Stepping-stone populations in linear landscape elements increase pollen dispersal between urban forest fragments

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Background and aims – Understanding how to ensure connectivity by gene flow between insect-pollinated plant populations is essential for designing functional ecological networks in a human-dominated matrix such as in urban settings. Linear landscape elements (LLEs) might contribute to such connectivity by increasing pollen transfer.

Methods – Pollen dispersal was investigated for the insect-pollinated herb *Primula elatior* in urban forest remnants, using fluorescent dye as a pollen analogue, in three study sites comprising population pairs located in fragments connected or not by a wooded LLE. We examined whether the presence of a small patch of plants of *P. elatior* placed within the LLE (stepping-stone population) might increase pollen dispersal compared to an LLE where *P. elatior* is absent.

Key results – Dye dispersal showed a leptokurtic decay distribution, with most dye transfer events at short distances (80% at less than 15.4–114.8 m) and a maximal distance of 647.5 m. Populations not connected by an LLE, separated by distances of 249 m or more, did not exchange dye, whereas populations that were connected by LLEs (up to 602 m) did show interpopulation dye transfer, suggesting that LLEs function as biological corridors facilitating dye dispersal. The presence of a stepping-stone population of *P. elatior* within the LLE increased between-population dye transfers (between 1.8 and 3.3 times the proportion of visited individuals) and deposition distances, compared to an LLE with no stepping stone, at least when pollen flow already existed.

Conclusions – These results provide evidence that LLEs may be more effective for pollen dispersal when serving as both movement conduit and habitat. In urban settings, developing a network of core populations connected by LLEs comprising small, even temporary, stepping-stone populations should be encouraged.

Key words – biological corridor, connectivity, dispersal kernel, fluorescent dye, insect pollination, linear landscape elements, pollen dispersal, *Primula elatior*, stepping stone, urban surrounding matrix.

INTRODUCTION

The restoration of connectivity by gene flow, especially by pollen, between fragmented remnants is often put forward as possibly contributing to ensure sustainable persistence of insect-pollinated plant species populations. Indeed, gene exchange may rescue small isolated populations from demographic, ecological and genetic stochasticity and inbreeding depression, and therefore promote long-term viability of populations (e.g. Wilcock & Neiland 2002). This may be particularly important in situations where it is not possible to restore large continuous wild habitat areas, such as in urban or intensive agricultural landscapes (e.g. Volis et al. 2005, Lundberg et al. 2008, Van Geert et al. 2010). The small linear elements of the landscape (linear landscape elements, further abbreviated LLEs), i.e. hedges, wooded strips, tree lines along lanes,

river and ditch banks and railways, provided they show high habitat quality (in particular appropriate floral resources, but see Haddad & Tewksbury 2005), may constitute a physical connection between wild habitat fragments. Such physical connection may be essential when the surrounding matrix is unfavourable to the species associated with these habitats. Indeed, the LLEs may reduce the landscape discontinuity by guiding pollinators to the next populations, and act as biological corridors enhancing pollen dispersal (e.g. Beier & Noss 1998, Haddad et al. 2003, Gilbert-Norton et al. 2010, Van Geert et al. 2010) and increasing plant fitness (Cranmer et al. 2012). In the absence of such conduits, pollinators may avoid crossing inhospitable or unfamiliar habitats, restricting their movement through the landscape, therefore reducing pollen flow. However, a network of LLEs does not necessarily en-

hance biological connectivity, and may even constitute barriers impeding pollinator movements in the landscape (Bhattacharya et al. 2003, Campagne et al. 2009).

A LLE may serve as a passing conduit for the pollinators moving from one to another fragment, but the pollinators may also forage in the LLE during their flight (Haddad & Tewksbury 2005, Schmucki & de Blois 2009). A small – possibly transitory – population of the foraged species may also be present in the LLE. For the foraging pollinators, the presence of conspecific flowers within the LLE may serve as a guiding bridge to the next patch of conspecific food plants and thus function as a stepping stone (Beier & Noss 1998, Hess & Fischer 2001, Schmucki & de Blois 2009). It can encourage foraging constancy to the visited plant species when they switch to another patch, thus reducing pollen loss by deposition on heterospecific flowers in the LLE (Kwak et al. 1998, Goulson 2003, Schmucki & de Blois 2009, Van Geert 2010). It may also contribute to increase secondary dye transfer by the remobilisation of pollen grains deposited on the flowers of the stepping-stone population, therefore favouring longer dispersal distances (e.g. Inouye et al. 1994). In contrast, if the LLE only consists of other flowering plant species, pollinators may change their foraging preferences. There may be pollen loss by deposition on other species and heterospecific pollen deposition on the target species in the next patch (Inouye et al. 1994, Kwak et al. 1998, Goulson 2003, Van Geert 2010). We can thus expect that the presence of a stepping-stone population of the foraged species within the LLE will facilitate pollen dispersal between populations, as well as increase dispersal distances. This may allow for longer LLEs to ensure connectivity between fragments (Kwak et al. 1998, Haddad & Tewksbury 2005).

Most studies that tested the efficacy of LLEs for allowing or facilitating pollen dispersal of insect-pollinated plant species were based on experimental designs with created LLEs (e.g. Tewksbury et al. 2002, Haddad et al. 2003, Townsend & Levey 2005, Gilbert-Norton et al. 2010), which is very difficult to apply in highly urbanized areas or in intensive farmlands because most of the land is used for human activities, or occupied by buildings. Only a few studies have attempted to estimate pollen dispersal through existing LLEs, and to our knowledge, very little is known about the facilitation potential of the presence of a small stepping-stone population in the LLE, and at what spatial scale pollen flow may benefit from these stepping-stone populations (Van Rossum 2009, Van Geert et al. 2010, Van Rossum & Triest 2010). However, this may be of great interest, since this may be of direct and concrete application for designing ecological networks allowing connectivity between wild habitat remnants in urban settings and intensive farmlands.

We investigated pollen dispersal patterns of the insect-pollinated herb *Primula elatior* (L.) Hill with respect to LLE connectivity in the Brussels urban area (Belgium). In Brussels, *P. elatior* shows an extremely fragmented distribution, occurring in small forest fragments surrounded by an often inhospitable urban matrix. Its small populations suffer from genetic erosion, increased inbreeding, reduced reproductive success and restricted gene flow (Van Rossum et al. 2002, Van Rossum 2008). Promoting pollen flow by increasing connectivity among forest fragments using wooded LLEs as corridors has been suggested. However, pollen dispersal appears to be restricted in *P. elatior* (e.g. Van Rossum & Triest 2006, Van Rossum et al. 2011), meaning that LLE length might be a critical factor in pollen flow restoration.

We used fluorescent powdered dye as a pollen analogue, which we demonstrated to be a reliable method to estimate realized pollen dispersal for *P. elatior* (Van Rossum et al. 2011), to examine the pollen dispersal patterns between pairs of populations located in urban forest fragments either connected or not by an LLE, and in the absence or presence of *P. elatior* plants in the LLE. We addressed the following questions: (1) What is the pattern of pollen (dye) dispersal between isolated and connected populations of *P. elatior*? (2) Does the presence of a small patch of plants (stepping-stone population) of *P. elatior* within the LLE facilitate pollen dispersal between fragments compared to an LLE where it is absent at the investigated spatial scale? We discuss the implications of our results for the management and conservation of insect-pollinated woodland species in urban landscapes.
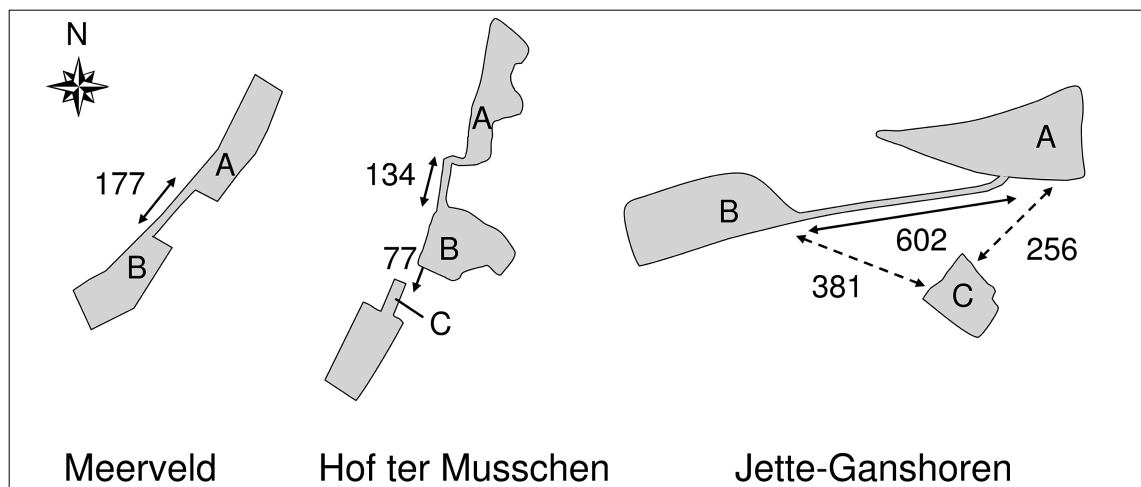

Figure 1 – Schematic presentation of the study sites (Meerveld, Hof-ter-Musschen, and Jette-Ganshoren) and location of populations A–C of *Primula elatior* in the forest fragments. The arrows indicate the LLE length or the distance (interrupted lines) between fragments (in m).

Table 1 – Study site details.
Coordinates (latitude, longitude), population size of *Primula elatior* populations (A–C), LLE length (m) between fragments A–B, distance of dye deposition (mean ± SE and at 80% of the dye transfers, in m), best fitting β parameter value of the dye dispersal kernel at the study site level in natural conditions (no stepping stone).

Site	Coordinates	Population size			LLE length (m)	Mean distance (± SE, m)	80% dye transfers (m)	β
		A	B	C				
Meerveld	50°49'N 4°15'E	200	90	-	177	62.8 (10.0)	114.8	0.49
Hof-ter-Musschen	50°51'N 4°27'E	51	84	32	134	30.5 (8.6)	24.2	0.45
Jette-Ganshoren	50°53'N 4°19'E	141	45	33	602	27.6 (17.8)	15.4	0.41

MATERIALS AND METHODS

The species

Primula elatior (Primulaceae) is a diploid, distylous long-lived perennial forest herb mainly occurring in western and central Europe (Taylor & Woodell 2008). In Belgium, it is common, but occurs in ancient, highly fragmented, moist deciduous forests. It is reported as rare and declining in the Brussels Capital Region, and most of the remaining populations are small, usually < 100 flowering individuals (Godefroid 2001, Van Rossum 2008). Plants flower in April, producing umbels with 5 to 20 flowers with five stamens and an ovary with one style and one stigma. Pollination depends on insects, primarily Hymenoptera. The main visitors are large bumblebee species, usually queens, such as *Bombus terrestris sensu lato, B. pascuorum, B. pratorum* and *B. hortorum*, and the solitary bees *Anthophora plumipes, Osmia rufa, Andrena bicolour, A. fulva* and *A. subopaca*. Diptera, mainly Syrphidae, also visit *P. elatior* flowers (Van Rossum et al. 2011 and references therein). Plants mainly reproduce by seeds. Seed dispersal and clonal growth are restricted to short distances (Van Rossum & Triest 2006, Taylor & Woodell 2008).

Study sites

Three study sites in the Brussels area (Meerveld, Hof-ter-Musschen and Jette-Ganshoren) were considered suitable for investigating pollen dispersal between urban forest fragments connected by an LLE. These showed similar surrounding matrix characteristics and were located at sufficient geographical distance from each other (7.35 to 13.75 km). The study sites consisted of two or three forest fragments (2.8–6.9 ha), each comprising one wild population of *P. elatior* (A, B or C; fig. 1, table 1). These fragments were surrounded by a matrix mainly composed of buildings and roads, only comprising a few meadows or lawns, where *P. elatior* was absent. In each site, populations A and B were connected by a wooded LLE, i.e. a wooded strip of about 5–10 m wide and 134–602 m long. A third, isolated population (population C), not connected by an LLE to the others, was present in Hof-ter-Musschen and Jette-Ganshoren (fig. 1, table 1). For both sites, the fragment with population C was separated from the other populations by a road and building matrix. Only a few other insect-pollinated species (cover < 10%) were co-flowering during the study.

To test for the effect of the presence of a stepping-stone population of *P. elatior* in the LLE on *P. elatior* pollen (dye) dispersal between forest fragments, two situations were tested, under similar weather conditions, and successively (with no delay), so that flower density and composition and pollinator guild composition and activity remained similar in the populations: (Case 1, "no stepping stone") no stepping-stone population, i.e. the studied species was absent in the LLE (only serving as a passage conduit for pollinators); (Case 2, "stepping stone") presence of a stepping-stone population of the studied species in the LLE (functioning as stepping stone). For case 2, two planters (window boxes) with 6-9 flowering plants of the two morphs (75–100 flowers) were installed halfway in the LLE. Given the ability of pollinators as bumblebees to learn floral visitation routes (see e.g. Goulson 2003), and thus to avoid that pollinators initially exposed to a stepping-stone population might retain that behaviour afterwards, case 1 was tested before case 2.

Estimating pollen dispersal using fluorescent dye

Five distinct colours (Radiant Color Corp., Series Radglo© R) were used as pollen analogues: orange, red, yellow, blue and UV blue. Marking flowers with dye was conducted at peak flowering, in April 2004, during two successive 4-day periods of similar dry sunny weather conditions (case 1 followed by case 2). On day 1, dyes were applied with wooden toothpicks to dehiscing anthers of about 50 open flowers (7–12 inflorescences) from a group of 3–5 individuals (= dye source plants), in populations A and B (except for Jette-Ganshoren, for which C was used as dye source for case 1 instead of B), and for case 2, also for one window box in the LLE (table 2). Within each site different dye colours were used for each dye source and case. Source plant location (different for case 1 and 2 to avoid interference with the previous dye colour, but close to each other) was selected so that a wide range of distances to the recipient individuals could be covered. The sample recipient plants were randomly chosen, covering the whole population area. The same recipient plants were sampled for case 1 and 2 if there were enough open flowers remaining, and those that could not be sampled again were replaced by close neighbours when possible. On day 4, receptive stigmas were harvested from 3–7 flowers on 8–42 recipient individuals per population and 3–6 plants per LLE. Repeating the experiment was not possible given the

Table 2 – Results for populations A–C of *Primula elatior* in three study sites (Meerveld, Hof-ter-Musschen and Jette-Ganshoren) in the absence and in the presence of a stepping-stone population in the LLE.
Number of sampled recipient individuals (*n*), mean distance of dye transfers in m (with ranges), proportion of recipient individuals showing dye deposition (Ind. with dye) and mean fraction of stigmas with dye (Stigmas with dye). Dye source populations: A, B, C, and LLE (stepping-stone population).
* Dye source = Jette-Ganshoren C.
In italics: dye transfers within-populations; in bold: dye transfers between populations A and B (connected by an LLE).

Population		Distance of dye transfers (m)		No stepping stone Ind. with dye from		Stigmas with dye from	
	n	A	B/C*	A	B/C*	A	B/C*
Meerveld							
Meerveld A	42	*20.5* (2.2–76.3)	**212.0** (194.9–226.7)	*0.67*	**0.07**	*0.30*	**0.02**
LLE	-						
Meerveld B	38	**209.5** (180.7–226.7)	*29.6* (0.3–114.8)	**0.26**	*0.58*	**0.05**	*0.31*
Hof-ter-Musschen							
Hof-ter-Musschen A	24	*12.8* (0.8–33.7)	-	*0.54*	**0.00**	*0.37*	**0.00**
LLE	-						
Hof-ter-Musschen B	34	**189.1** (174.2–214.8)	*7.5* (0.7–29.8)	**0.09**	*0.56*	**0.03**	*0.31*
Hof-ter-Musschen C	9	-	93.5 (93.3–93.7)	0.00	0.33	0.00	0.11
Jette-Ganshoren							
Jette-Ganshoren A	27	*9.6* (0.2–27.9)	-	*0.85*	0.00*	*0.56*	0.00*
LLE	-						
Jette-Ganshoren B	8	**646.8** (646.1–647.5)	-	**0.17**	0.00*	**0.03**	0.00*
Jette-Ganshoren C	21	-	*10.3** (0.3–23.1)	0.00	*0.57**	0.00	*0.40**

restricted number of distinguishable dye colours, the number of flowers per plant and the short flowering period.

In total 1996 stigmas were embedded in a semi-permanent mount of glycerine jelly on a microscope slide directly after harvesting (Van Rossum 2010) and examined for presence or absence of fluorescent dye particles at 40x under a fluorescence microscope. For each recipient individual and dye colour, the fraction of stigmas with dye was calculated as the number of stigmas with dye divided by the total number of collected stigmas. The fraction of stigmas with dye can be considered as a reliable estimate of dye dispersal for *P. elatior* as it highly correlates ($P < 0.001$) with mean dye abundance on stigmas (Stiers 2005). The mean fraction of stigmas with dye and the proportion of recipient individuals with dye on at least one stigma were also calculated for each recipient

population and dye source. For each recipient individual, the distance to the dye source was calculated using map coordinates. The potential distances (range and mean) from dye source to recipient individuals are given for each population and dye source in electronic appendix 1.

Data analysis

Dye dispersal patterns in natural conditions – The shape of the dye dispersal distribution was described for each site in natural conditions (no stepping stone, data sets pooled together for all populations) based on the method described by Van Rossum et al. (2011). The dye deposition events estimated by the fraction of stigmas with dye were used to fit an exponential power function characterizing dye dispersal in each site, the dye dispersal kernel, $f(\alpha, \beta, r) = \beta * \exp(-(r/\alpha)^\beta)/$

Table 2 (continued) – **Results for populations A–C of *Primula elatior* in three study sites (Meerveld, Hof-ter-Musschen and Jette-Ganshoren) in the absence and in the presence of a stepping-stone population in the LLE.**
Number of sampled recipient individuals (*n*), mean distance of dye transfers in m (with ranges), proportion of recipient individuals showing dye deposition (Ind. with dye) and mean fraction of stigmas with dye (Stigmas with dye). Dye source populations: A, B, C, and LLE (stepping-stone population).
* Dye source = Jette-Ganshoren C.
In italics: dye transfers within-populations; in bold: dye transfers between populations A and B (connected by an LLE).

Population	Stepping stone									
	n	Distance of dye transfers (m)			Ind. with dye from			Stigmas with dye from		
		A	B	LLE	A	B	LLE	A	B	LLE
Meerveld										
Meerveld A	39	*16.2*	**215.5**	101.3	*0.77*	**0.21**	0.13	*0.46*	**0.05**	0.02
		(0.6–57.8)	**(192.9–241.2)**	(79.0–121.8)						
LLE	3	110.7	139.4	*1.6*	0.67	0.67	*0.67*	0.10	0.13	*0.18*
		(110.4–110.9)	(139.1–139.6)	*(1.4–1.8)*						
Meerveld B	29	**223.5**	*28.3*	120.8	**0.48**	*0.93*	0.21	**0.08**	*0.64*	0.02
		(199.2–284.7)	*(0.2–110.8)*	(109.3–139.4)						
Hof-ter-Musschen										
Hof-ter-Musschen A	12	*8.7*	-	79.3	*0.42*	**0.00**	0.08	*0.22*	**0.00**	0.02
		(0.9–17.0)		(79.3)						
LLE	6	93.1	-	*2.2*	0.17	0.00	*0.33*	0.03	0.00	*0.16*
				(2.1–2.4)						
Hof-ter-Musschen B	23	**207.6**	*10.4*	-	**0.30**	*0.61*	0.00	**0.10**	*0.39*	0.00
		(173.3–228.1)	*(4.7–55.1)*							
Hof-ter-Musschen C	9	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00
Jette-Ganshoren										
Jette-Ganshoren A	25	*28.4*	**649.1**	391.4	*0.80*	**0.08**	0.04	*0.46*	**0.02**	0.01
		(7.4–42.9)	**(647.7–650.5)**	(391.4)						
LLE	4	420.7	260.9	*8.0*	1.00	0.25	*1.00*	0.36	0.03	*0.94*
		(419.4–422.0)	(260.9)	*(4.1–11.9)*						
Jette-Ganshoren B	18	**680.7**	*29.3*	264.1	**0.39**	*0.83*	0.22	**0.09**	*0.58*	0.07
		(665.0–696.1)	*(0.4–49.0)*	(248.5–292.3)						
Jette-Ganshoren C	19	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00

$(2\pi\alpha^2\Gamma(2/\beta))$. In this equation, *r* is the distance crossed, α and β are the parameters to be fitted, and Γ is the Gamma function (for more details, see Hardy et al. 2004, Van Rossum et al. 2011). The dispersal kernel is fat-tailed (leptokurtic distribution) when $\beta < 1$, and thin-tailed when $\beta > 1$ (e.g. Hardy et al. 2004). A maximum likelihood estimate approximated by a chi-square test was used to fit the α and β parameters using Excel Solver (for more details, see Van Rossum et al. 2011).

Effect of the presence of a stepping-stone population in the LLE – We examined whether the presence of a stepping-stone population of *P. elatior* in an LLE (case 2) connecting two forest fragments might facilitate pollen transfer between populations compared to an LLE where *P. elatior* is absent (case 1). We tested whether between-population dye deposition was higher for case 2 than case 1 at the population level, and for each recipient population separately. First, at the population level, we performed Wilcoxon matched pairs tests (five population pairs, A–B/B–A only, excluding Jette-Ganshoren BC dye sources), on the mean and maximal distance of dye transfer, the proportion of recipient individuals with dye and the mean fraction of stigmas with dye. Secondly, for each recipient population separately, we performed a test of homogeneity of slopes, with the fraction of stigmas with dye (data sets restricted to the effective –successful– dye transfers, i.e. only the recipient plants where at least one stigma had received dye) as dependent variable, the distance to dye source as independent variable and case as grouping variable. This could however only be performed for population B in Meerveld (receiving dye from A), as the sample size of effective dye transfers in case 1 was too small in the

other populations ($n = 1–3$). The shape of the dye dispersal distribution based on the best-fitting β parameter of the dye dispersal kernel was estimated for each study site (populations A–B, data sets pooled), for case 1 and 2 separately (excluding the LLE data).

To exclude the possibility that the observed patterns might result from other factors (e.g. plant spatial distribution, pollinator activity) than the presence or absence of a stepping-stone population in the LLE, we performed two additional analyses. First, we tested for each site whether plant spatial distribution might differ between the two cases, using a nonparametric Kolmogorov-Smirnov (K-S) test for goodness of fit. This tested whether the general shape of the distribution of the potential distances to dye source was similar between cases (for this purpose the distances were not grouped into classes; Sokal & Rohlf 2000). Second, pollinator activity might differ between the two cases, despite similar weather conditions and flowering density and composition, as a result of varying pollinator abundance and guilds. This might affect pollen dispersal, also within populations (e.g. Aizen 2001, Hayter & Cresswell 2006). Therefore we compared within-population dye dispersal patterns between cases (for the same range of distances to dye source), using a test of homogeneity of slopes (GLM, logistic function) on the fraction of stigmas with dye (transformed as an ordinal multinomial variable), with the distance to dye source as independent variable and case as grouping variable. Significance was determined using a likelihood ratio χ^2 test. The analyses were performed using STATISTICA version 10 (Statsoft 2010).

<div align="center">RESULTS</div>

Dye dispersal patterns in natural conditions

In natural conditions (no stepping stone), dye transfers were found between most dye source and recipient populations, with the proportion of recipient individuals receiving dye ranging from 0.01 to 0.33. Across all populations, the fraction of stigmas with dye ranged from 0.04 to 1.00 for recipient individuals showing dye deposition (table 2). At the study site level, the mean dye transfer distance varied between 27.6 and 62.8 m, with 80% of the dye transfers occurring at less than 15.4–114.8 m (table 1, electronic appendix 2). The maximum distance over which dye was transported was 647.5 m. The best-fitting β parameter describing the shape of the distribution curves (electronic appendix 2), with the fraction of stigmas with dye as a function of the distance to the dye source, was $\beta = 0.41–0.49$ (table 1).

Dye dispersal patterns between connected and isolated populations

All populations connected by an LLE showed bi-directional between-population dye transfers for 7 to 48% of the recipient individuals, in both case experiments (table 2), with one exception, from Hof-ter-Musschen B to A. No dye transfers were found between the isolated populations (Jette-Ganshoren C and A–B, and Hof-ter-Musschen A to C), except for Hof-ter-Musschen B to C in case 1 (table 2).

Effect of the presence of a stepping-stone population of *P. elatior* in the LLE

The LLE stepping-stone population in the three study sites showed within-population dye deposition, but also dye transfers from at least one of the dye source populations A–B (table 2). All recipient populations, except Hof-ter-Musschen B-C and Jette-Ganshoren C, received dye from the LLE stepping-stone population (proportion of recipient individuals and mean fraction of stigmas showing dye deposition ranging from 0.04 to 0.22 and from 0.01 to 0.07, respectively).

Overall, the mean and maximal distances of dye transfers, the proportion of recipient individuals showing dye and the mean fraction of stigmas with dye in the recipient populations were significantly higher when a stepping-stone population was present in the LLE (for all Wilcoxon matched pairs tests $T = 0$, $P < 0.05$; table 2, fig. 2), except for Hof-ter-Musschen A (no dye transfer from B in both cases). The proportion of recipient individuals showing dye increased 1.8 to 3.3 times (from 0.26 to 0.48 and 0.09 to 0.30 in Meerveld B and Hof-ter-Musschen B, respectively). The maximum distance over which dye was transported was 696.1 m. When comparing the two cases for the recipient individuals of Meerveld B, the test of homogeneity of slopes indicated a trend for a higher between-population fraction of stigmas with dye for case 2, for a negative effect of the distance to dye source and for the interaction between case and distance ($F_{1,20} = 3.86$, 3.28 and 4.26, $P = 0.063$, 0.085 and 0.052, respectively). For the three study sites, in both cases, the dye distribution curves were leptokurtic ($\beta < 1$, ranging from 0.23 to 0.93).

A significant difference (K-S test: $P < 0.05$) in shape of the distribution of the potential distance to dye source was found between case 1 and 2 in Jette-Ganshoren (with higher distances in case 2). No significant difference (K-S test: $P > 0.05$) in curve shape was found for the other study sites. The GLM test of homogeneity of slopes investigating whether pollinator activity might differ between cases indicated a significantly higher within-population fraction of stigmas with dye (for the same distance range) for case 2 (stepping stone) in Meerveld B ($\chi^2 = 7.02$, $P = 0.008$), but no significant interaction between case and distance ($\chi^2 = 0.13$, $P > 0.10$). No significant difference ($\chi^2 < 3.11$, $P > 0.05$) between cases was found for the other populations. There was a significantly negative effect of the distance to dye source for all populations ($\chi^2 = 8.75–28.89$, $P \leq 0.003$).

<div align="center">DISCUSSION</div>

Pollen dispersal between populations

The present study gives evidence of fluorescent dye transfer between some populations of *P. elatior* located in urban forest fragments, indicating pollinator movements between these populations. Dye dispersal, which may be considered as a reliable estimate of realized pollen dispersal for *P. elatior* (Van Rossum et al. 2011), shows a leptokurtic distribution ($\beta < 1$) with respect to spatial distance, with most dye (pollen) deposition occurring at short distances but also with some long-distance events, up to 647.5 m (696.1 m for the stepping-stone case). Between-population long-distance dye deposition has also been observed for other insect-pollinated

herbs in the same urban context, to 524 m for the wet meadow *Lychnis flos-cuculi*, 743 m for the early-successional *Centaurium erythraea* and 2.58 km for the hay-meadow species *Centaurea jacea* (Van Rossum 2009, 2010, Van Rossum & Triest 2010).

Dye transfer appears to be bi-directional (A to B and B to A), except in Hof-ter-Musschen (for both cases), where some preferential direction of dye dispersal is observed (A to B but not B to A). This might be related to the spatial distribution of the floral resources in the study site with respect to the

Figure 2 – Distribution of dye deposition (fraction of stigmas with dye) for case 1 (no stepping stone, black dots) and case 2 (stepping-stone population in the LLE, white dots) as a function of the distance to dye source for population pairs A–B and B–A in (A) Meerveld, (B) Hof-ter-Musschen and (C) Jette-Ganshoren. The line represents the distribution expected under the exponential power dispersal model for best-fitting parameters (case 1, continuous line; case 2, broken line).

location of the nesting sites or colonies of the foraging pollinators, which may lead to preferential flight routes followed by the insects (Osborne et al. 1999, Ghazoul 2005, Wolf & Moritz 2008).

Effect of the presence or absence of an LLE between fragments

When populations are embedded in an inhospitable matrix, we may expect that separation of the fragments by human structures such as roads and buildings may restrict pollinator movements, reducing pollen dispersal distances (Bhattacharya et al. 2003, Andrieu et al. 2009, but see Leidner & Haddad 2010). By contrast, the presence of LLEs connecting fragments may enhance pollen dispersal (e.g. Tewksbury et al. 2002, Gilbert-Norton et al. 2010, Van Geert et al. 2010). Our results on *P. elatior* indicate that all population pairs connected by an LLE showed between-population dye deposition, whereas the unconnected populations did not exchange dye, even at lower distances (table 2). This suggests that the urban LLEs might form biological corridors facilitating pollinator movements and pollen dispersal for *P. elatior*, at least wooded strips or hedges connecting urban forest fragments. However, population Hof-ter-Musschen C received dye from population B (at 77 m) despite the absence of a connecting LLE, indicating that there may be a minimal threshold for preventing pollinator movements. These findings certainly merit further investigation with additional populations isolated by various distance ranges. Existing LLEs were also found to facilitate pollen (dye) dispersal of the congener *Primula vulgaris* in an intensive farmland (Van Geert et al. 2010).

Effect of the presence of a stepping-stone population of *P. elatior* in the LLE

The present findings on *P. elatior* populations in an urban landscape confirm that the pollinators use the wooded LLEs as movement conduits between forest fragments. They also possibly forage in it, at least on *P. elatior* when present, as indicated by the dye deposited on the plants placed in the corridor and by the dye transfers from the stepping-stone population to the populations located in the connected fragments. Small floral arrays of the forest herb *Trillium grandiflorum* placed in hedgerow corridors have also been found to be visited by foraging pollinators (Schmucki & de Blois 2009). Moreover, placing a small patch of *P. elatior* plants in the LLE increases dye transfers (in proportion of individuals and stigmas visited) between the fragmented populations in the three study sites. The distances of dye transfers also appear to be higher. However, the potential distances to dye source appear slightly higher when there is a stepping stone in Jette-Ganshoren, and might thus also have contributed to the higher distances of dye transfers observed. But, despite these, for which we may have expected less dye deposition as a result of the decaying dispersal distribution pattern, the proportion of visited individuals increased by 1.8 to 3.3 times in the populations showing interpopulation dye transfers. To our knowledge, this study is the first to show that the presence of a small stepping-stone population in an LLE may facilitate pollen dispersal between insect-pollinated popula-
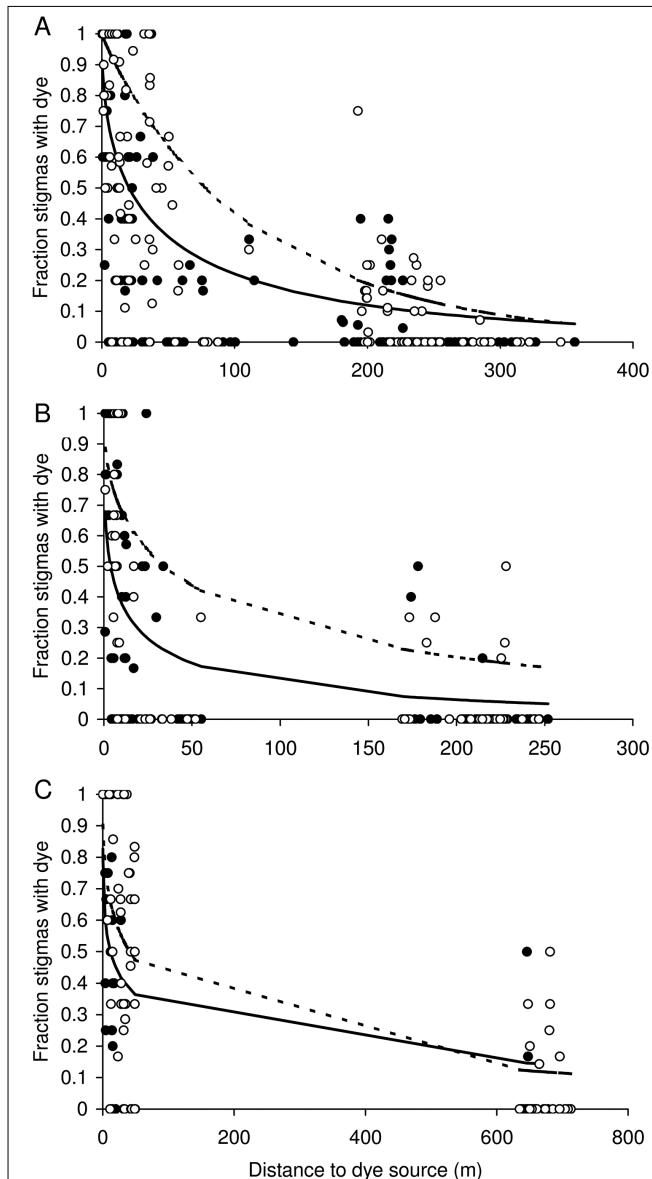
tions compared to a "passage" LLE, at least when pollen dispersal already exists. This confirms that LLEs for the same length might be more effective as corridors for dispersal if they can serve as both movement conduit and habitat (Kwak et al. 1998, Haddad & Tewksbury 2005).

The higher within-population fraction of stigmas with dye found for case 2 (stepping stone) in Meerveld B suggests that pollinator activity might have been higher in this particular population during the second phase of the experiment. A higher pollinator activity might affect pollen dispersal, e.g. by increasing pollen removal and deposition (e.g. Aizen 2001, Hayter & Cresswell 2006). Other factors, such as population size and isolation may affect pollinator behaviour and visitation rate (e.g. Kwak et al. 1998, Cresswell & Osborne 2004, Ghazoul 2005). However, for the population sizes investigated here, dye dispersal patterns were found to be similar in urban populations of *P. elatior* for the same range of distances to the dye source (Van Rossum et al. 2011).

Implications for management

Ensuring pollen flow between fragmented plant populations may contribute to increase population reproductive success and long-term viability (e.g. Wilcock & Neiland 2002, Cranmer et al. 2012). It is therefore essential to understand how in practice we can ensure connectivity by pollen flow between small isolated habitat patches, using LLEs as corridors, especially when they are embedded in a human-dominated matrix such as urban settings. This can contribute to design functional ecological networks for insect-pollinated plant species (e.g. Volis et al. 2005, Van Geert et al. 2010, Van Rossum & Triest 2010). Our studied species, *P. elatior*, is rare and declining in Brussels, occurring in small forest fragments surrounded by an urban matrix, mostly existing as small populations that suffer from genetic erosion, increased inbreeding, reduced reproductive success and restricted gene flow (Godefroid 2001, Van Rossum et al. 2002, Van Rossum 2008). Our present findings highlight the potential role of existing wooded LLEs for restoring connectivity and pollen dispersal between these urban forest fragments, and of the stepping-stone populations to contribute to increase LLE effectiveness.

Developing a network of core populations connected by LLEs comprising (small) stepping-stone populations should thus be encouraged, involving the creation of stepping-stone populations, either temporary or sustained, in the connecting LLEs, especially when their length might exceed a critical distance threshold for pollen dispersal (depending on pollinator foraging abilities). This may be realised with seeds collected from large local populations (to avoid outbreeding depression), and by sowing them, or transplanting flowering individuals (after plant rearing in a garden or greenhouse) into the LLEs. Improving LLE habitat quality by ecological management might contribute to sustain the stepping-stone population. As a sufficient pollinator service may be essential for ensuring sufficient visitation rates and pollen dispersal (Wilcock & Neiland 2002, Ghazoul 2005, Schmucki & de Blois 2009), further knowledge on pollinator requirements (e.g. concerning nesting and floral resources) and

movements through the landscape matrix may contribute to improve the effectiveness of the network connectivity.

SUPPLEMENTARY DATA

Supplementary data are available in pdf format at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingentaconnect.com/content/botbel/plecevo/supp-data), and consist of the following: (1) mean potential distance to dye source for populations A–C of *P. elatior* in the three study sites; and (2) overall distribution of dye deposition as a function of the distance to dye source at the study site level.

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