánchez-Lafuente et al. 2012). Visit duration, although different among the three groups, did

not translate to differences in pollen transfer. The shortest visits were by bumblebees and *Anthophora* males such that either of them can service approximately 6–7 times more flowers per unit time than *Andrena* bees. Longer visits of the latter, however, do not produce larger pollen deposition. We note that the results for *Andrena* and *Anthophora* are based on very low sample size, which limits the scope of inference.

Contrasting our results on pollen deposition to data on pollen carried on insect body from the natural population, we argue that body pollen loads, at least for Fritillary, are not necessarily good estimates of insect effectiveness in pollination. Small *Andrena* bees foraged for pollen in pendant flowers of *F. meleagris*, and sometimes, they leave the flower completely covered with pollen, which could explain large body pollen loads found by Zych and Stpiczynska (2012) on these insects. This high pollen carriage did not imply significantly larger pollen deposition but, as suggested by these authors, could result in more self-pollen grains being delivered to the stigma. Also, in the field conditions, *Andrena* individuals (and honey bees) are reported as more opportunistic foragers, carrying more heterogeneous body pollen loads (over 82 % of non-Fritillary pollen; Zych and Stpiczynska 2012). On the other hand, bumblebees and *Anthophora* seem to provide more direct transfer of pollen on thorax, which is left upon arrival in the next flower's stigma, and as shown by Zych and Stpiczynska (2012) for in situ plants, body pollen loads carried by *Bombus* individuals are composed in more than 91 % of Fritillary pollen. In the present study, however, we did not address the issue of the quality of pollen (its viability, compatibility, etc.) delivered by a given pollinator, which itself deserves further study.

In our study, pollinator effectiveness was similar for all flower visitors regardless of body size, and pollen transfer efficiency was only insignificantly higher for the smallest *Andrena*. Interestingly, Adler and Irwin (2006) also could not confirm their prediction that bee size is positively correlated with its pollen transfer ability. This, however, is not an universal trend, as the opposite was, for instance, reported by Snow and Roubik (1987) for *Cassia* and Howlett et al. (2011) for *Brassica rapa* var. *chinensis*. Pollen-collecting behaviour of *Andrena* can also, at least partly, explain the long duration of their visits, when compared to faster movements of large-bodied bumblebees and *Anthophora* foraging for nectar. The latter, however, are absent from all the natural populations studied so far, where, in turn, honeybees foraging on *F. meleagris* were observed (Hedström 1983; Zych and Stpiczynska 2012). In the present study, although we placed a small beehive close to our experimental array, not a single honeybee visited *F. meleagris* flowers. This was probably because our experiment was conducted in early spring, so the colony

included mostly experienced workers that would rather choose floral resources they were familiar with. Last, but not least, our experimental population could be too small for bees to switch from other kind of flowers.

Fritillaria meleagris is a rare and threatened species throughout its range (Schnittler and Günther 1999) and its populations are generally decreasing (Zych and Stpiczynska 2012; and literature cited). Our results show that bumblebees are the most effective pollinators of *F. meleagris*, when both quality and quantity component of pollination is concerned; furthermore, they visit flowers (and therefore pollinate) even in bad weather conditions (Hedström 1983; Zych and Stpiczynska 2012). Although these insects are generally declining in Central Europe (Kosior et al. 2007; Goulson et al. 2008a), the taxa present in our study belong to common species and the plant can be successfully pollinated also by other floral visitors, which suggests that its pollination biology cannot alone explain its rarity.

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