

DOI: 10.5586/asbp.3489

Publication history

Received: 2015-11-04

Accepted: 2016-02-23

Published: 2016-04-28

Handling editor

Aleksandra Samecka-Cymerman, Faculty of Biological Sciences, University of Wrocław, Poland

Authors' contributions

MZ: designed the study, analyzed data and wrote the manuscript; MAZ: collected field data and performed lab analyses

Funding

The study was financed as part of the Statutory Research Programme of the Faculty of Biology, University of Warsaw.

Competing interests

No competing interests have been declared.

Copyright notice

© The Author(s) 2016. This is an Open Access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits redistribution, commercial and non-commercial, provided that the article is properly cited.

Citation

Ziemiański MA, Zych M. Pollination biology of the urban populations of an ancient forest, spring ephemeral plant. *Acta Soc Bot Pol.* 2016;85(2):3489. <http://dx.doi.org/10.5586/asbp.3489>

Digital signature

This PDF has been certified using digital signature with a trusted timestamp to assure its origin and integrity. A verification trust dialog appears on the PDF document when it is opened in a compatible PDF reader. Certificate properties provide further details such as certification time and a signing reason in case any alterations made to the final content. If the certificate is missing or invalid it is recommended to verify the article on the journal website.

ORIGINAL RESEARCH PAPER

Pollination biology of the urban populations of an ancient forest, spring ephemeral plant

Maciej A. Ziemiański^{1,2}, Marcin Zych^{1*}

¹ Botanic Garden, Faculty of Biology, University of Warsaw, Al. Ujazdowskie 4, 00-478 Warsaw, Poland

² Białowieża Geobotanical Station, University of Warsaw, Sportowa 19, 17-230 Białowieża, Poland

* Corresponding author. Email: mzych@biol.uw.edu.pl

Abstract

Habitat fragmentation, caused by, among all, agriculture and urbanization, is one of the most important drivers of plant biodiversity decline worldwide. One of the signs of deteriorating zoogamous plant reproduction is pollen limitation, often associated with a decline in pollinator diversity and abundance. Various authors predict that the most vulnerable taxa are outbreeding plant species characterized by specialist pollination systems. We have, therefore, focused on self-incompatible *Corydalis solida*, an ancient forest, spring ephemeral plant, growing in three remnant urban populations in the city of Warsaw (Poland). Over two years, we checked for pollen limitation and recorded insect diversity and abundance for *C. solida* flowers. Our study populations composed of self-incompatible individuals were mainly visited by generalist pollinators, and produced more seeds when supplementally pollinated. Pollen limitation, however, was greater during 1 year with an early spring onset, when we observed a decline in floral visitors diversity and activity. This was probably an effect of phenological mismatch between plants and their pollinators, in this case, mostly social bees, i.e., over-wintered bumblebee queens and *Apis mellifera*. We conclude that for outbreeding zoogamous spring ephemerals, such as *C. solida* serviced by generalist pollinators, changing climatic conditions may override the effects of habitat fragmentation and influence their reproductive success.

Keywords

bees; *Corydalis*; forest remnants; pollen limitation; pollination; self-incompatibility; urbanization; Warsaw

Introduction

Habitat fragmentation, caused by, among all, agriculture and urbanization, is one of the most important drivers of plant biodiversity decline worldwide. It is estimated that by now, 50% of the global human population live in urbanized areas [1]. Already, rapid expansion of human altered habitats, especially the expansion of cities, has led to a situation where urbanized areas have become prevalent in some landscapes [2], resulting not only in the isolation of natural ecosystems, but also in the interruption of many important ecological processes [3]. As shown recently, mutualistic interactions may be especially sensitive to the negative effects of fragmentation [4]. One such interaction is the animal-mediated pollination of flowers which, according to various estimates, involves the majority of flowering plants and countless animal (mostly insect) species [5]. Pollen limitation that may be associated with habitat fragmentation [6] and a general decline in pollinator diversity and abundance [7], can negatively affect plant reproductive success and, therefore, influence plant population viability [8–11].

The susceptibility of a plant population to habitat fragmentation may depend on various aspects of the biology of the species such as its reproductive biology, which may range from complete outbreeding to autonomous self-pollination [12]. Furthermore, flowers are pollinated by taxonomically variable pollinator assemblages (generalists) or by a small number of taxonomically related animals (specialists), with many intermediate stages between the two extremes [5], and the pollinator community may even differ for two color forms of the same species [13]. This may have a profound effect on the survival of certain plant populations and species, because, according to theoretical predictions, specialist-outbreeders are perceived as the most vulnerable [14,15]. This is because a decrease in population size reduces the possibility of encountering a suitable mating partner and it impedes compensation for the lack of the most effective pollinators by substituting another alternative species. The empirical data, however, are somewhat equivocal. For example, Aizen et al. [15], who analyzed a large dataset of plant species, suggest that there is no clear relationship between compatibility system, pollination specialization and susceptibility to fragmentation. Furthermore, Corbet [16] showed that for the British flora, there is little association between increasing floral specialization and decreasing species range. On the other hand, Aguilar et al. [17], having meta-analyzed the responses to habitat fragmentation of 89 plant species, found that fragmentation had a general negative effect on pollination and plant reproduction, but showed that the type of breeding system (self-compatible SC vs. self-incompatible SI) alone explains differences in the magnitude of the response, possibly in relation to pollen limitation.

Spring ephemeral taxa, which exhibit specialist pollination ecology, seem especially prone to pollen limitation. This is mainly because of a short blooming season, flowering during unpredictable and often inclement weather conditions, and competition with other co-flowering plant species for generally rare pollinators (predominantly overwintered bumblebee queens) [18]. The same reasons make spring ephemeral plants likely candidates for the phenological mismatch between flowers and pollinators, caused for example by climatic changes [19]. Therefore, in order to contribute to the above discussion, we focused on the reproductive biology of a spring-flowering perennial, *Corydalis solida* (L.) Clairv. growing under urban conditions. Our study plant is a typical ancient forest species [20]. It is zoogamous, and reproduces by seeds, indicating that pollination is a crucial step in the survival of its populations. *Corydalis solida* produces morphologically specialized flowers (euphilous sensu [16]) with deeply concealed nectar available only to long-tongued, specialized flower visitors, and is reported to be self-incompatible [21,22]. This species inhabits mainly natural or semi-natural forest communities, but can also persist for long periods in small forest patches, despite poor dissemination capacity to colonize disturbed habitats [23]. A few remnant populations of the species have been reported from Warsaw, the largest city in Poland, persisting in isolated forest patches within the urban landscape. Given the above, we were interested in (i) its pollinators and their activity under urban conditions, (ii) plant seed production in highly fragmented urban populations, especially evidence of pollen limitation, and (iii) confirmation of the reproductive system of *C. solida*.

Material and methods

The plant

Corydalis solida (L.) Clairv. is a spring-flowering, tuberous forest perennial species and a member of the family Papaveraceae (Fumariaceae). It is also known to be a spring ephemeral species (vernal geophyte), blooming usually for 1–2 weeks in April before the spring period of leaf expansion by canopy trees growing at high light intensities [24]. Its morphologically specialized, pinkish-purple flowers are zygomorphic and produced in dense erect racemes (Fig. 1) composed of 4–16 flowers [25]. Although the flowers are reported to be self-incompatible [21], we found no account of experimental testing of the breeding system of this species in the literature. The main floral reward is nectar, produced in a long spur, which is frequently robbed, but



Fig. 1 *Corydalis solida* plant in full bloom. Photograph by M. Zych.

bee visitors also collect pollen [24]. In general, many members of the genus *Corydalis* are thought to be specialized for pollination by newly emerged bumblebee queens [26–28]. According to Knuth [21], floral visitors to *C. solida* include bees [*Anthophora plumipes* (Pall.), *Apis mellifera* L., *Bombus terrestris* L.] and, rarely, bee flies (*Bombylius* L.). This author regarded *A. plumipes* to be a legitimate pollinator, *A. mellifera* and *B. terrestris* nectar robbers, and *Bombylius* flies as non-pollinating floral visitors. So far, in Poland, only *A. mellifera* and *Bombus* queens have been reported to visit flowers of *C. solida* (in the eastern part of the country [24]). This species is also the larval host plant for the red-listed clouded Apollo butterfly *Parnassius mnemosyne* (L.) [29]. Its seeds bear elaiosomes (oil bodies) and are dispersed by ants [30].

Corydalis solida is an indicator of ancient, broad-leaved forests. In Poland, it is generally a common species (except in the western part of the country) and occurs in old, lowland hornbeam-lime and beech stands.

Study area

Warsaw is the capital of Poland, and the largest city in the country. It is situated in Central Europe, covering 517.2 km², with a population of approximately 1.7 million people (based on 2008 data [31]). We investigated three unique natural populations of *C. solida* located along the Vistula River valley escarpment within the city borders: two occurring in isolated natural forest fragments (Natoliński Forest and Bielański Forest), and the other growing in the arboretum of the Warsaw University Botanic Garden. Although our study was limited only to three populations we believe that our study sites captured a wide range of natural variation within the urban environment so that the obtained results are due to our manipulations rather than underlying natural variation.

Natoliński Forest (hereafter NAT), 52°08'27.8" N, 21°04'31.8" E, 89 m a.s.l., is situated in the southern part of the city, covering an area of 105 ha. It was created in the former royal hunting park established in the seventeenth century in the then forested suburban region. The site encompasses a fragment of the natural escarpment of the Vistula River together with the lower terrace at the foot of the escarpment, surrounded by wastelands, fallow lands and housing estates with no direct connection to other forests (the nearest forested fragment is approximately 1 km south). Although the forest was not designated a nature reserve until 1991 (now also a Natura 2000 site), public access to the site has been largely restricted ever since the end of WWII, which has allowed the area to retain its natural character. The Natoliński Forest is almost exclusively covered by various lowland forms of hornbeam-lime (*Tilio-Carpinetum*) and elm-ash (*Fraxino-Ulmetum*) forests [32]. Our experimental plants grew in moist hornbeam-lime forest patches. During the study period, the following co-flowering plant species were recorded in the experiment site: *Anemone nemorosa* L., *Anemone ranunculoides* L., *Gagea lutea* (L.) Ker Gawl., *Pulmonaria obscura* Dumort. In May 2012 our study plot was partly destroyed by wild boars searching most likely for *C. solida* tubers.

The Warsaw University Botanic Garden (UBG), 52°13'03.2" N, 21°01'40.6" E, 109 m a.s.l., is one of the oldest institutions of this kind in Poland. It was founded in 1818 and created from part of the Royal Park Łazienki. Once larger, it now covers an area of 5.16 ha [33]. The garden is situated in the very heart of the city, neighboring the large Łazienki Park, with no direct connection to other green areas. Both parks (which jointly form a continuous area of approximately 81 ha) are surrounded by the dense matrix of buildings and roads. Our study population, growing in naturalized parts of the dendrological collection (arboretum) under lime (*Tilia* sp.) and beech (*Fagus sylvatica* L.) trees, is a remnant of the natural vegetation of the site. In addition to natural elements of woodland spring flora (*A. nemorosa*, *G. lutea*), during the study period the following co-flowering cultivated plant species were recorded for the experimental site: *Cornus mas* L., *Crocus* sp., *Galanthus nivalis* L., *Hepatica nobilis* Mill., *Scilla siberica* Andrews.

Bielański Forest (BIE), 52°17'16.1" N, 20°58'13.9" E, 83 m a.s.l., is the only surviving remnant of the once large Mazowiecka Primeval Forest located in the northern part of Warsaw city agglomeration. Like Natoliński Forest, it is partly surrounded by housing estates, but is separated from the nearest forested fragments (semi-natural river bank vegetation) only by a motorway. It covers an area of 130.35 ha and much of it is occupied not only by natural lowland forms of hornbeam-lime (*Tilio-Carpinetum*) and elm-ash (*Fraxino-Ulmetum*) forest communities, but also by other drier forest types [34]. Although BIE has been a nature reserve since 1973, and was recently also made a Natura 2000 site (since 2011), the area has rather intensively been subject to human activity since it has been a popular walking area for the citizens of Warsaw for decades. Our experimental plants grew in moist forest fragment of hornbeam-lime stand. During the study period the following co-flowering plant species were recorded for the experimental site: *A. nemorosa*, *A. ranunculoides*, *Chrysosplenium alternifolium* L., *G. lutea*.

The distance from UBG to both BIE and NAT was approximately 9 km, and between BIE and NAT approximately 18 km.

Population density

For each site we estimated *C. solida* population density in 100 m² plots measuring the distance from 25 randomly chosen plants to their nearest neighbor and using the formula $D = A/(1.67d)^2$, where D is the population density, A is the plot size (100 m²) and d is the distance measured for a random plant to its nearest neighbor plant of the same species [35]. The results were then averaged for each population and year.

Population characteristics associated with reproduction

We randomly marked 100 individual plants per population. In 2012 and 2013, we scored plant height (from the soil surface to the tip of the flowering spike; measured to the nearest 1 mm) and inflorescence size (total number of flowers per inflorescence). Additionally, in 2013, we measured for each population, the depth of the leaf litter layer to the nearest 1 mm, at 10 randomly chosen spots within the area occupied by *C. solida* plants.

Insect observations

For two flowering seasons (2012–2013), for each population, we recorded insect visitors to flowers of *C. solida* using video cameras (Panasonic NV-GS75 or Sony DCR-SR15E). This was performed during peak flowering season (mid-April), and only during good weather conditions (sunny and minimal wind). The standard methodology included making 12 recordings of each population per flowering season (four recordings, respectively, for morning, noon and afternoon), totaling 720 min over 2 years. Each recording lasted 10 min and was preceded by random selection of the *C. solida* plant patch (usually 5–10 inflorescences in full bloom). Later in the lab, we noted all insect visits and calculated the frequency of visits by each recognizable group of insect visitors. Names of insect taxa are given according to Bogdanowicz et al. [36,37].

Pollen limitation and breeding system experiment

Each year, in order to check for pollen limitation, for each study population, we randomly marked 30 inflorescences and divided them into two groups which were subjected to different treatments: (A) supplemental pollination, and (B) open-pollination (control). Since we found no experimental evidence for self-incompatibility in *C. solida* in the literature, in order to test the breeding system of this species, an additional 30 plants per population were marked and subjected to two further treatments: (C) autonomous self-pollination (inflorescence bagged with a fine silk mesh to prevent insect visitation; 15 plants), and (D) induced geitonogamous pollination (inflorescence bagged; flowers pollinated with pollen from the neighboring flower of the same individual plant; 15 plants). We used the three lowermost flowers from any individual plant for all experimental treatments, always subjecting each to the same treatment. In doing so we ensured that the obtained result could be attributed solely to pollen limitation, and not to resource limitation because in *Corydalis* investment in female function diminishes acropetally along the inflorescence [26]. For supplemental pollination, we cross-pollinated a plant using a small brush and the pollen of a single flower from another individual growing at a distance of at least 3 m from the first. Before each attempt at pollination, the brush was cleaned using 70% EtOH. The experimental flowers were left in the field and then harvested after a further 3–4 weeks, by which time the capsules had started to ripen. In the lab, we scored the number of seeds per plant. When scoring, we excluded fruit showing signs of herbivore attack (caterpillars present or droppings, holes in capsules, etc.). Flowers with dry ovaries that clearly did not form fruit were scored as containing no seed.

For each site and year, following Larson and Barrett [38], we calculated pollen limitation index $PL = 1 - (P_o/P_{sp})$, where P_o is the average number of seeds produced

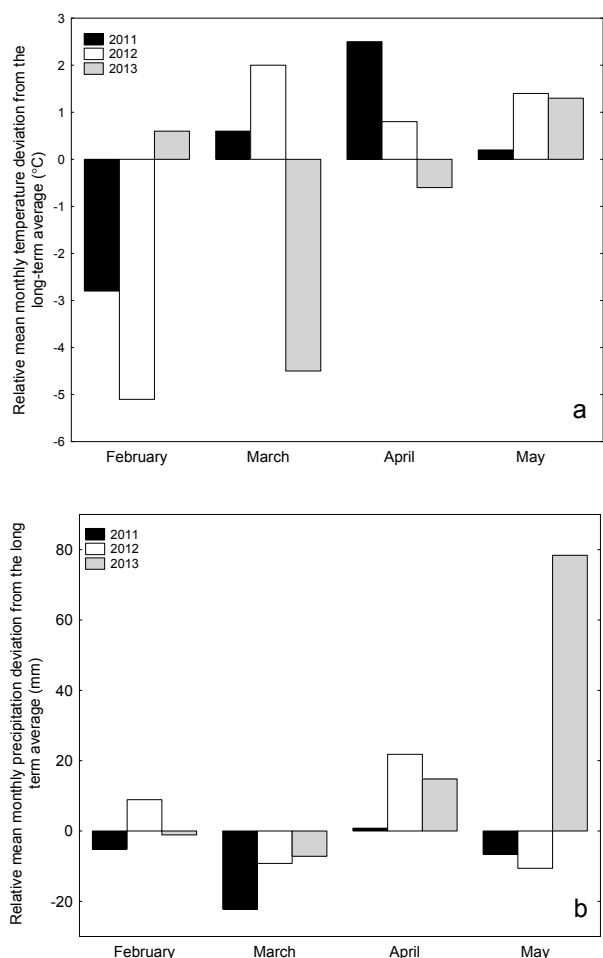


Fig. 2 Climatic conditions during February–May 2011–2013 for meteorological station Warsaw [71–73] shown as relative differences from the 30 year average (1981–2011). Mean monthly air temperature (a) and precipitation (b).

in open pollinated flowers, and P_{sp} the average number of seeds produced in supplementally pollinated flowers; consequently $PL = 0$ indicates no pollen limitation and $PL = 1$ no seed production and maximum pollen limitation.

Weather conditions

According to the Institute of Meteorology and Water Management, long-term average temperature/precipitation data (years 1981–2010) for Warsaw were as follows: February – 1.0°C / 26.1 mm; March – 2.7°C / 30.2 mm; April – 8.6°C / 33.9 mm; May – 14.2°C / 54.6 mm. Spring weather was variable among the study years. The spring of 2012 was early with very little snow cover in February, mild temperatures in March and a rather dry May, whereas the spring of 2013 was a later one, with less insolation, temperatures below freezing in March, heavy snowfall during the first week of April and an extremely wet May. The spring of 2011, which preceded the experiments, was very similar to that of 2012 in terms of temperature, but much drier. Monthly average temperature and precipitation deviation from the long-term mean for meteorological station Warsaw for February–May 2011–2013 are presented in Fig. 2.

Statistics

Statistica 9.0 was used for all statistical calculations. Before performing any statistical tests, the data were checked for normality. Subsequently, ANOVA was employed for normally distributed data (transformed when necessary) or Kruskal–Wallis ANOVA when transformation was not possible. In order to account for natural variation between our study populations and the resulting error that could affect the model [39], in case of supplemental pollination experiment data we used the GLM approach, treating populations as random factor. When necessary, the calculations were followed by appropriate post-hoc tests.

Results

Population density and characters associated with reproduction

Corydalis solida population density was high in all sites and ranged from 15 410 ± 32 150 (NAT in 2013) to 293 385 ± 889 646 individuals per 100 m² (mean ± SD). Generally we found no differences among populations except in 2013, when population density in NAT was significantly smaller from that in OB [Kruskal–Wallis ANOVA₂₀₁₃: $H(2, N = 75) = 6.083548, p < 0.05$].

Our study populations differed in traits associated with reproduction: plant height ($p < 0.001$) and inflorescence size / number of flowers per spike ($p < 0.005$; two-way ANOVAs, data for number of flowers were square root-transformed prior to analysis; Tab. 1).

For both study years, the smallest plants were recorded in UBG (97 ± 29 and 116 ± 26 mm, respectively, in 2012 and 2013; Fig. 3). They were significantly shorter than those in BIE (111 ± 25 and 132 ± 28 mm; $p < 0.01$, HSD post-hoc test for uneven N), and

Tab. 1 Results of two-way ANOVA on plant height and number of flowers per spike of *Corydalis solida*.

Source	df	SS	MS	F	p
Plant height					
Year	1	42701.0	42701.0	66.960	0.000
Population	2	19304.0	9652.0	15.136	0.000
Year × Population	2	686.0	343.0	0.538	0.585
Error	500	318850.0	638.0		
No. of flowers					
Year	1	6.3	6.3	8.291	0.004
Population	2	18.0	9.0	11.772	0.000
Year × Population	2	6.1	3.0	3.982	0.019
Error	500	381.7	0.8		

Year denotes the effect of study year (2012 vs. 2013), and *population* the effect of experimental population (see characters associated with reproduction in “Material and methods”). In order to obtain normal distribution, data for *No. of flowers* were square root-transformed.

did not statistically differ from plants in NAT. Greater plant height, however, did not result in larger inflorescences, since during both years, UBG plants produced, on average, more flowers per spike. These differences were, however, statistically significant only for 2012, when the BIE plants differed from both those in UBG and NAT. Overall, in 2012, our study plants produced significantly shorter shoots (105 ± 26 vs. 123 ± 26 mm; $p < 0.001$; Tab. 1 – data pooled over all populations) but bore larger inflorescences (13 ± 7 vs. 11 ± 6 ; data pooled over all populations) than during 2013.

Leaf litter depth, measured in 2013, differed between populations (ANOVA $N = 30$, $F = 31.947$, $p < 0.001$), and was significantly greater in NAT and BIE (respectively, 21.1 ± 3.7 and 20.2 ± 2.9 mm) compared to UBG (11.9 ± 1.5 mm; $p < 0.001$, post hoc Fisher’s NIR).

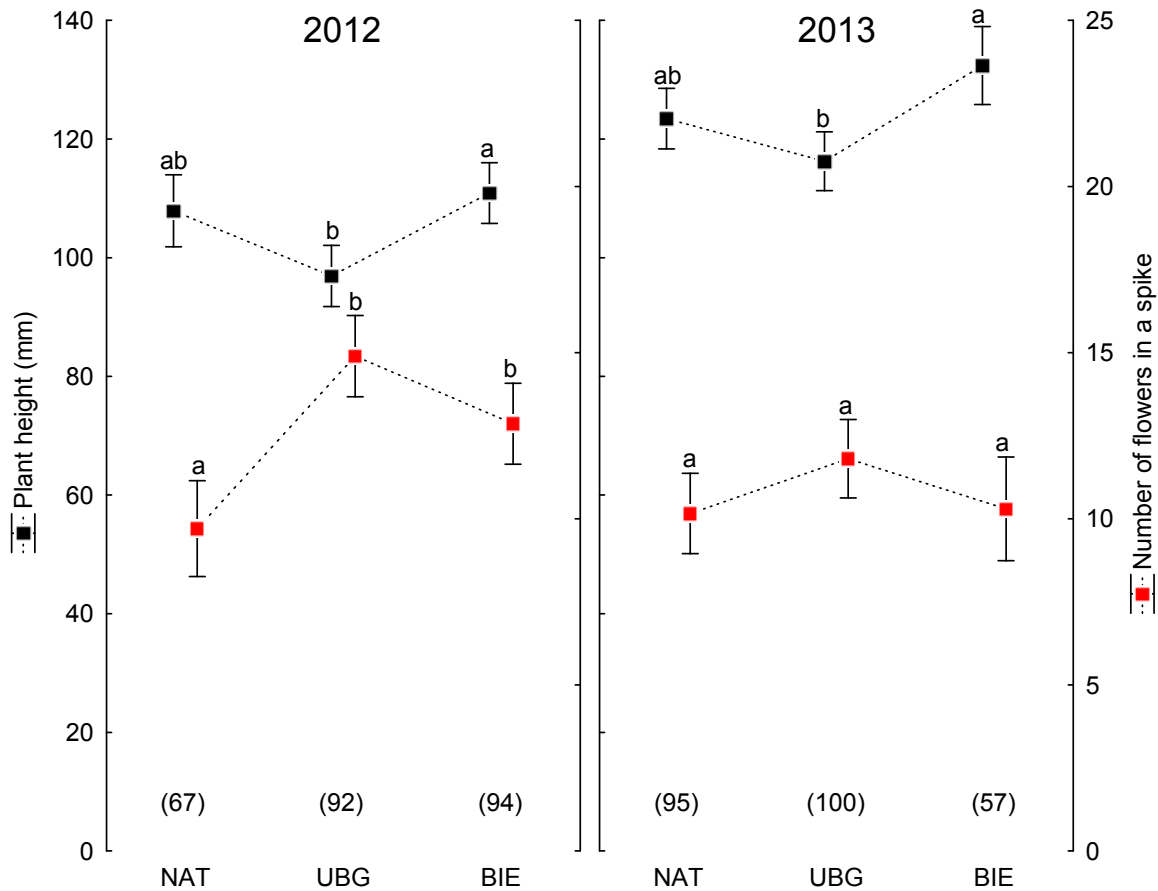


Fig. 3 Average plant height in mm (left scale) and number of flowers (right scale) per inflorescence (spike) in three *Corydalis solida* populations NAT, UBG, and BIE over two growing seasons 2012 and 2013. Error bars indicate 0.95 confidence limits of the mean and different letters next to the mean indicate statistically significant results for a given trait between populations for the same year ($p < 0.01$, HSD post-hoc test for uneven N). Numbers in brackets show sample size.

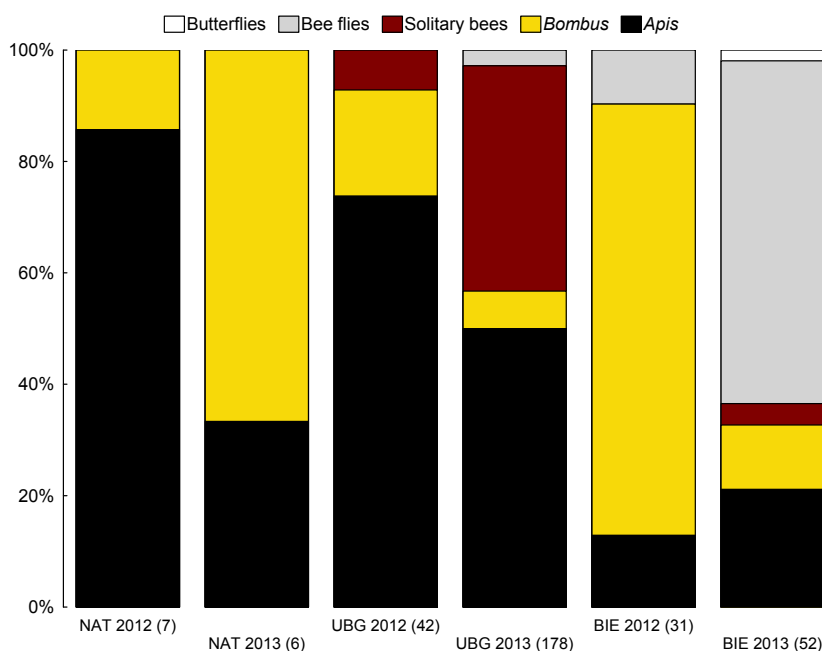


Fig. 4 Relative abundance (%) of floral visitors for three studied *Corydalis solida* populations over two growing seasons (2012–2013). NAT – Natolin Forest; UBG – Warsaw University Botanic Garden; BIE – Bielański Forest. Numbers in brackets indicate total number of recorded visits over 120 min of observations.

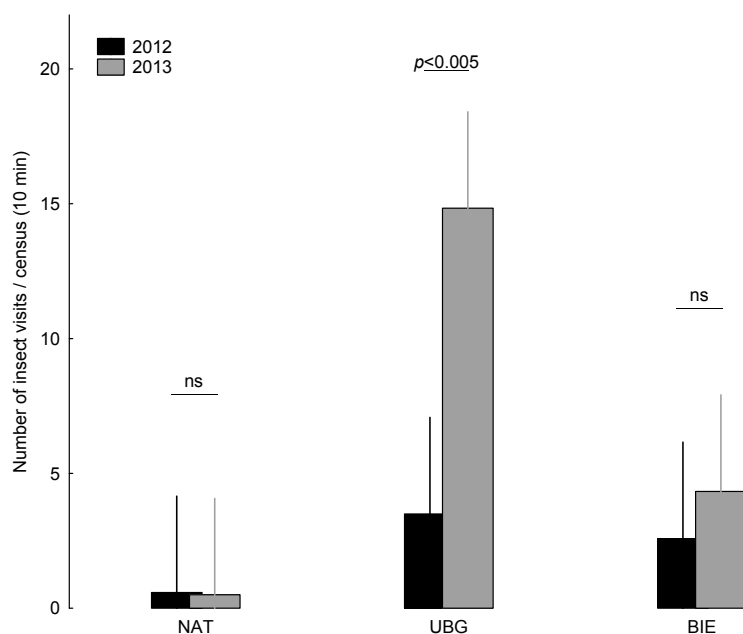


Fig. 5 Mean number of insect visits per census (10 min) to flowers of three studied populations of *Corydalis solida* during the years 2012–2013. NAT – Natolin Forest; UBG – Warsaw University Botanic Garden; BIE – Bielański Forest. Error bars indicate 0.95 confidence limits of the mean, for each data bar $N = 12$ observations, data for an individual population compared across years using Kruskal–Wallis ANOVA.

Insect observations

During study period we recorded 316 insect visits to flowers of *C. solida*. In 2012 only 80 visits were observed, whereas in 2013, this number increased by a factor of 3. We identified eight taxa of predominantly generalist floral visitors: *Andrena* Fabr. (Hymenoptera: Andrenidae), *Anthophora* Latr. (Hymenoptera: Apidae), *Apis mellifera* L. (Hymenoptera: Apidae), *Bombus lapidarius* (L.) queens (Hymenoptera: Apidae), *B. pratorum* (L.) queens (Hymenoptera: Apidae), *B. terrestris* L. queens (Hymenoptera: Apidae), *Bombylius* L. (Diptera: Bombyliidae), and *Parnassius mnemosyne* (L.) (Lepidoptera: Papilionidae). Based on size and morphological characters, we assigned them to five distinct morphogroups: bumble bees, honeybees, solitary bees, bee flies, and butterflies. Most visits were performed by honeybees and bumblebee queens (except for BIE in 2013, when bee flies were predominant). These insects were continuously recorded for all three populations over both study years, and they were the sole floral visitors observed in NAT. Solitary bees and bee flies were observed in UBG and BIE. In some cases, however, the records were confined to only one of the study years. Butterflies (*P. mnemosyne*) were recorded once, in BIE in 2012 (Fig. 4).

Data for insect visit frequency could not be successfully transformed, and was compared using non-parametric tests. Overall visit frequency was moderate (4.4 ± 7.7 visits per census; mean and SD, data pooled across study years and populations), and on average, 3 times lower in 2012, compared to 2013 (2.2 ± 3.1 vs. 6.6 ± 10.1 ; $p = 0.069$, Mann–Whitney U test). In both study years, visit frequency recorded for NAT (0.6 ± 1.2 in 2012 and 0.5 ± 0.8 in 2013) was the

lowest for the three populations studied [Kruskal–Wallis ANOVA₂₀₁₂: $H(2, N = 36) = 10.17631$, $p < 0.01$; Kruskal–Wallis ANOVA₂₀₁₃: $H(2, N = 36) = 21.42557$, $p < 0.001$]. In 2012, it differed from BIE and UBG, whereas in 2013, it differed only from that for UBG. For individual populations, we observed significant annual differences only for UBG, with 2013 scores significantly exceeding those for 2012 (Fig. 5).

Tab. 2 Results of three-way mixed model ANOVA on *Corydalis solida* seed set.

Source	df	SS	MS	F	p
Year	1	2279.07	2279.07	62.329	0.000
Treatment	1	1144.97	1144.97	31.313	0.000
Population	2	342.98	171.49	4.690	
Year × Treatment	1	156.92	156.92	4.291	0.040
Year × Population	2	181.30	90.65	2.479	
Treatment × Population	2	198.86	99.43	2.719	
Year × Treatment × Population	2	227.17	113.58	3.106	
Error	150	5484.76	36.57		

Year (fixed factor) denotes the effect of study year (2012 vs. 2013), *treatment* (fixed factor) denotes the effect of experimental manipulation (supplemental pollination vs. control), and *population* (random factor) denotes the effect of experimental population (NAT, UBG, and BIE).

outcrossing variants (A–B), mixed model ANOVA showed that study year and experimental treatment significantly affected seed set (Tab. 2). In general, regardless of experimental manipulation, seed set was higher during 2013 than during 2012 ($p < 0.001$; here and subsequently post-hoc HSD Tukey's test for uneven N), and, depending on population and study year, seed production was 8–71% greater in supplementally pollinated flowers vs. control plants (Fig. 6). The latter difference, however, was greater in 2012 (18 ± 8 vs. 10 ± 6 seeds per plant; $p < 0.001$, data pooled for three sites) than in 2013 (24 ± 6 vs. 21 ± 5 seeds per plant; $p = 0.03$, data pooled for three sites). Pollen limitation index (PL) was smaller in 2012 (overall mean 0.14 ± 0.07) than in 2013 (0.39 ± 0.21).

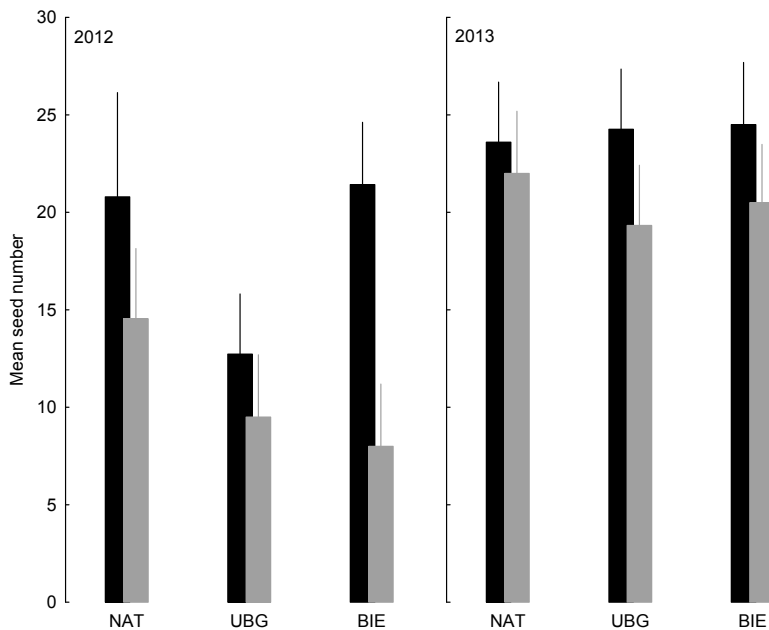


Fig. 6 Mean seed production in three *Corydalis solida* populations from Warsaw by supplementally pollinated (black) and control plants (grey). Seeds were scored from the three lowermost capsules. NAT – Natolin Forest; UBG – Warsaw University Botanic Garden; BIE – Bielański Forest. Error lines indicate 0.95 confidence limits of the mean.

Seed set

For all study populations, significant seed production was generally observed only in supplementally pollinated (experimental variant A) and control plants (variant B). For both selfing variants [isolated flowers (C) and flowers pollinated with geitonogamous pollen (D)], we found that either no seeds at all had been produced (in 2013) or, on average, no more than a single seed per three lowermost capsules (in 2012), the last result probably having been caused by inadequate cleaning of the brush. For

Discussion

According to our expectations *Corydalis solida* studied in urban conditions showed reduced seed set in open pollinated flowers when compared to pollen-supplemented plants. However, the extent of pollen limitation varied in our study populations (0.07–0.63) and was generally greater in 2012, a year which coincided with overall lower seed production in all populations and lower floral visitors' diversity and activity. Similar temporal and spatial fluctuations in pollen limitation are observed in many plant species [40], possibly due to a stochastic pollination environment [41]. In spring wildflower communities, for example, stochasticity may be caused by early spring onset resulting in a phenological mismatch between plants and pollinators. We observed that in 2012, when March and April air temperature was higher

than in 2013, when the last snow melted at the beginning of April. This coincided with earlier flowering and a three-fold reduction in the number of recorded insect visits and nearly 3-fold increase in pollen limitation index. A similar early onset of flowering in *C. solida* was observed in 2012 for other parts of the species' range [24].

Such a phenomenon was previously observed for other bumblebee-pollinated vernal geophytes (e.g., [18]) and resulted in a reduction in *C. ambigua* seed production [19,42]. This clear relationship, as suggested by Kudo and Ida [19], is due to the plant depending on a very narrow pollinator guild, and the phenological window for reciprocal interactions which is temporarily limited to a very short period in spring. Vegetation and flowering in spring ephemeral plant species appears to be mainly controlled by the date of snowmelt, the cumulative temperature, and the prevailing temperature 1–2 months before flowering [43–45], whereas emergence of young queen bumblebees is influenced more by soil temperature [19,46]. Therefore, during a particularly early spring, conditions are likely to favor the growth of spring ephemerals before the emergence of bumblebee queens has occurred. During warm days, the nights may still be rather cold, maintaining cool soil temperatures.

The core of floral visitors to all our study populations consisted of over-wintered and freshly-emerged bumblebee queens. Since they are able to operate even during harsh weather, they are one of the most important pollinators of temperate regions during the initial weeks of spring (see, e.g., [13,18,24,47–50]). Queens have been observed searching mainly for nectar on some spring-flowering plant species, and appear to express less floral constancy than workers [18,47,50]. Bumblebee queens can therefore probably pollinate flowers in plant populations consisting of only a small number of individuals. Apart from bumblebees, our study plants were visited by honeybees, and populations in UBG and BIE were also visited by representatives of a few other insect groups. As shown for honey bees, in Polish conditions early advance of spring speeds up the first emergence of workers, the so called “cleansing flight” [51], therefore in years with particularly early spring onset the absence of *Bombus* pollinators may be compensated by the presence of *A. mellifera* individuals. In urban conditions, however, this would be restricted to populations in the proximity to the apiaries.

In *Corydalis* flowers, honeybees usually forage for pollen, whereas bumblebees forage for nectar [24]. Owing to their specialized floral structure [21,24,27], pollen can be extracted only with great difficulty from *Corydalis* flowers without touching their reproductive parts, resulting in highly effective pollination by honeybees. *Corydalis solida* flowers produce nectar concealed in a long floral spur [21,24] and, when visited legitimately, they are pollinated by long-tongued insects, such as bumblebees. This species, and its congeners are also frequently visited by nectar robbers [21,24,27,52]. In all three populations, we observed signs of floral larceny (pierced floral spurs), which could indicate the inferior performance of bumblebees as pollinators. This is not necessarily the case, since in *C. solida*, pollination by *Bombus* queens can probably be ensured, even during robber visits, because of a mechanism described by Higashi et al. [52] for the closely related *C. ambigua*. Furthermore, as shown for *C. ambigua* and *C. caseana*, a high proportion of robber visits may actually benefit the population, since the increased movement of pollinators between plants caused by lower reward increases the distance of pollen transfer within the population, thereby reducing geitonogamous pollination and pollen discounting [26,53].

Social bees (*A. mellifera* and *Bombus* queens), seem the most effective pollinators of *C. solida*, which agrees with earlier observations from Poland [24], and is similar to the pollination system of closely related *C. cava* [13]. Our results, however, contradict Knuth's [21] suggestions that its key pollinator is the solitary bee *Anthophora plumipes*. Indeed, we observed *Anthophora* bees visiting flowers in BIE and UBG, and greater floral visitor diversity and numbers observed in 2013 resulted in overall increase in seed set (this solitary bee was also observed visiting *C. cava* flowers [13]). But this means that other floral visitors, i.e., solitary bees and bee flies, also contribute to seed production, and pollination system of *C. solida* is more generalized than could be predicted from its morphologically specialized flowers. Unfortunately, we did not perform any analysis of insect effectiveness, so the contribution of certain groups remains unclear. However, our results for 2013 in BIE, when very few bee visits were observed, and a large proportion of insect visits was made by bee flies show that Knuth's

[21] opinion disregarding Bombyliidae as legitimate pollinators of *C. solida* was at least premature. Also the role of solitary bees seems equivocal. As shown for other spring ephemerals, although capable of transferring large pollen loads, solitary bees show less floral constancy than bumblebees and honeybees, leading to more hetero-specific pollination [18,49]. This may explain our results for UBG population, where increased visitation (especially in 2013; Fig. 4), possibly caused by larger floral displays and magnet roles of neighboring plant species, did not translate into markedly greater seed production. Increased inflorescence size in this population was probably simply derived in response to local growing conditions. In order to attract pollinators, plants growing in the forests of NAT and BIE produce longer scapes that lift their flowers above the thick layer of leaf litter that is characteristic of these communities. Their production of smaller floral displays, when compared to the UBG population, seems a simple trade-off for this adaptation (Tab. 2).

Apart from pollen limitation in 2012, our survey generally showed low seed production in supplementally pollinated plants that year. Since *C. solida* is a tuberous ephemeral, every spring producing annual shoots that last only for a few weeks, this could be caused by a change in the availability of resources during experimental years. As shown for another representative of the genus, *C. ambigua*, seed production in such plants is partly supported by carbohydrate reserves in the old tuber tissue, whereas nectar production relies on current photosynthesis. This, in effect, indicates that these two processes do not necessarily compete for the same carbon pool [54]. It is possible, as suggested by these authors, that adverse spring conditions for photosynthesis may have a negative effect on reproductive performance during the following year, since seed production partly depends on resources stored in tubers the preceding spring. Given that 2011, the year preceding our experiments, was dry with very little precipitation, it is likely that due to lower photosynthetic efficiency during that season, plants laid down less carbohydrate resources for use in seed production during 2012, thus resulting in an overall reduced lower seed set that year.

Contrary to our expectations, the present study demonstrated that pollen limitation, although constantly observed in our urban populations of specialist, spring ephemeral and self-incompatible *C. solida*, is mostly related to phenological mismatch between flowers and pollinators. This seems rather surprising given the predictions based on literature [14,17] and the specialized floral morphology of *C. solida*. However, our results show that, to use the most recent terminology [55,56], being a morphological specialist our study plant is in fact a functional generalist pollinated by mostly generalist insects from several functional groups (guilds). Such a generalist pollination system can explain relatively low pollen-limitation of urban populations of *C. solida* in predictable weather conditions. Although urban habitats may still be places of considerable pollinator diversity [57–59], many studies have shown that urban pollinator assemblages mostly include ecological generalists [59–61]. This also seems to be true for spring wild-flower communities [18,47]. Plant-animal interactions are mostly asymmetrical, and pollination networks studied so far indicate that specialist insects tend to interact with generalist plants, whereas both generalist and specialist plants are pollinated by generalist insects [62–64]. Thus, as has been proposed by Ashworth et al. [65], generalist plants have a greater number of mutualistic partners (both generalist and specialist species) than do specialists, but the loss of specialist pollinators affects only generalist plants, which like specialist plants, retain only generalist mutualists. This, too, was reflected in our study populations, mostly serviced by honeybees and common bumblebee species. In the most isolated and homogenous (in terms of floral resources) population in NAT, they were the sole visitors during both study years. Rare, more specialized visitors (solitary bees, bee flies and butterflies) were recorded in the largest population (in terms of area occupied) in BIE, and the most diversified population (in terms of floral resources) in UBG. Some authors have shown that plant species serviced by generalist pollinators, such as bumblebees, appear to be resistant to the effect of urbanization. Recently, a lack of associated pollen limitation was, for example, reported for the predominantly bumblebee-pollinated plants *Digitalis purpurea* L. [66] and *Trifolium repens* L. [9]. This effect, however, may be species-specific, since conversely, a significant decline in seed production was found for self-sterile bumblebee pollinated *Lotus corniculatus* L. [67], and the ornithogamous tree, *Bruguiera gymnorhiza* (L.) Lam. [68].

It is thus evident that plants do not react to habitat fragmentation in only one way, but that their response in terms of their reproductive output is highly species- and context-dependent. For some plants, local-scale habitat and ecosystem characteristics, including pollinator availability, co-flowering plant species or green/impervious area ratio, may be more important for successful seed production than habitat loss on landscape scale [67,69]. For spring ephemerals, such as *C. solida*, habitat fragmentation may be less important, but changes in weather conditions may dramatically affect their reproductive success, indicating the great vulnerability of such plant species to the climatic changes that are currently occurring. For *C. solida* one of the possible scenarios may well include increased pollen limitation of urban populations. Alternatively, provided that absence of natural pollinators can be compensated by introduced taxa, such as honeybees, such populations could be relatively stable in terms of their reproductive output. Lately urban apiculture has been increasingly popular [70], which results, for instance, in saturation of the urban environment with extra pollinators. Coupling that with information on temperature-induced earlier appearance of *A. mellifera* workers [51] suggests that this process may alleviate negative consequences of phenological mismatch at least in some urban plant populations. This however awaits further studies.

Acknowledgments

We thank Kamil Kwiecień for help in the field, Katarzyna Roguz for translating Knuth's (1898) description of *Corydalis*, and anonymous reviewers for valuable suggestions to improve the manuscript. Field work in nature reserves was conducted with the permission of the Regional Nature Conservator Office in Warsaw.

References

1. Grimm NB, Faeth SH, Golubiewski NE, Redman CL, Wu JG, Bai XM, et al. Global change and the ecology of cities. *Science*. 2008;319(5864):756–760. <http://dx.doi.org/10.1126/science.1150195>
2. Seto KC, Fragkias M, Guneralp B, Reilly MK. A meta-analysis of global urban land expansion. *PLoS One*. 2011;6(8):e23777. <http://dx.doi.org/10.1371/journal.pone.0023777>
3. Goddard MA, Dougill AJ, Benton TG. Scaling up from gardens: biodiversity conservation in urban environments. *Trends Ecol Evol*. 2010;25(2):90–98. <http://dx.doi.org/10.1016/j.tree.2009.07.016>
4. Magrach A, Laurance WF, Larrinaga AR, Santamaria L. Meta-analysis of the effects of forest fragmentation on interspecific interactions. *Conserv Biol*. 2014;28(5):1342–1348. <http://dx.doi.org/10.1111/cobi.12304>
5. Willmer P. *Pollination and floral ecology*. Princeton, NJ: Princeton University Press; 2011. <http://dx.doi.org/10.1515/9781400838943>
6. Knight TM, Steets JA, Vamasi JC, Mazer SJ, Burd M, Campbell DR, et al. Pollen limitation of plant reproduction: pattern and process. *Ann Rev Ecol Evol Syst*. 2005;36:467–497. <http://dx.doi.org/10.1146/annurev.ecolsys.36.102403.115320>
7. Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol*. 2010;25(6):345–353. <http://dx.doi.org/10.1016/j.tree.2010.01.007>
8. Newman BJ, Ladd P, Brundrett M, Dixon KW. Effects of habitat fragmentation on plant reproductive success and population viability at the landscape and habitat scale. *Biol Conserv*. 2013;159:16–23. <http://dx.doi.org/10.1016/j.biocon.2012.10.009>
9. Verboven HAF, Aertsen W, Brys R, Hermy M. Pollination and seed set of an obligatory outcrossing plant in an urban–peri-urban gradient. *Perspect Plant Ecol Evol Syst*. 2014;16(3):121–131. <http://dx.doi.org/10.1016/j.ppees.2014.03.002>
10. Eckert CG, Kalisz S, Geber MA, Sargent R, Elle E, Cheptou PO, et al. Plant mating systems in a changing world. *Trends Ecol Evol*. 2010;25(1):35–43. <http://dx.doi.org/10.1016/j.tree.2009.06.013>
11. Thomann M, Imbert E, Devaux C, Cheptou PO. Flowering plants under global

- pollinator decline. *Trends Plant Sci.* 2013;18(7):353–359. <http://dx.doi.org/10.1016/j.tplants.2013.04.002>
12. Richards AJ. *Plant breeding systems*. Oxford: Bios Scientific; 1997. <http://dx.doi.org/10.1007/978-1-4899-3043-9>
 13. Myczko Ł, Banaszak-Cibicka W, Sparks T, Tryjanowski P. Do queens of bumblebee species differ in their choice of flower colour morphs of *Corydalis cava* (Fumariaceae)? *Apidologie*. 2015;46(3):337–345. <http://dx.doi.org/10.1007/s13592-014-0326-x>
 14. Johnson SD, Steiner KE. Generalization versus specialization in plant pollination systems. *Trends Ecol Evol.* 2000;15(4):140–143. [http://dx.doi.org/10.1016/S0169-5347\(99\)01811-X](http://dx.doi.org/10.1016/S0169-5347(99)01811-X)
 15. Aizen MA, Ashworth L, Galetto L. Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter? *J Veg Sci.* 2002;13(6):885–892. <http://dx.doi.org/10.1111/j.1654-1103.2002.tb02118.x>
 16. Corbet SA. A typology of pollination systems: implications for crop management and the conservation of wild plants. In: Waser NM, Ollerton J, editors. *Plant-pollinator interactions from specialization to generalization*. Chicago, IL: The University of Chicago Press; 2006. p. 315–340.
 17. Aguilar R, Ashworth L, Galetto L, Aizen MA. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecol Lett.* 2006;9(8):968–980. <http://dx.doi.org/10.1111/j.1461-0248.2006.00927.x>
 18. Motten AF. Pollination ecology of the spring wildflower community of a temperate deciduous forest. *Ecol Monogr.* 1986;56(1):21–42. <http://dx.doi.org/10.2307/2937269>
 19. Kudo G, Ida TY. Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology.* 2013;94(10):2311–2320. <http://dx.doi.org/10.1890/12-2003.1>
 20. Hermy M, Honnay O, Firbank L, Grashof-Bokdam C, Lawesson JE. An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. *Biol Conserv.* 1999;91(1):9–22. [http://dx.doi.org/10.1016/S0006-3207\(99\)00045-2](http://dx.doi.org/10.1016/S0006-3207(99)00045-2)
 21. Knuth P. *Handbuch der Blütenbiologie, II Band, 1. Teil: Ranunculaceae bis Compositae*. Leipzig: Verlag von Wilhelm Engelmann; 1898.
 22. Fryxell PA. Mode of reproduction of higher plants. *Bot Rev.* 1957;23(3):135–233. <http://dx.doi.org/10.1007/BF02869758>
 23. Sikorski P. Wpływ naturyzacji parku miejskiego na różnorodność florystyczną runa i trawników parkowych. Warsaw: Wieś Jutra Sp.z o.o.; 2013.
 24. Denisow B, Strzałkowska-Abramek M, Bożek M, Jeżak A. Early spring nectar and pollen and insect visitor behavior in two *Corydalis* species (Papaveraceae). *J Apic Sci.* 2014;58(1):93–102. <http://dx.doi.org/10.2478/jas-2014-0009>
 25. Zając EU. *Papaveraceae*. In: Jasiewicz A, editor. *Dwuliścienne wolnopłatkowe – dwuokwiatowe*. 2nd ed. Warszawa: Państwowe Wydawnictwo Naukowe; 1985. p. 102–126. (Flora Polska. Rośliny Naczyniowe; vol 4).
 26. Kudo G, Maeda T, Narita K. Variation in floral sex allocation and reproductive success within inflorescences of *Corydalis ambigua* (Fumariaceae): pollination efficiency or resource limitation? *J Ecol.* 2001;89(1):48–56. <http://dx.doi.org/10.1046/j.1365-2745.2001.00512.x>
 27. Maloof JE. Reproductive biology of a North American subalpine plant: *Corydalis caseana* A. Gray ssp. *brandegei* (S. Watson) G.B. Ownbey. *Plant Species Biol.* 2000;15(3):281–288. <http://dx.doi.org/10.1111/j.1442-1984.2000.00047.x>
 28. Ohara M, Higashi S. Effects of inflorescence size on visits from pollinators and seed set of *Corydalis ambigua* (Papaveraceae). *Oecologia.* 1994;98(1):25–30. <http://dx.doi.org/10.1007/bf00326086>
 29. Luoto M, Kuussaari M, Rita H, Salminen J, von Bonsdorff T. Determinants of distribution and abundance in the clouded Apollo butterfly: a landscape ecological approach. *Ecography.* 2001;24(5):601–617. <http://dx.doi.org/10.1034/j.1600-0587.2001.d01-215.x>
 30. Ehlers BK. Geographic variation for elaiosome-seed size ratio and its allometric relationship in two closely related *Corydalis* species. *Plant Ecol Divers.* 2012;5(3):395–401. <http://dx.doi.org/10.1080/17550874.2012.695812>
 31. Czerwińska-Jędrusiak B. *Ludność i powierzchnia Warszawy w latach 1921–2008*. Warsaw: Urząd Statystyczny w Warszawie; 2009.
 32. Sikorski P, Borowski J, Sikorska D, Wierzba D, Kehl J, Włodarczyk S. Mało znane parki i zieleńce Warszawy jako rezerwuar dzikiej przyrody. In: Obidziński A, editor. *Z Mazowsza*

- na Polesie i Wileńszczyznę Zróżnicowanie i ochrona szaty roślinnej pogranicza Europy Środkowej i Północno-Wschodniej. Warsaw: Polish Botanical Society; 2010. p. 103–117.
33. Werblan-Jakubiec H, Zych M. University of Warsaw Botanic Garden. A guidebook. Warsaw: Muza SA; 2013.
 34. Solińska-Górnicka B, Symonides E. Effect of a large city on the structure of coenoelements in a natural woodland in Warsaw. *Vegetatio*. 1990;88:163–176. <http://dx.doi.org/10.1007/BF00044833>
 35. Elzinga CL, Salzer DW, Willoughby JW. Measuring and monitoring plant populations. Denver, CO: USDI Bureau of Land Management; 1998.
 36. Bogdanowicz W, Chudzicka E, Pilipiuk I, Skibińska E. Fauna of Poland. Characteristics and checklist of species. Warsaw: Museum and Institute of Zoology, Polish Academy of Sciences; 2004.
 37. Bogdanowicz W, Chudzicka E, Pilipiuk I, Skibińska E. Fauna of Poland. Characteristics and checklist of species. Warsaw: Museum and Institute of Zoology, Polish Academy of Sciences; 2007.
 38. Larson BMH, Barrett SCH. A comparative analysis of pollen limitation in flowering plants. *Biol J Linn Soc Lond*. 2000;69(4):503–520. <http://dx.doi.org/10.1111/j.1095-8312.2000.tb01221.x>
 39. Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, et al. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol*. 2009;24(3):127–135. <http://dx.doi.org/10.1016/j.tree.2008.10.008>
 40. Burd M. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Bot Rev*. 1994;60:83–139. <http://dx.doi.org/10.1007/BF02856594>
 41. Ashman TL, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, et al. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*. 2004;85(9):2408–2421. <http://dx.doi.org/10.1890/03-8024>
 42. Kudo G, Nishikawa Y, Kasagi T, Kosuge S. Does seed production of spring ephemerals decrease when spring comes early? *Ecol Res*. 2004;19(2):255–259. <http://dx.doi.org/10.1111/j.1440-1703.2003.00630.x>
 43. Diekmann M. Relationship between flowering phenology of perennial herbs and meteorological data in deciduous forests of Sweden. *Can J Bot*. 1996;74(4):528–537. <http://dx.doi.org/10.1139/b96-067>
 44. Fitter AH, Fitter RSR, Harris ITB, Williamson MH. Relationships between first flowering date and temperature in the flora of a locality in central England. *Funct Ecol*. 1995;9(1):55–60. <http://dx.doi.org/10.2307/2390090>
 45. Thórhallsdóttir TE. Flowering phenology in the central highland of Iceland and implications for climatic warming in the Arctic. *Oecologia*. 1998;114(1):43–49. <http://dx.doi.org/10.1007/s004420050418>
 46. Alford DV. A study of the hibernation of bumblebees (Hymenoptera: Bombidae) in southern England. *J Anim Ecol*. 1969;38(1):149–170. <http://dx.doi.org/10.2307/2743>
 47. Macior LW. Pollination ecology of vernal angiosperms. *Oikos*. 1978;30(3):452–460. <http://dx.doi.org/10.2307/3543340>
 48. Kudo G. Vulnerability of phenological synchrony between plants and pollinators in an alpine ecosystem. *Ecol Res*. 2014;29(4):571–581. <http://dx.doi.org/10.1007/s11284-013-1108-z>
 49. Zych M, Stpiczyńska M. Neither protogynous nor obligatory out-crossed: pollination biology and breeding system of the European red list *Fritillaria meleagris* L. (Liliaceae). *Plant Biol*. 2012;14:285–294. <http://dx.doi.org/10.1111/j.1438-8677.2011.00510.x>
 50. Zych M, Goldstein J, Roguz K, Stpiczyńska M. The most effective pollinator revisited: pollen dynamics in a spring-flowering herb. *Arthropod Plant Interact*. 2013;7(3):315–322. <http://dx.doi.org/10.1007/s11829-013-9246-3>
 51. Sparks TH, Langowska A, Głazaczow A, Wilkaniec Z, Bieńkowska M, Tryjanowski P. Advances in the timing of spring cleaning by the honeybee *Apis mellifera* in Poland. *Ecol Entomol*. 2010;35(6):788–791. <http://dx.doi.org/10.1111/j.1365-2311.2010.01226.x>
 52. Higashi S, Ohara M, Arai H, Matsuo K. Robber-like pollinators – overwintered queen bumblebees foraging on *Corydalis ambigua*. *Ecol Entomol*. 1988;13(4):411–418. <http://dx.doi.org/10.1111/j.1365-2311.1988.tb00373.x>

53. Maloof JE. The effects of a bumble bee nectar robber on plant reproductive success and pollinator behavior. *Am J Bot.* 2001;88(11):1960–1965. <http://dx.doi.org/10.2307/3558423>
54. Kudo G, Ida TY. Carbon source for reproduction in a spring ephemeral herb, *Corydalis ambigua* (Papaveraceae). *Funct Ecol.* 2010;24(1):62–69. <http://dx.doi.org/10.1111/j.1365-2435.2009.01601.x>
55. Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. Pollination syndromes and floral specialization. *Annu Rev Ecol Evol Syst.* 2004;35:375–403. <http://dx.doi.org/10.1146/annurev.ecolsys.34.011802.132347>
56. Ollerton J, Killick A, Lamborn E, Watts S, Whiston M. Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon.* 2007;56(3):717–728. <http://dx.doi.org/10.2307/25065856>
57. Bates AJ, Sadler JP, Fairbrass AJ, Falk SJ, Hale JD, Matthews TJ. Changing bee and hoverfly pollinator assemblages along an urban-rural gradient. *PLoS One.* 2011;6(8):e23459. <http://dx.doi.org/10.1371/journal.pone.0023459>
58. Hennig E, Ghazoul J. Pollinating animals in the urban environment. *Urban Ecosyst.* 2012;15:149–166. <http://dx.doi.org/10.1007/s11252-011-0202-7>
59. Jędrzejewska-Szmek K, Zych M. Flower-visitor and pollen transport networks in a large city: structure and properties. *Arthropod Plant Interact.* 2013;7(5):503–516. <http://dx.doi.org/10.1007/s11829-013-9274-z>
60. Banaszak-Cibicka W, Żmihorski M. Wild bees along an urban gradient: winners and losers. *J Insect Conserv.* 2012;16(3):331–343. <http://dx.doi.org/10.1007/s10841-011-9419-2>
61. Geslin B, Gauzens B, Thébault E, Dajoz I. Plant-pollinator networks along a gradient of urbanisation. *PLoS One.* 2013;8(5):e63421. <http://dx.doi.org/10.1371/journal.pone.0063421>
62. Bascompte J, Jordano P, Melian CJ, Olesen JM. The nested assembly of plant-animal mutualistic networks. *Proc Natl Acad Sci USA.* 2003;100(16):9383–9387. <http://dx.doi.org/10.1073/pnas.1633576100>
63. Bascompte J, Jordano P, Olesen JM. Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science.* 2006;312(5772):431–433. <http://dx.doi.org/10.1126/science.1123412>
64. Stang M, Klinkhamer PGL, van der Meijden E. Asymmetric specialization and extinction risk in plant-flower visitor webs: a matter of morphology or abundance? *Oecologia.* 2007;151(3):442–453. <http://dx.doi.org/10.1007/s00442-006-0585-y>
65. Ashworth L, Aguilar R, Galetto L, Aizen MA. Why do pollination generalist and specialist plant species show similar reproductive susceptibility to habitat fragmentation? *J Ecol.* 2004;92(4):717–719. <http://dx.doi.org/10.1111/j.0022-0477.2004.00910.x>
66. Verboven HAF, Brys R, Hermy M. Sex in the city: reproductive success of *Digitalis purpurea* in a gradient from urban to rural sites. *Landsc Urban Plan.* 2012;106(2):158–164. <http://dx.doi.org/10.1016/j.landurbplan.2012.02.015>
67. Pellissier V, Muratet A, Verfaillie F, Machon N. Pollination success of *Lotus corniculatus* (L.) in an urban context. *Acta Oecol (Montrouge).* 2012;39:94–100. <http://dx.doi.org/10.1016/j.actao.2012.01.008>
68. Wee AKS, Low SY, Webb EL. Pollen limitation affects reproductive outcome in the bird-pollinated mangrove *Bruguiera gymnorrhiza* (Lam.) in a highly urbanized environment. *Aquat Bot.* 2015;120:240–243. <http://dx.doi.org/10.1016/j.aquabot.2014.09.001>
69. Williams NM, Winfree R. Local habitat characteristics but not landscape urbanization drive pollinator visitation and native plant pollination in forest remnants. *Biol Conserv.* 2013;160:10–18. <http://dx.doi.org/10.1016/j.biocon.2012.12.035>
70. Moore LJ, Kosut M. Buzz: urban beekeeping and the power of the bee. New York, NY: New York University Press; 2013.
71. Central Statistical Office. Statistical Bulletin. Warsaw: Central Statistical Office; 2012.
72. Central Statistical Office. Statistical Bulletin. Warsaw: Central Statistical Office; 2013.
73. Central Statistical Office. Statistical Bulletin. Warsaw: Central Statistical Office; 2014.