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## Assessing spatial mating patterns in translocated populations of *Campanula glomerata*

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### ABSTRACT

Plant translocations represent a solution for rescuing nonviable populations and recreating population networks. An essential criterion for translocation success is genetic diversity enhancement or maintenance in post-translocation generations. Genetic mixing, i.e. individual relatedness and spatial structure, also needs to be considered. We investigated the processes involved in the spatial patterns of genetic diversity in the first post-translocation generation (recruits and seed progeny) of translocated populations of the insect-pollinated, self-incompatible herb *Campanula glomerata*. We combined direct and indirect estimates of contemporary mating and gene dispersal using 15 microsatellite loci to estimate clonal extent, and genetic diversity and structure, to reconstruct sibship and parentage and to estimate pollen and seed dispersal distances. Genetic diversity in the first post-translocation generation is representative of the natural seed-source populations. Combining sources enhanced the effective number of breeders ( $N_e = 102\text{--}175$ ). Clonal propagation was only marginal. Pollen and seed dispersal occurred at short distances within populations (up to 18 m), favouring fine-scale spatial genetic structure (SGS). This means that the enhanced genetic diversity, random mating and high admixture levels are ascribed to the mixed spatial arrangement of transplants. Stronger SGS might lead to biparental inbreeding and reduced seed set due to a lack of compatible neighbours, and impact the long-term population genetic sustainability. Increasing seed-dispersal distances within and among sites might be mediated by grazing livestock used for grassland management. Some long-distance pollen flow events (0.68–2.35 km) between populations were also detected, suggesting the potential of creating large translocated populations to restore connectivity by gene flow.

### 1. Introduction

Plant translocation is the intentional introduction of new individuals (seeds, cuttings or young plants). It represents a solution for rescuing inbred or genetically eroded populations, for re-establishing extirpated populations and for recreating an interconnected network of new genetically diverse populations, when restoring connectivity by gene flow among nonviable populations by habitat restoration or by biological corridors is not possible (Zimmer et al., 2019; De Vitis et al., 2022). Translocating a highly variable gene pool is recommended to optimize transplant establishment, the number of compatible mates (in case of a self-incompatibility system or

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dioecy) and evolutionary resilience (Sgrò et al., 2011; Commander et al., 2018; Schäfer et al., 2020). An essential criterion for translocation success is whether genetic diversity has been enhanced (or maintained) in the next generations after translocation (e.g., Van Rossum and Hardy, 2022). Not only the global amount of genetic diversity in the population is important, but also the efficiency of genetic mixing in the post-translocation generations (seed progeny quality). Seed progeny quality relates to individual relatedness (e.g., sibship and the number of breeders contributing to parentage) and to the way genetic diversity is distributed among individuals and spatially structured (Aguilar et al., 2019; Thomas et al., 2021; Van Rossum, 2023).

The patterns of genetic mixing in the new generations will depend on genetic drift effects, on the breeding system and on mating patterns, i.e. pollen and seed flow patterns (and so on their dispersal vectors), but also on clonal extent and translocation design, and on site management regimes (e.g., DiLeo et al., 2018; Plue et al., 2019; Van Rossum et al., 2020; Monks et al., 2021; Van Rossum and Le Pajolec, 2021). A sufficient contemporary pollen flow can optimize genetic mixing, prevent inbreeding (and so inbreeding depression) and increase plant fitness through heterosis (e.g., Frankham et al., 2019). When seed and/or pollen dispersal is restricted to short distances, a spatial patchy distribution of genetically related offspring (full sibs and half sibs) may be expected, which may increase (biparental) inbreeding in the next generations (Aguilar et al., 2019; Thomas et al., 2021; Van Rossum and Le Pajolec, 2021). Extensive clonal propagation can promote selfing in populations of self-compatible species, and reduce seed set through Allee effect for self-incompatible species (Tierney et al., 2020; Van Rossum, 2023). By contrast, implementing grazing by livestock (e.g., sheep, goats, cattle, horses) as post-translocation management practice may contribute to long-distance seed dispersal within and among sites through epi- or endozoochory, in particular in grassland habitats (Benthien et al., 2016; Plue et al., 2019; Kapás et al., 2020).

Many frameworks can be found in the literature, which propose to use genetic diversity as a guiding tool for conservation and management actions and as an indicator for restoration success (e.g., Frankham et al., 2019; Hoban et al., 2020; De Vitis et al., 2022). Besides, assessment of genetic diversity is increasingly considered in monitoring aiming at evaluating the success of plant translocations (e.g., Zavodna et al., 2015; Gargiulo et al., 2019; Van Rossum et al., 2020; Monks et al., 2021). However, mating processes and the processes responsible of spatial genetic structuration of the new post-translocation generations remain largely overlooked (de Souza et al., 2022; Ritchie and Krauss, 2012; Van Rossum, 2023). Indirect methods are commonly used for investigating spatial genetic structure (e.g., spatial autocorrelation analyses, Bayesian clustering), but they only provide insights of gene dispersal (e.g., Pritchard et al., 2000; Hardy and Vekemans, 2002). Additional computing tools have been recently developed to directly quantify spatial mating patterns. These direct methods offer opportunities for detailed pedigree and parentage reconstruction, and for quantifying seed and pollen dispersal kernels, selfing (inbreeding) rates, and the effective population size (number of breeders), provided both offspring and their parent generations have been sampled and mapped (e.g., Hadfield et al., 2006; Jones and Wang, 2010; Chybicki, 2018; Flanagan and Jones, 2019).

In this paper, we identified the processes involved in the spatial patterns of genetic diversity in the first post-translocation generation of populations created by plant translocations. Such a short-term genetic monitoring of the early post-translocation generations can give insights into the contemporary processes important for population establishment (recruitment, mating, pollination, and seed dispersal), and identify those that could lead to early failure, so that management adjustments can be quickly done (Van Rossum and Le Pajolec, 2021; Van Rossum and Hardy, 2022). In particular, we performed genetic analyses using 15 microsatellite loci (1) to estimate clonal extent and genetic diversity; (2) to estimate genetic structure within and among populations based on indirect methods; and (3) to reconstruct sibship and parentage and to estimate pollen and seed dispersal distances based on direct methods. The study was carried out on *Campanula glomerata* L. (Campanulaceae), an insect-pollinated herbaceous species that has been included in a restoration project of calcareous grasslands (Godefroid et al., 2016; Van Rossum et al., 2022).

## 2. Materials and methods

### 2.1. Study species

*Campanula glomerata* L. (Campanulaceae) is a self-incompatible, insect-pollinated herb species that is found on calcareous habitats (grasslands, scrub, open woodlands and sand dunes) (Denisow and Wrzesień, 2015). In northwestern Europe, at the margin of its wide continental Eurasian distribution range, the species becomes rare and declining, with small and fragmented populations (Hill et al., 2004; Bachmann and Hensen, 2007; Godefroid et al., 2016). In Belgium, the species is critically endangered, with a few small remaining populations. Therefore, a species recovery plan involving plant translocations in four sites (restored calcareous grasslands) from Belgian Lorraine (southern Belgium) has been implemented in the framework of a EU Life project. Small populations (census and effective population sizes ranging 7–28 and 6–40, respectively) were used as mixed seed sources for translocating 500 transplants in each site in September 2015 (Godefroid et al., 2016; Van Rossum et al., 2022).

Flowering occurs from June to August. The violet-blue protandrous, nectar-producing flowers are mainly pollinated by bumble bees, solitary bees, honey bees and hoverflies (Albrecht et al., 2007; Denisow and Wrzesień, 2015). Seeds are tiny and numerous, and dispersed over short distances by capsule movement. Grassland habitats of *C. glomerata* were traditionally grazed in the past in Germany, and zoochory by grazing sheep might occur for *C. glomerata* (Bachmann and Hensen, 2006, 2007). Epi- and endozoochorous dispersal by grazing sheep has been reported for the tiny seeds of *Campanula patula* and *C. rotundifolia*, respectively (Albert et al., 2015; Plue et al., 2019). There is no persistent seed bank in the soil. Vegetative spread can occur by rhizomes, over short distances (0.01–0.25 m/year) (Klotz et al., 2002).

## 2.2. Study populations and sampling

The study was conducted in two translocated populations, Ficherulle (FICH) and Sainte-Cécile (CEC2) (Fig. S1). The transplants of the other two translocated populations (located in Torgny, 49°30'39"N, 5°29'E) were heavily grazed (by snails and rodents), and could not be sampled. Five small natural populations were used as seed sources: Chassepierre (CHA), Emond (EMO), Fontenoille (FON), Lambermont (LAM) and Watrinsart (WAT) (Van Rossum et al., 2022). FICH and CEC2 were separated by 1.53 km and located at 0.68–4.12 km from the natural populations (Fig. S1). The number of flowering transplants for CEC2 and FICH was 445 and 481 in 2016 (Godefroid and Van Rossum, unpublished data), and 427 and 346 in 2017 (Godefroid, unpublished data), respectively.

Seeds were collected in 2016 on 95 and 93 transplants in CEC2 and FICH, respectively (16–20 per source origin), and were stored in dry-cold environment (15 °C, 15 % relative moisture) until germination in Petri dishes on 1 % agar (10 g/L) in incubators (20 °C, 8/16 light/dark photoperiods) in April 2017 (after 1 month at 5 °C to break the dormancy). Leaves were collected in CEC2 and FICH, respectively, on 136 and 166 transplants (Van Rossum and Godé, 2021), and on the first post-translocation generations: on 122 and 125 seed progenies (1–3 offspring per maternal plant, obtained from seed germination) and on 64 and 102 newly established individuals (recruits) in 2018 (Table 1). Leaf samples were dried in silica gel. Transplants and sampled recruits were mapped in the field so that spatial distances between individuals could be calculated (Fig. S2).

## 2.3. DNA extraction and microsatellite analysis

DNA was extracted from ca. 5–10 mg of dried leaf material using the NucleoMag 96 Plant kit (Macherey Nagel, Duren, Germany) following the manufacturer's protocol. The concentration of genomic DNA in the extracts was measured using the Qubit Quantitation Platform (Invitrogen), and its concentration standardized to 2 ng/μL.

We used 15 polymorphic microsatellite loci (excluding CAM19) previously developed by Van Rossum and Godé (2022) and followed the protocol adapted in Van Rossum et al. (2022) for PCR amplifications and cycling. Multiplexes were electrophoresed on an ABI PRISM 3130 sequencer (Applied Biosystems), with 1.5 μL of PCR product diluted with dH<sub>2</sub>O (1:10), and 9.75 μL of Hi-Di Formamide (Life Technologies, USA) and 0.25 μL of MapMarker 500 labelled with DY-632 (Eurogentec). Alleles were scored with software GeneMapper version 5 (Applied Biosystems) and Geneious Prime (Biomatters).

## 2.4. Data analysis

### 2.4.1. Changes in genetic variation across generations

To assess changes in genetic variation across generations, estimates of genetic variation were calculated for each population for transplants, recruits and seed progeny using GEN-SURVEY (Vekemans and Lefèbvre, 1997) or FSTAT (Goudet, 2003) as follows: allelic richness ( $A_{[N]}$ ) for a fixed sample size ( $N = 57$ ), observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity and Wright's inbreeding coefficient ( $F_{IS}$ ), corrected for small sample size. We tested the significance of the  $F_{IS}$  values (over all loci) by randomization tests and sequential Bonferroni-type correction. Given that null alleles were detected at a few loci (Van Rossum and Godé, 2022), mean inbreeding coefficient values ( $F_{ISnull}$ ) and their 95 % highest posterior density intervals (HPDI) were calculated after adjusting allele frequencies with INEST (Chybicki and Burczyk, 2009) to take the effect of null alleles into account on  $F_{IS}$  values, using the Bayesian approach (IIM) with  $10^6$  Markov Chain Monte Carlo iterations, of which the first  $10^5$  were discarded as burnin phase. Two models were tested: a full model ( $nfb$ , including null alleles, inbreeding and genotyping failures), and a model ( $nb$ ) where inbreeding was set at 0. The best fitting model was selected based on the lowest value of the deviation information criterion (DIC). Whether genetic diversity was higher or maintained and whether inbreeding levels changed across generations (transplant, seed progeny and recruit) were tested by performing pairwise Wilcoxon matched pairs tests by locus on  $A_{[57]}$ ,  $H_o$ ,  $H_e$ , and  $F_{IS}$  values. Analyses were performed using STATISTICA version 12 (Dell Inc.).

**Table 1**

Estimates of within-population genetic variation for two translocated populations of *Campanula glomerata* at 15 nuclear microsatellite loci.

Population	<i>N</i>	<i>n</i>	<i>n<sub>a</sub></i>	$A_{[57]}$	$H_o$	$H_e$	$F_{IS}$	$F_{ISnull}$	IIM model
CEC2									
Transplants	500	166	251	14.5	0.740	0.849	0.125*	0.061 (0.029–0.094)	inbr.
Seed progeny		122	229	13.9	0.813	0.852	0.043*	0.007 (0.000–0.017)	no inbr.
Recruits	246	64	214	14.0	0.820	0.847	0.028ns	0.005 (0.000–0.016)	no inbr.
FICH									
Transplants	500	136	245	14.5	0.758	0.850	0.103*	0.056 (0.084–0.035)	inbr.
Seed progeny		125	224	13.8	0.820	0.853	0.036*	0.006 (0.000–0.015)	no inbr.
Recruits	2783	102	236	14.4	0.803	0.842	0.041*	0.007 (0.000–0.019)	no inbr.

Abbreviations: *N*, total number of transplants or recruits; *n*, sample size; *n<sub>a</sub>*, total number of alleles;  $A_{[57]}$ , allelic richness;  $H_o$ , observed heterozygosity;  $H_e$ , expected heterozygosity;  $F_{IS}$ , Wright's inbreeding coefficient;  $F_{ISnull}$ , inbreeding coefficient corrected for null alleles (mean value and 95 % highest posterior density intervals); IIM model, best fitting model in the IIM analysis, including inbreeding (inbr, *nfb*) or not (no inbr, *nb*); ns, not significant;

\*  $P < 0.05$ .

#### 2.4.2. Extent of clonal propagation in recruits

Whether recruits were produced by clonal propagation of transplants was estimated in each population using GenAEx 6.5 (Peakall and Smouse, 2012). Identical multilocus genotypes were likely to be putative clones when the probability ( $p_{se}$ ) of finding the same multilocus genotype a second time  $< 0.05$ , and to result from sexual reproduction when  $p_{se} > 0.05$  (Parks and Werth, 1993). Given that not all transplants were sampled and genotyped, ClonEstiMate version 1.02 was also used to infer the rate of clonality in each population. This Bayesian method calculates posterior probability distributions of clonality rate by comparing transitions of genotype frequencies between two successive generations (Becheler et al., 2017), thus the transplants and the recruits. As *C. glomerata* is self-incompatible, selfing rate value was set at 0 and inbreeding rate at  $F_{ISnull}$  value calculated for the recruits using INEST. Values of mutation rate and of clonality rate (from 0 to 1.0) were set as recommended. The locus CAM27 that showed high null allele frequency (Van Rossum and Godé, 2022) and so missing data and nine individuals with missing data in the other loci were excluded from the analyses.

#### 2.4.3. Indirect estimates of genetic structure and contemporary gene flow

To investigate the changes in genetic differentiation across generations, thus between transplants (reflecting source populations) and the F1 generation (seed progeny and recruits, which are expected to reflect (no) genetic drift effects and mating in translocated populations), we calculated pairwise  $F_{ST}$  values (Weir and Cockerham, 1984) using FSTAT and pairwise  $G''_{ST}$  values (Meirmans and Hedrick, 2011) using GenAEx within and between populations. The significance of the  $F_{ST}$  and  $G''_{ST}$  values was tested by randomization tests and Bonferroni correction.

To infer changes in population spatial structure across generations in translocated populations, we estimated fine-scale spatial genetic structure (SGS) using spatial autocorrelation analyses for transplants and recruits separately, as well as between the two generations using SPAGeDi (Hardy and Vekemans, 2002). The slope ( $b$ ) of the linear regression between pairwise multilocus kinship coefficients between individuals (Loiselle et al., 1995) and the logarithm of the geographic distance between them represents a good estimator of the degree of SGS (Hardy and Vekemans, 2002). For graphical illustration, we computed average multilocus kinship coefficients per distance intervals ( $F_i$ ) for eight distance classes (upper bound distance in meters): 1, 2, 3, 4, 5, 7, 10, and 30. The significance of the kinship coefficients and of the  $b$  estimates was tested by comparing the observed values with those obtained after 1000 random permutations of the individuals among positions. Differences in SGS between transplants and recruits and within/between generations were tested by Wilcoxon matched pairs tests on the  $b$  values, with the 15 loci as replicates.

To investigate whether seed progeny and recruits (new F1 generation) were obtained from crosses between transplants of different source origins, genotypic data were analyzed using the software STRUCTURE version 2.3.4 (Pritchard et al., 2000), a Bayesian clustering method. Analyses were performed after downloading the genotypic data on Galaxy web platform, using the public server at UseGalaxy.be (The Galaxy Community, 2022). In a former study (Van Rossum et al., 2022), STRUCTURE identified  $K = 6$  clusters of gene pools differing by a set of allele frequencies at each locus, allowing distinguishing of the five source populations used for plant translocations (FON transplants were assigned to two clusters). Each transplant was (probabilistically) assigned to the cluster of its source population with a high membership ( $Q$ ) value ( $> 80\%$ ). The two clusters of FON were summed, as admixed individuals between the two clusters corresponded to within-source crosses. We might therefore expect that individuals of the F1 generation (recruits, seed progeny) resulting from within-source crosses will show a high  $Q$  value at one cluster and from between-source crosses  $Q$  values closer to 0.5 (Van Rossum et al., 2020; Van Rossum and Le Pajolec, 2021). We conducted an analysis with  $K = 6$  (for distinguishing the five sources) for the two translocated populations, computing 30 different runs using an admixture ancestry model with correlated allele frequencies (run length and burn-in period of  $2 \cdot 10^6$  and  $10^6$  Markov Chain Monte Carlo repetitions, respectively). We grouped the 10 best independent runs using STRUCTURE HARVESTER (Earl and vonHoldt, 2012) to calculate the most likely  $Q$  values using CLUMPP version 1.1.2 (Jakobsson and Rosenberg, 2007), and visualized them on plots using DISTRUCT version 1.1 (Rosenberg, 2004).

#### 2.4.4. Direct estimate of contemporary gene flow

Sibship (full sibs, half sibs, and nonsibs), parentage (recruits, excluding the four putative clones in FICH, see Section 3.2) or paternity (seed progeny) and contemporary  $N_e$ , were inferred on seed progeny and recruits (corresponding to a single generation cohort of offspring) for each population, using the full-pedigree likelihood approach implemented in COLONY version 2.0.6.6 (Wang, 2009; Jones and Wang, 2010). Contemporary  $N_e$  estimated from sibship frequency of a single cohort in species with overlapping generations gives an estimate of the effective number of breeders (Wang et al., 2010; Wang, 2016). The analyses were conducted using a full-likelihood approach with high likelihood precision, no prior sibship size, and long length of run. Estimations of allelic dropout rates (null allele frequencies for loci with 95 % HPDI different from 0, calculated with INEST for each cohort in each population) and genotypic error rates were included in the analyses (Wang, 2018). The transplants were included as candidate (maternal and paternal) parent genotypes, with probability of an actual sire or mother being included in candidates being guessed at 0.2, 0.5 and 0.8 (keeping the pedigree reconstruction with the highest maximum likelihood). For seed progeny, probability of finding a mother in candidate parent genotypes was estimated as the proportion of offspring with known maternal genotypes. The other parameters were set as by default or at 0. For  $N_e$ , 95 % confidence intervals were obtained from bootstrapping. Estimates of  $N_e$  may lack accuracy when the upper confidence limit is large or when sample size/ $N_e$  ratio is  $\leq 0.015$  (Wang, 2009, 2016). To search for contemporary pollen flow between populations (Fig. S1), we also performed the analyses considering the transplants of both CEC2 and FICH and the 88 adult genotypes of the six natural populations (Van Rossum and Godé, 2021) as candidate paternal genotypes (excluding them as maternal genotypes for recruits), for a total of 390 genotypes. The best configuration (with the maximum likelihood) of the parentage or paternity was illustrated by a pedigree graph using Pedigree Viewer version 6.5 f (Kingham and Kinghorn, 2015). To obtain an estimation of seed and pollen dispersal distances, we only kept the parents identified in the candidate genotypes with paternity or maternity assignments with

90 % likelihood. Distances between recruits and parent genotypes reflected both seed and pollen dispersal, whereas distance between parents corresponded to pollen dispersal.

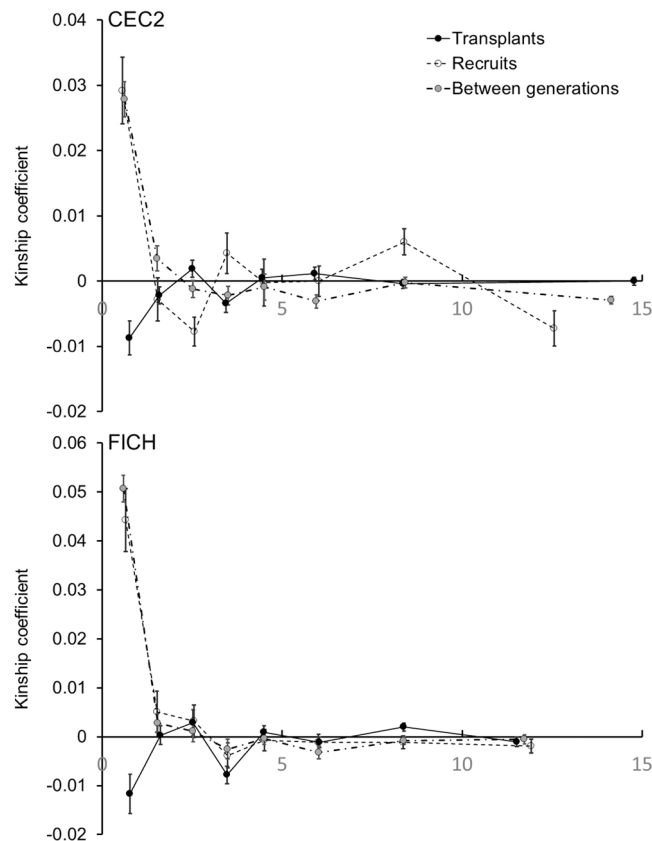
### 3. Results

#### 3.1. Genetic variation within populations across generations

Values of allelic richness,  $H_o$  and  $H_e$  of the F1 generation (seed progeny and recruits) were higher than or similar to those of the transplants, except allelic richness of the seed progeny that was slightly lower than the value of the transplants (Table 1, Table S1). Significantly positive  $F_{IS}$  values were found (Table 1,  $P < 0.05$ ) in the transplants and in the F1 generation (except in the recruits of CEC2), but the  $F_{ISnull}$  values were lower, indicating that the presence of null alleles contributed to the positive  $F_{IS}$  values. The IIM analysis indicated no inbreeding (lowest DIC for the *nb* model) for the F1 generation, with non-significant  $F_{ISnull}$  values. For the transplants, the best fitting model included inbreeding (*nyb*), but the significantly positive  $F_{ISnull}$  values could also be at least partly ascribed to genetic differentiation between source populations ( $F_{ST}$  values ranging 0.035–0.154; Van Rossum et al., 2022).  $F_{IS}$  values were lower for the F1 generation than for the transplants, and were similar between recruits and seed progeny (Table 1, Table S1).

#### 3.2. Extent of clonal propagation in recruits

Out of 459 sampled recruits and transplants with no missing data, 451 were associated with a single multilocus genotype. Only four recruits showed identical multilocus genotypes to a transplant in FICH, at a distance of 0.11–0.15 m. Therefore, they could be considered as putative clones ( $p_{se} < 0.05$ ). All multilocus genotypes of the recruits were distinct from those of the sampled transplants in CEC2. There was also indication of low clonality rate when using the Bayesian method of ClonEstiMate, with the highest posterior probabilities corresponding to a clonality rate of 0.05 in CEC2 and 0.1 in FICH, whatever the mutation rate (posterior probabilities = 0.77 and 0.94, respectively).



**Fig. 1.** Correlograms showing the spatial genetic structure, with mean Loiselle kinship coefficients over all loci as a function of the geographic distance in meters within and between generations (transplants and recruits) in two translocated populations of *Campanula glomerata*.

3.3. Indirect estimates of genetic structure and contemporary gene flow

There was no or low genetic differentiation between generations within and between the translocated populations (pairwise  $F_{ST}$  values varying from 0.000 to 0.004; Table S2). The patterns between generations were similar for  $G''_{ST}$  values (Table S2).

The relationship between pairwise kinship coefficients and the logarithm of geographic distance was significantly negative for the recruits and between generations ( $b$  varying from  $-0.0044$  to  $-0.0087$ ,  $P < 0.001$ ; Fig. 1, Table S3). The slope ( $b$ ) of the regression was not significant for the transplants ( $b = 0.0012$  and  $0.0022$  for CEC2 and FICH, respectively,  $P > 0.05$ ), as expected with the planting design (Fig. S2). The SGS was stronger for recruits and between generations than for transplants (Wilcoxon test  $Z = 2.67-3.41$ ,  $P < 0.05$ , Table S3).

In the STRUCTURE analysis, the transplants were distinguished according to their source origin in each translocated population (membership  $Q$  value  $> 80\%$  for 91–85 % of CHA transplants, 92–94 % of EMO transplants, 89–94 % of FON transplants after summing both clusters, 100–88 % of LAM transplants, and 81–72 % of WAT transplants) in CEC2 and FICH, respectively (Fig. 2a). There were wider ranges of  $Q$  values for the new F1 generation than for transplants: for the recruits, the highest (maximum)  $Q$  value amongst the six clusters ranged from 36 % to 98 % in CEC2 and from 37 % to 97 % in FICH; for the seed progeny, the maximum  $Q$  value ranged from 44 % to 97 % in CEC2 and from 36 % to 97 % in FICH. For both life stages, the maximum  $Q$  values showed a peak at the class of 50–60 %, with a second small peak at 90–100 % for FICH (Fig. 2b). According to transplant and F1 generation distribution patterns, we distinguished within-source crosses when maximum  $Q$  value was high ( $> 80\%$ ) and between-source crosses (admixed individuals) when maximum  $Q$  value was lower. The admixed individuals that were jointly assigned to two clusters showed mixing – and so pollen dispersal – between all five sources (Fig. 2a).

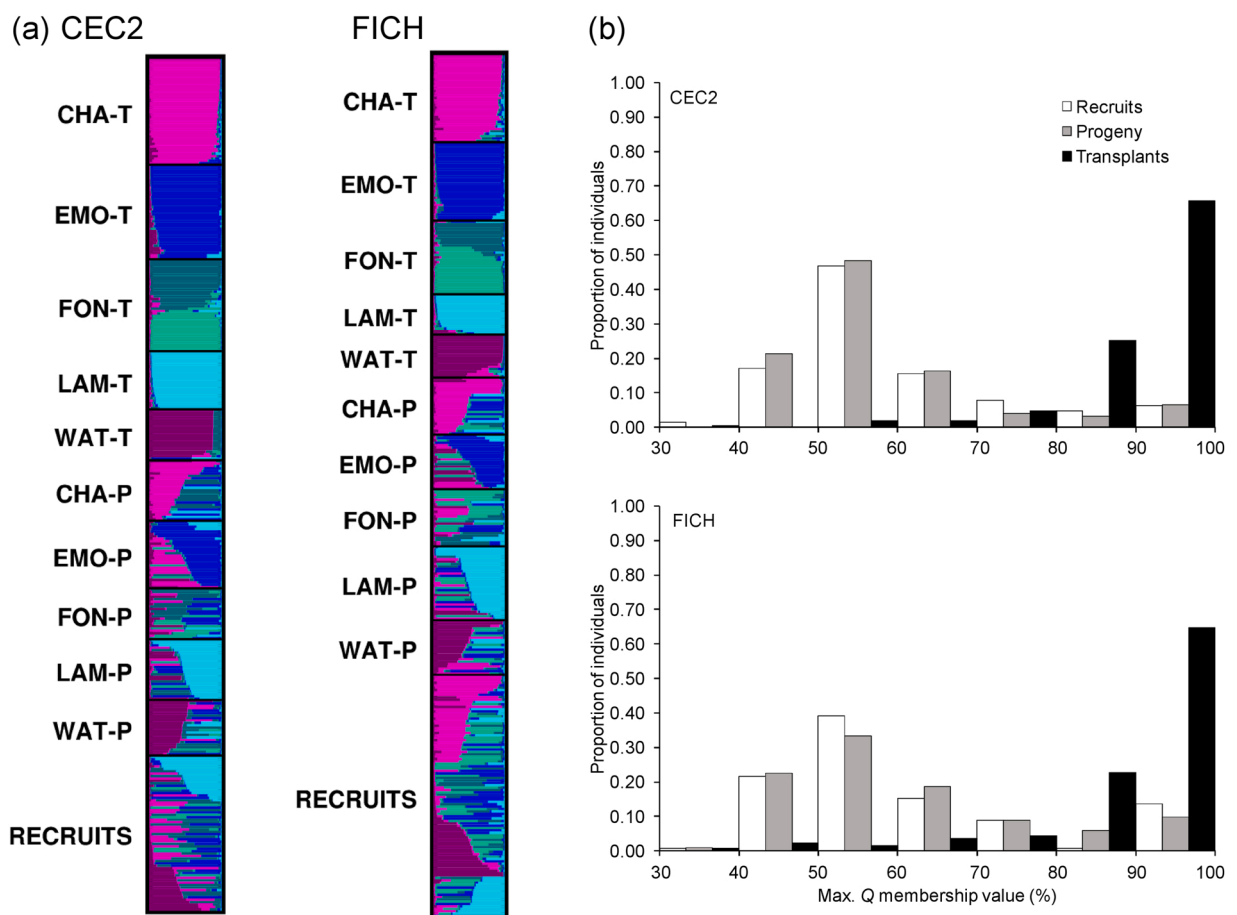


Fig. 2. Bayesian clustering ( $K = 6$ ) of two translocated populations of *Campanula glomerata* (CEC2, FICH): (a) barplot with the distribution of the probability of membership ( $Q$ ) values for each group (T, Transplants; P, seed progeny; Recruits; sources: CHA, EMO, FON, LAM, WAT); and (b) distribution of the transplants, recruits and seed progeny according to their maximum  $Q$  values. In the barplot, each individual is represented by a horizontal bar, showing the membership ( $Q$ ) to each of the 6 modal clusters.

### 3.4. Direct estimate of contemporary gene flow

The proportion of full sibs and half sibs was very low (Table 2, Fig. S2), so that most of the sampled offspring were inferred as nonsibs (96.18–97.56 %). Full-sib family sizes and the number of offspring per inferred (maternal or paternal) parent genotype were small (Table 2, Fig. S3), indicating high multiple paternity. The total number of inferred parent genotypes (sampled and unsampled) ranged from 43 to 71 (Table S4). The proportion of offspring whose at least one maternal or paternal parent was assigned from sampled adult genotypes ranged from 36.7 % to 76.6 % (Table S4), which was not surprising, given that transplant sampling was non-exhaustive. Contemporary  $N_e$  estimated from sibship assignments were 102 and 175 for recruits and 156 and 148 for seed progeny, in CEC2 and FICH, respectively (Table 2). The sample size/ $N_e$  ratios ranged from 0.56 to 0.84, and 95 % confidence intervals were relatively narrow, so that  $N_e$  estimates might be considered as accurate.

Dispersal distances within populations calculated with candidate parents assigned to offspring with  $\geq 90$  % likelihood, however based on small sample sizes for recruits (Table S5), showed restricted pollen and seed dispersal, with most dispersal events occurring at less than 2–4 m, reaching distances up to 18.11 or 13.51 m, which did not cover the distance ranges between transplants (Fig. 3, Table S5). Admixture between seed sources was identified for 79–91 % of the pollination events assigned with  $\geq 90$  % likelihood. Some between-population pollen flow events (considering candidate paternal plants assigned to offspring with  $\geq 90$  % likelihood) were inferred from the paternity analyses (for 18–15 seed progenies and 3–22 recruits for CEC2 and FICH, respectively), in particular between the two translocated populations (CEC2 and FICH), but also from CHA, EMO, FON or WAT to the translocated populations, which were distant at 0.68–2.35 km (Table S6). The number of offspring per sire ranged from 1 to 4, with 1–9 potential sires per pollen donor population. Only two seed progeny in CEC2 and three recruits in FICH had an already assigned sire for the within-population analysis.

## 4. Discussion

Translocating a highly variable genetic pool usually improves the genetic status of the restored populations (e.g., Ritchie and Krauss, 2012; St. Clair et al., 2020; Van Rossum and Hardy, 2022). The natural populations of *C. glomerata* used as seed sources for translocations were small and fragmented, occurring in relict habitats, but still held high –likely pre-fragmentation– genetic diversity in the adult generation (Van Rossum et al., 2022). However, we can expect mating patterns such as pollination and seed dispersal to be already impacted, resulting in gene flow disruption in the newly recruited generations (e.g., Aguilar et al., 2008, 2019; Cristóbal-Pérez et al., 2021). Negative consequences of habitat fragmentation (genetic drift leading to high genetic differentiation among populations, biparental inbreeding and inbreeding depression) were indeed detected in the seed progeny, which corresponded to the transplants used for plant translocations in CEC2 and FICH (Van Rossum et al., 2022). Genetic diversity in the first post-translocation generation is representative of the natural source populations, and combining these sources allowed for random mating (no inbreeding found in the seed progeny or recruits), and for enhancing the number of breeders (contemporary  $N_e$ ). The  $N_e$  estimates for FICH and CEC2 are even higher than the sum of  $N_e$  estimates of the source populations (126), except for recruits in CEC2 (based however on a sample size of 64 recruits). These short-term findings are encouraging for further population establishment (Schäfer et al., 2020).

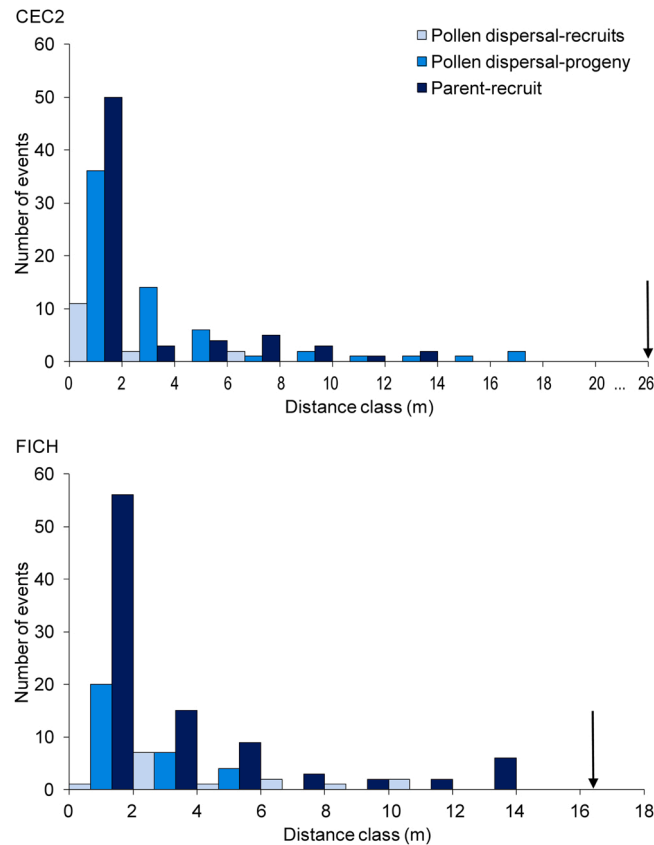
Indirect and direct estimates of contemporary mating and gene dispersal patterns in the first post-translocation generation of translocated populations of *C. glomerata* were consistent with each other, revealing successful crosses between transplants of different seed source origins. These patterns can be related to sexual reproduction (pollen and seed dispersal), as clonal propagation only plays a very marginal role in the beginning of post-translocation population expansion. However, parentage and paternity analyses revealed that pollen and seed dispersal mostly occurred at short distances within populations, favouring fine-scale SGS in the newly established generation as shown by the spatial autocorrelation analyses (Figs. 1 and 3). Even pollen dispersal distances were short (far below the maximal distances between parents, which is quite surprising, given the potential pollen dispersal ability of wild solitary bees,

**Table 2**  
Estimates of effective population size ( $N_e$ ), sibship and parentage for two translocated populations of *Campanula glomerata*.

Population	$n_{\text{off}}$	$n_{\text{par}}$	$N_e$	Full sibs (%)	Half sibs (%)	Full-sib family size	Offspring	
							Parent 1	Parent 2
CEC2								
Recruits	64	166	102 (74–150)	0.10	3.72	2 (2–2)	1.5 (1–5)	1.4 (1–3)
Seed progeny	122	166	156 (121–203)	0.09	2.37	2 (2–2)	F: 1.7 (1–6)	M: 1.5 (1–2)
FICH								
Recruits	98	136	175 (134–234)	0.04	2.40	2 (2–2)	1.5 (1–3)	1.5 (1–4)
Seed progeny	125	136	148 (114–194)	0.05	2.65	2 (2–2)	S: 1.8 (1–6)	M: 2.0 (1–3)

Abbreviations:  $n_{\text{off}}$ , number of sampled offspring in a single cohort (excluding four putative clones for FICH recruits);  $n_{\text{par}}$ , number of sampled candidate parent genotypes; full sibs and half sibs (%): percentage of full-sib and half-sib offspring pairs, respectively; full-sib family size: mean number of offspring per full-sib family, with range; offspring: mean number of offspring per maternal and paternal parent (parent 1 or 2), with ranges; M, mother, and S, sire (paternity analyses).  $N_e$  values are shown with their 95 % confidence intervals.





**Fig. 3.** Distance class distribution of seed and pollen dispersal inferred from parentage and paternity analysis for two translocated populations (CEC2 and FICH) of *Campanula glomerata*. Pollen dispersal distance is the distance between maternal and paternal parent (in seed progeny and in recruits), and parent-recruit distance is the distance between recruit and maternal or paternal parent. The arrows indicate the maximal distance between sampled transplants in the translocated populations.

bumblebees and honey bees (e.g., Van Geert et al., 2010; Van Rossum, 2010; Zurbuchen et al., 2010), which are the main pollinators of *C. glomerata* (Albrecht et al., 2007; Denisow and Wrzesień, 2015), and the extensive pollen dispersal found in the natural populations (Van Rossum et al., 2022). Translocated (restored) and natural populations may differ in several characteristics, which may affect pollination processes, such as floral density, presence of other flowering resources, differences in pollinator abundance, and disturbance related to restoration management (e.g. soil scraping) (Ghazoul, 2005; DiLeo et al., 2018; Carvalho et al., 2022). For instance, natural populations *C. glomerata* were small, and so pollinators might visit all plants once they have found the population, favouring extensive within-population pollen dispersal (e.g., Van Rossum and Triest, 2010). In the large (and dense; Fig. S2) translocated populations, pollinators might spend more time visiting flowers within dense flowering patches, increasing short-distance pollen dispersal among neighbours (Ghazoul, 2005; Llaurens et al., 2008). Our findings thus mean that the high admixture between seed sources found both by STRUCTURE and sibship assignment methods and the resulting enhanced genetic diversity, random mating (no inbreeding) and high number of breeders (contemporary  $N_e$ ) in the F1 post-translocation generation may be rather ascribed to the spatial arrangement of transplants optimizing source mixing sources (Fig. S2) than to extensive pollen flow across the translocated populations.

The role of the transplants is mainly to provide genetically diverse seeds that will produce the next generations. When they will disappear, future changes in genetic patterns will only depend on local contemporary mating and evolutionary processes. If very short gene dispersal distances remain in subsequent generations, the resulting stronger SGS might lead on the long term to biparental inbreeding and reduced seed set due to a lack of compatible neighbours (Tierney et al., 2020; Thomas et al., 2021; Monks et al., 2021). In the first years of the experiment, the translocated area was fenced to prevent herbivory and to optimize transplant establishment, flowering and seed production. Allowing the entrance of the grazing flocks of sheep and goats used for grassland management during the fruiting season may contribute to increase seed dispersal distances. Indeed, livestock can be a seed dispersal vector for many plant species (including *Campanula patula* and *C. rotundifolia*), via epizoochory –their fur is known to carry seeds–, or endozoochory (Albert et al., 2015; Benthien et al., 2016; Kapás et al., 2020). Migrating herds of grazing sheep have been reported to increase long-distance gene flow and connectivity between populations of *C. rotundifolia* via endozoochorous dispersal (Plue et al., 2019; Lehman et al., 2020). Zoochory by grazing sheep is also hypothesized as an explanation for the low genetic differentiation between *C. glomerata* populations in Germany (Bachmann and Hensen, 2007). Moving grazing flocks among sites might therefore be a solution to facilitate

gene flow by seeds between populations of *C. glomerata*.

Parentage analyses also suggest the existence of some long-distance events (up to a few kilometres) of pollen flow between translocated populations as well as from natural populations (Table S6). Such contrasted pattern of short-distance pollen dispersal within populations and of long-distance pollen flow at landscape scale was also found for *Pulsatilla vulgaris*, also pollinated by wild bees (DiLeo et al., 2018). Small bees often carry pollen over a few hundred meters. However, large bees such as bumble bees and honey bees, are able to carry pollen over longer distances (Gathmann and Tschardt, 2002; Van Geert et al., 2010; Van Rossum, 2010; Zurbuchen et al., 2010). Only one pollen flow event was found between two natural populations based on parentage assignments, suggesting that the small, fragmented, natural populations were isolated from each other (Van Rossum et al., 2022). The large flowering size of the two translocated populations might have increased the visibility and attractiveness of *C. glomerata* for the pollinators in this area, favouring pollen flow between populations (Ghazoul, 2005). This enlightens the potential of creating new large populations by plant translocations to restore population connectivity by gene flow. However, the relatedness of individuals between natural populations and transplants (adults and their seed progeny, respectively), and the nonexhaustive sampling of the potential sires might also have led to some possible false assignments (Wang, 2018). Therefore, these findings need to be confirmed, for instance, by investigating pollen dispersal among populations using fluorescent dyes as pollen analogues (Van Geert et al., 2010; Van Rossum, 2010). Whether there is pollen flow from translocated populations to small natural populations, counteracting genetic erosion, biparental inbreeding and inbreeding depression which may be expected in the coming generations (Van Rossum et al., 2022), also needs to be investigated.

In conclusion, estimating mating patterns and pollen and seed dispersal kernels in the early post-translocated generations permitted to identify spatially important issues to consider for population establishment after translocation, in particular the spatial design of transplants, post-translocation management, and plant-pollinator interactions, as they can impact the long-term genetic sustainability of the translocated populations.

### CRedit authorship contribution statement

FVR conceived and designed the research; FVR and SLP conducted the field sampling; SLP conducted the germination experiment; CG conducted the microsatellite analyses and genotyping; FVR analyzed the data and wrote the manuscript and all authors critically reviewed the manuscript.

### Declaration of Competing Interest

None.

### Data Availability

The data are available on Zenodo repository at <https://doi.org/10.5281/zenodo.5676286> (Van Rossum and Godé, 2021) and <https://doi.org/xxx> (Van Rossum and Godé, 2023).

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.gecco.2023.e02548](https://doi.org/10.1016/j.gecco.2023.e02548).

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