



Evaluating pollen flow indicators for an insect-pollinated plant species

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Abstract

For insect-pollinated plant species, reproductive success and genetic exchange via the transfer of pollen between flowers depends (i.a.) on the efficiency, abundance and behaviour of floral visitors. These in turn are expected to respond to plant population size and flower density. High floral densities for example usually attract large numbers of pollinators that visit more flowers per plant or patch, which increases pollen deposition at short distances. Thus, population characteristics might serve as indicators for pollen dispersal patterns and help to identify suitable habitat size and quality for conservation measures. To test this hypothesis, we observed floral visitors of a generalist, entomophilous species, *Comarum palustre*, and compared their abundance and visitation rates in populations of different sizes and flower densities. At the same time, we mimicked pollen flow using fluorescent dye. In the large and dense populations, pollinator abundance and visitation rates were high and dye was dispersed to the edges of the populations (up to 200 m). In the medium-sized population with high flower density, insect abundance and visitation rates were unexpectedly low and dye dispersal declined very quickly. On the contrary, in the smallest population with scattered flowers, especially bumble bee abundance was similar to the large populations and dye dispersal mirrored this high bumble bee activity. Thus, our results indicate that in smaller habitat fragments, the mere size of a population might be insufficient to suggest pollen flow for a plant species. Instead, the abundance of its major pollinators should be considered.

Zusammenfassung

Die Ausbreitung von Pollen hängt bei insektenbestäubten Pflanzen u.a. von der Effizienz, der Häufigkeit und dem Verhalten der Blütenbesucher ab. Diese wiederum werden häufig durch die Größe der Pflanzenpopulationen sowie deren Dichte an Blüten bedingt. Zum Beispiel locken hohe Blütendichten meist auch eine große Bestäuberzahl an, die dann mehr Blüten pro Pflanze bzw. Standort besuchen. Folglich wird Pollen zwar in großen Mengen, aber nur über kurze Distanzen ausgebreitet. Populationsgröße und Blütenangebot könnten daher als gute Indikatoren für Pollenfluss dienen, was im Naturschutz helfen könnte, die geeignete Größe und Qualität von Lebensräumen zu definieren. Um diese Hypothese zu testen, beobachteten wir die Blütenbesucher einer insektenbestäubten, generalistischen Pflanzenart (*Comarum palustre*) und verglichen deren Häufigkeit und Besuchsraten in Populationen von unterschiedlicher Größe und Blütendichte. Gleichzeitig imitierten wir den Pollentransfer mit fluoreszierendem Puder. In den großen und dichten Populationen waren die Häufigkeit und Besuchsraten der Bestäuber erwartungsgemäß hoch, und der Puder wurde über die gesamte Fläche der Populationen ausgebreitet (bis zu 200 m). Überraschenderweise waren die Abundanz und Besuchsraten von Insekten in einer mittelgroßen Population mit hoher Blütendichte sehr niedrig und die Übertragung von Puder nahm schnell ab. In der kleinsten Population dagegen mit nur geringem Blütenaufkommen, war besonders die Anzahl der Hummeln vergleichbar mit den großen Populationen, was sich in der Verteilung des Puders widerspiegelte.

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Unsere Ergebnisse legen den Schluss nahe, dass in kleineren Habitatfragmenten, die Größe und Dichte einer Population allein nur unzureichende Rückschlüsse auf den Pollenfluss einer Pflanze erlaubt. Stattdessen sollte auch die Häufigkeit der effizientesten Bestäuber in Betracht gezogen werden.

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Keywords: Fluorescent dye; Pollen dispersal; Flower visitation rate; Insect abundance; Flower density; *Bombus* spp.

Introduction

Besides propagation via seeds, pollen dispersal is one of the sole opportunities for plants to achieve gene flow within and among populations (Levin & Kerster 1974; Barrett 2003). For insect-pollinated species, pollen flow between flowers is greatly influenced by the spatial distribution of plants, floral characteristics (e.g. size, colour, rewards) as well as the efficiency and behaviour of the different floral visitors (Waser 1983; Cresswell, Bassom, Bell, Collins, & Kelly 1995; Barrett 2003; Young, Dunning, & Hasseln 2007). In general, most pollen is deposited at short distances on the first few flowers visited and only occasionally over long ranges (leptocurtic distribution, e.g. Thomson & Plowright 1980; Holmquist, Mitchell, & Karron 2011; Van Rossum, Stiers, Van Geert, Triest, & Hardy 2011). The amount of pollen a flower receives may accumulate with higher abundances of pollinators (Thomson, Price, Waser, & Stratton 1986; Aizen 2001; Elliott & Irwin 2009). Visitor abundances in turn often increase with patch or population size, flower density or both (Sowig 1989; Kunin 1997; Bernhardt, Mitchell, & Michaels 2008). At high floral densities, pollinators usually probe more flowers per foraging bout, which may increase pollen deposition at short distances (Harder & Barrett 1996; Karron, Holmquist, Flanagan, & Mitchell 2009). We may thus conclude that both abundance and distance of pollen flow is closely linked to flower density and plant population size (Karron, Thumser, Tucker, & Hessenauer 1995; Duncan, Nicotra, Wood, & Cunningham 2004; Wailes & Ågren 2004). Yet, decreases in plant population size through habitat loss and fragmentation can disrupt plant–pollinator interactions and impact on pollen flow (Kwak, Velterop, & van Andel, 1998; Ghazoul 2005). Habitat fragmentation through human activities and the appropriate size and quality of remaining fragments are of primary concern for the conservation of species (Fahrig 2003; Mayer et al. 2011). Better knowledge on pollen dispersal patterns in fragmented habitats is therefore needed to define appropriate management strategies (Volis, Bohrer, Oostermeijer, & Van Tienderen 2005; Menz et al. 2011).

Pollinator visitation and pollen deposition have often been studied, but research that combines spatial patterns of pollen flow with simultaneous observations on pollinators is rare (Mitchell, Irwin, Flanagan, & Karron 2009). We recorded floral visitors during standardised flower observations and mimicked pollen flow with fluorescent powdered dye in four populations of the Marsh Cinquefoil, *Comarum palustre* (Rosaceae). The use of fluorescent dye particles as pollen

analogue for entomophilous plants is a successful, convenient and cheap method to track pollinator movements and pollen dispersal, even at the landscape scale (Campagne, Affre, Baumel, Roche, & Taton 2009; Van Geert, Van Rossum, & Triest 2010). Our model species, *C. palustre*, is an insect-pollinated herb visited by a large variety of insects, including bumble bees that are known to be efficient pollinators (Olesen & Warncke 1992; Goulson 2003; C.M. pers. obs.). *C. palustre* grows in peat bogs, fens, wet meadows and edges of water bodies. These biotopes have been largely destroyed and fragmented in Belgium since the middle of the 19th century (Verté 2007). The remaining habitat fragments provide an ideal scenario with populations of variable sizes and densities. In this study, we tested the hypothesis that population size, flower density or both serve as predictors for the amount and distance of pollen dispersal by influencing the abundance of insect visitors (especially bumble bees) and flower visitation rates.

Materials and methods

Plant species

C. palustre L. (Rosaceae) is a clonal, perennial plant species flowering from May to July. An inflorescence consists of a sympodial cyme with an average of seven purple flowers (Olesen & Warncke 1992). The flowers are bowl-shaped with a mean diameter of 25 mm (± 4 mm, mean $\pm$ SD, $n = 60$, C.M. unpublished data). The centre of the hypanthium is formed by a spongy receptacle and carries large numbers of single-ovuled carpels (227 ± 71 , $n = 113$). A flower has about 22 (± 3) anthers each producing over 16,000 pollen grains (± 4800 , $n = 60$). Nectar is secreted between the perianth and the stamens (Olesen et al. 1992).

Study sites

We observed insect visitors and pollen dispersal in four populations (two large ones, a medium and a small one) located in protected fen areas in southern Belgium (Table 1). The geographic distance between neighbouring populations ranged from 1.3 to 3.7 km. With the help of GPS coordinates, we estimated the area (m²) of a habitat fragment that was covered with *C. palustre* plants. This area, including patches without the focal plant, is hereafter referred to as “population size” (Table 1). Flower density (number of open flowers per m², Table 1) was determined on 1-m² plots ($n = 20$ per population).

Table 1. Characteristics of four study populations of *Comarum palustre*: geographic coordinates, population size (in m²), number of flowers m⁻² (flower density), number of insect and bumble bee individuals, flower visitation rate (=number of visited flowers per number of open flowers) per 20 min flower observation, and proportion of flowers visited by bumble bee individuals per single foraging bout.

Population	Geographic coordinates Latitude (N) Longitude (E)	Population size (m ²)	Flower density (m ⁻²)	All insects		Bumble bees	
				Number of individuals	Flower visitation rate	Number of individuals	Proportion of flowers visited
Vance	49°40'14" 5°40'40"	7450	38 ^A (30–48)	15 ^A (10–23)	1.35 ^A (0.96–2.14)	7 ^A (4.3–11.5)	0.08 ^A (0.04–0.17)
Chantemelle	49°39'34" 5°39'54"	6422	41 ^A (32–51)	14.5 ^A (7.3–18)	1.36 ^A (0.89–2.02)	7.5 ^A (4.3–11.8)	0.08 ^A (0.04–0.15)
Plate ^a	49°41'52" 5°32'12"	3708	34 ^{AB} (24–48)	4.5 ^B (3–7.8)	0.32 ^B (0.16–0.46)	1 ^B (0.3–3.8)	0.08 ^A (0.04–0.15)
Stockem	49°40'30" 5°46'17"	1241	25 ^B (23–32)	8.5 ^B (2.5–12.8)	0.95 ^{AB} (0.26–1.55)	4 ^{AB} (0.3–8.8)	0.08 ^A (0.04–0.21)

Different capital letters indicate statistical differences according to Mann–Whitney *U* tests ($P < 0.05$, Bonferroni corrected); Data are presented as medians with lower and upper quartiles.

^a Plate-dessous-le-Monts⁺.

Floral visitors

We conducted flower observations on several sunny, warm days during the peak of flowering (between the 12th and 26th of June 2009). All open flowers were observed for 20 min on the same twenty 1-m² plots (established for flower density estimates), resulting in 20 × 20 min of flower observation in each population. The total observation time was 26.7 h. The minimum distance between two plots was ~3 m on one observation day and care was taken to spread the plots over the entire population. All flower visitors touching stigmas or anthers were recorded as well as the number of flowers they visited. We calculated flower visitation rates per 20 min, i.e. the number of flowers visited divided by the number of open flowers per m². Abundance (number of individuals) and visitation rates per observation were analysed for all insect species together as well as for bumble bees only. We further compared the number of flowers visited per bumble bee individual during a single foraging bout within a plot.

Estimating pollen dispersal using fluorescent dye

To estimate pollen dispersal, we used a different colour of fluorescent dye per population as pollen analogue (orange, yellow, pink or blue: Radiant Color Corp., Series Radglo[®] R). Flowers were marked with dye during a 3-day period (14th–16th June 2009). On day 1, dye was applied with wooden toothpicks to dehiscing anthers of 15 flowers (3–4 inflorescences) within an area of ~1 m² (=dye source). The dye source was located within the central third of the population to offer a large range of potential distances between the source and the recipient flowering ramets (Table 2). On day 3, stigmas from 2 to 5 receptive flowers were collected from 50 recipient ramets per population. In each population, the recipient ramets were randomly sampled following a transect across the entire population. The dye source and recipient ramets were mapped using a 50 m tape measure and a laser distance meter (Leica Disto A5). Stigmas were embedded in glycerine jelly on a microscope slide (Van Rossum 2010) and examined for dye particles at 250× under a fluorescence microscope. The abundance of dye on the stigmas was estimated for each sample according to four classes (0: no dye particles observed; 1: <5 particles; 2: 6–50 particles; 3: >50 particles).

Statistical analyses

We compared the investigated variables (flower density, number of insect and bumble bee individuals, visitation rates, proportion of flowers visited) among populations using non-parametric Kruskal–Wallis analyses. In case of significant results ($P < 0.05$), pairwise comparisons between populations were conducted with Mann–Whitney *U* tests and significance levels corrected following the conservative Bonferroni method (Dytham 2003).

Table 2. Dye dispersal results within four populations of *Comarum palustre*: potential distance to dye source in m (mean with ranges), observed distance of dye transfers in m (mean with ranges), distance of 80% of recipient ramets showing dye, proportion of recipient ramets showing dye, and dye abundance over the whole population (median with lower and upper quartiles).

Population	Potential distance to dye source (m)	Observed distance of dye transfers (m)	80% of ramets with dye (m)	Proportion of ramets with dye	Dye abundance
Vance	82.6 (0.4–199.4)	74.0 (0.4–199.4)	174.1	0.84	1 (1–2.25)
Chantemelle	55.5 (0.5–174.2)	46.6 (0.5–169.8)	61.1	0.90	2 (1–3)
Plate ^a	43.6 (0.1–137.0)	14.1 (0.1–96.8)	12.8	0.60	1 (0–2.25)
Stockem	20.9 (0.1–52.8)	20.4 (0.1–52.8)	38.0	0.98	1 (1–2)

^a“Plate-dessous-les-Monts”.

Since no dye transfers were observed between populations, the analyses only concerned within-population dye dispersal patterns. The relationship between dye abundance per recipient ramet (ordinal multinomial variable) and distance to dye source (Box Cox transformed) was examined for each population using a logistic regression analysis. The shape of the dye dispersal distribution was described using the best-fitting parameter β of the dye dispersal kernel, an exponential power function with mean dye abundance used to fit the function (for more details, see Hardy et al. 2004). The dispersal kernel is fat-tailed (leptokurtic distribution) when $\beta < 1$, and thin-tailed when $\beta > 1$. To test for differences in dispersal patterns between populations, we performed pairwise tests of homogeneity of slopes on dye abundance with the distance to dye source as independent variable (Box Cox transformed) and population as grouping variable. We used a logit link function for the model and significance was determined with a likelihood ratio Chi-square test. Depending on the size of the smaller population, pairwise comparisons were conducted for one, two or three distance ranges (up to 174, 139 or 59 m from the dye source). Gamma (Γ) correlation coefficients were calculated between dye abundance and the distance to dye source for each population and distance range. Analyses on dye were performed using STATISTICA, all other analyses were computed with R (R Development Core Team 2009).

Results

Abundance and visitation rates of insect visitors

In total, 892 insects from 46 different species were observed visiting flowers of *C. palustre* (Appendix A-Table A1). The main visitors were Hymenoptera ($n = 633$, between 52% and 89% per population, Fig. 1A), almost exclusively bees (Apoidea, 99%). Bumble bees (*Bombus* spp.) were the most abundant in all populations ($n = 460$, between 66% and 99% of all bees per population, Fig. 1B). In the two large populations, a quarter of all bees were honey bees (*Apis mellifera*, Appendix A and Fig. 1B). Other visitors included Diptera ($n = 172$, with 47% syrphid flies), Lepidoptera ($n = 43$), Coleoptera ($n = 39$) and Mecoptera ($n = 5$).

Flower density was similar in three populations while the smallest population, Stockem, had significantly lower flower numbers per m^2 than the two large ones ($\chi^2 = 17.23$, $df = 3$, $P < 0.001$; Table 1). The abundance of all insects differed significantly among populations ($\chi^2 = 31.03$, $df = 3$, $P < 0.001$; Table 1). Higher numbers of insects were counted in the large and dense populations (Vance and Chantemelle) than in the two smaller populations (Plate and Stockem). Flower visitation rates were similar in the smallest (Stockem) and the large populations. In Plate, a medium-sized population with high flower density, flower visitation rates of all insects were significantly lower than in the large populations ($\chi^2 = 25.93$, $df = 3$, $P < 0.001$; Table 1).

Regarding the main flower visitors, bumble bees, the highest numbers of individuals were counted in the two large, dense populations (Vance and Chantemelle). However, comparable abundances were observed in the smallest population

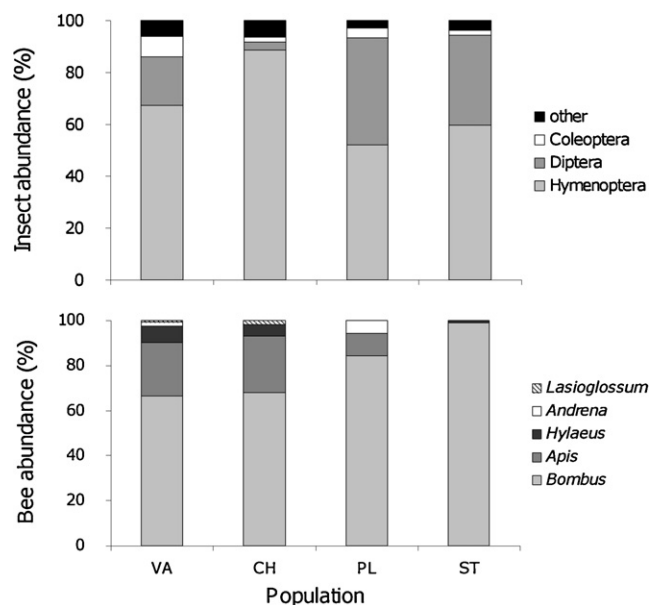


Fig. 1. Visitor abundance (%) observed on flowers of *Comarum palustre* for the different insect orders (A) and bee genera (B) during 20 observations of 20 min within each population (Legend: VA, Vance; CH, Chantemelle; PL, Plate-dessous-les-Monts; ST, Stockem).

Table 3. Differences in dye abundance patterns between pairs of populations for the same distance ranges (up to 174, 139 or 59 m; analyses of homogeneity of slopes with a logit link function and a likelihood ratio χ^2 test).

Comparison between	Vance		Chantemelle		Plate	
	χ^2	P	χ^2	P	χ^2	P
Chantemelle (up to 174 m)						
Population	2.41	0.121				
Distance to dye source	122.46	<0.001				
Population $\times$ distance to dye source	0.50	0.481				
Plate ^a (up to 139 m)						
Population	3.47	0.062	14.01	<0.001		
Distance to dye source	78.10	<0.001	82.10	<0.001		
Population $\times$ distance to dye source	6.39	0.011	1.89	0.169		
Stockem (up to 59 m)						
Population	18.22	<0.001	8.65	0.003	3.84	0.050
Distance to dye source	86.61	<0.001	59.64	<0.001	119.09	<0.001
Population $\times$ distance to dye source	4.25	0.039	1.02	0.313	21.57	<0.001

^a“Plate-dessous-les-Monts”.

with the lowest flower density (Stockem). Number of bumble bee individuals was the lowest in Plate ($\chi^2 = 19.76$, $df = 3$, $P < 0.001$; Table 1). Similarly, bumble bee visitation rates were significantly higher in Vance and Chantemelle compared to Plate but not to Stockem (Table 1). The proportion of flowers visited by bumble bees during a single foraging bout was equal in all populations ($\chi^2 = 4.02$, $df = 3$, $P = 0.26$; Table 1).

Dye dispersal patterns

The proportion of recipient ramets with dye varied from 0.60 in Plate to 0.98 in Stockem (Table 2). Dye was transferred from 0.1 to 199.4 m. This distance usually corresponded to the entire population range, except for Plate, where dye was dispersed over only 70% of the population area (Table 2). In this population, 80% of the recipient ramets with dye occurred within the first 12.8 m (=1/10 of the range of the population). In the other three populations, 80% of the recipient ramets with dye were distributed over 1/3 and 3/4 of the population area (Table 2).

Dye abundance and the distance to dye source were negatively related in all four populations (logistic regression: $\chi^2 = 51.84$ – 75.31 , $\Gamma = -0.78$ to -0.94 , $P < 0.001$). Dye dispersal followed a fat-tailed or leptokurtic distribution (Fig. 2), as the best-fitting β parameter describing the shape of the distribution curves was <1 (0.12–0.93). In the two large populations (Vance and Chantemelle), dye dispersal patterns did not differ significantly within 174 m distance from the dye source (test of homogeneity of slopes, Table 3). On the contrary, when comparing with the smaller populations for the same distance ranges (139 or 59 m), significant differences in dye dispersal patterns could be found (Table 3). Within 139 m, dye abundance in Plate (1; 0–2 = median; lower and upper quartile) was significantly lower than in Chantemelle (2; 1–3). Compared to Vance ($\Gamma = -0.86$), the slope of the

curve in Plate was significantly steeper ($\Gamma = -0.94$), i.e. dye abundance declined much faster (significant interaction population $\times$ distance to dye source, Table 3). Within 59 m, dye abundance in the smallest population was lower (Stockem; 1; 1–2) than in the two large populations (3; 1–3 for Vance and 3; 2–3 for Chantemelle). However, the slope of the curve ($\Gamma = -0.88$) in Stockem was significantly shallower than in Plate ($\Gamma = -0.97$) and Vance ($\Gamma = -1.00$; significant interaction population $\times$ distance to dye source, Table 3). Thus, the abundance of dye declined more slowly in this small population and long-distance dispersal was more efficient than in Plate and Vance.

Discussion

The open, bowl-shaped flowers of *C. palustre* attracted a large variety of insects. Bumble bees (*Bombus* spp.) were the most abundant floral visitors in all populations. They are usually considered to be efficient pollinators since they transfer high amounts of pollen and are capable to fly long distances (up to 2 km) (Young et al. 2007; Osborne et al. 2008). The highest numbers of insect individuals in general and bumble bee individuals in particular were counted in the two large and dense populations that also received the highest visitation rates per flower. Probably, greater amounts of resources in such large populations attract more insects (Wailes & Ågren 2004; Makino, Ohashi, & Sakai 2007; Elliott & Irwin 2009). Moreover, in the large and dense populations of *C. palustre*, high amounts of dye were dispersed over the entire range of the populations (up to 200 m). In short, in such large habitat fragments, large population size could stand for high pollinator activities and eventually efficient pollen (dye) flow. Previous studies have reported that pollinator activity as well as dye or pollen dispersal are positively affected by plant population size, density or both (e.g. Bernhardt et al. 2008; Van Rossum & Triest 2010; Nattero, Malerba, Medel, & Cocucci

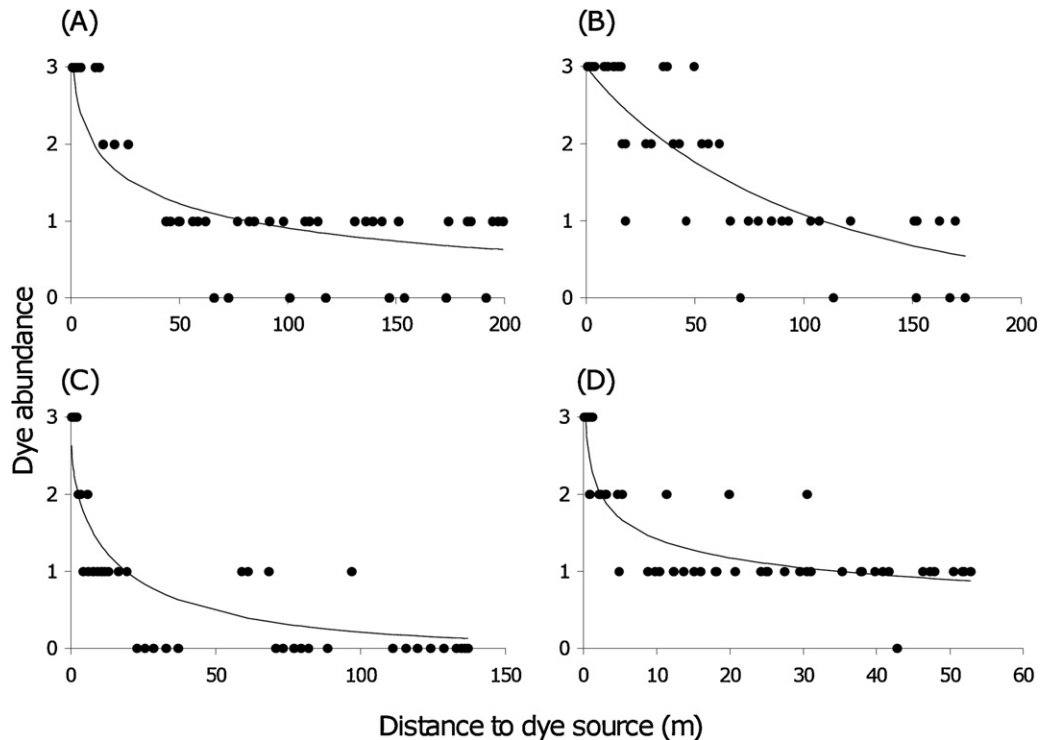


Fig. 2. Distribution of dye deposition (dye abundance) as a function of the distance to dye source in four study populations of *Comarum palustre*. (A) Vance, (B) Chantemelle, (C) Plate (=“Plate-dessous-les-Monts”), and (D) Stockem. The line represents the distribution expected under the exponential power dispersal model for best-fitting parameters.

2011), but studies combining such observations remain rare (Mitchell et al. 2009).

However, when habitat fragment size decreased, the observed differences of dye dispersal patterns between the populations seemed no longer linked to plant population size or flower density. Indeed, in a medium-sized population with high flower density (Plate), dye was dispersed to very short distances (<15 m on average). Long-distance dispersal was even less efficient than in the smallest population with the lowest flower density (Stockem). At the same time, we observed low abundances and visitation rates of insects and particularly of bumble bees in this medium-sized population, suggesting that this population might be pollinator-limited due to other factors than those investigated. In contrast, in the smallest population (Stockem), the number of bumble bee individuals and visitation rates were similar to the large populations. Dye was dispersed to all but one of the sampled recipient ramets and reached the extremities of the population. Dye abundance in Stockem was lower than in the larger populations, but it declined less rapidly and was efficiently transported over long distances. Honey bees, which are considered good pollen dispersers during nectar collection (Young et al. 2007), were not present at Stockem. These results add to our conclusion that bumble bees were the major pollen vectors for *C. palustre* and that they were responsible for the observed dye transfer patterns. Population size and flower density might therefore serve as indicators for

pollen flow in large populations, but not necessarily in smaller fen fragments. Other factors, such as vegetation structure (height) or heterospecific richness of the surrounding vegetation, could impact on insect attraction and translate into pollen dispersal (Van Geert et al. 2010; Holzschuh, Dormann, Tschantke, & Steffan-Dewenter 2011). Given the small number of studied populations, this certainly deserves further investigation in additional populations with different combinations of size and density for confirmation and general conclusions.

Conclusion

Flower visitation rates can be a good proxy for pollen deposition (Engel & Irwin 2003). We found that it can also be a good indicator for pollen abundance and dispersal distance. However, our findings also indicate that plant population size and flower density might not in all cases serve as reliable indicators for pollen flow patterns. Still, habitat size is often used as crucial factor in conservation projects. We recommend looking one step further at abundance and visitation rates of the most efficient pollinators in small plant populations. Our study also emphasizes the value of even small habitat fragments for the conservation of rare plant species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.baae.2012.09.012>.

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