Maternal effects and inbreeding depression in post-translocation progeny of *Campanula glomerata*

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Key message of the paper:
Inbreeding depression and strong maternal effects were detected in post-translocation progeny fitness related to maternal transplant seed source origin and lineage in translocated populations of *Campanula glomerata*, which might affect global genetic diversity patterns in the long term.

Abstract

- Evaluation of plant translocation success based on fitness-related quantitative traits combined with molecular markers may contribute to a finer assessment of inbreeding, selective and rescue processes, which may have long-term consequences for population dynamics and viability.
- We investigated fitness traits (seed germination, seedling viability, and juvenile growth and mortality) combined with 15 microsatellite loci of the first post-translocation seed progeny from two translocated populations of *Campanula glomerata*, an insect-pollinated, self-incompatible perennial herb. We examined whether inbreeding, heterosis through admixture, translocation site and maternal transplant seed source origin and lineage might affect seed quality and juvenile growth in controlled cultivation conditions.
- Flower production and seed germination of the transplants was higher in one of the two translocation sites, which might be related to differences in soil and vegetation composition and cover. Strong maternal effects related to seed source origin and lineage were found on progeny size, with the largest transplants producing the largest progenies. The differences in rosette diameter were maintained across the whole growth period measured. There was inbreeding depression (rather than heterosis) related to biparental inbreeding at early progeny growth stage, also expressed through juvenile mortality.
- Our findings highlighted that maternal transplant origin, especially when seed sources consisted of small fragmented remnants, might have a selective value on fitness in the post-translocation generations. If maternal effects and inbreeding depression persist, they might affect global genetic diversity patterns in the long term. Further admixture in the next generations might buffer maternal and inbreeding effects or lead to outbreeding depression.

INTRODUCTION

Reintroductions in extirpated populations and reinforcements of inbred and genetically impoverished populations through plant translocations has become a popular restoration practice for the recovery of viable and evolutionarily resilient populations of critically endangered species (e.g., Zimmer *et al.* 2019; Fenu *et al.* 2023). Genetic monitoring based on neutral molecular markers (microsatellites, SNPs) can evaluate whether genetic restoration has been effective in reinforced populations and whether translocation has recreated populations with a highly diverse gene pool (e.g., Van Rossum *et al.* 2020; Van Rossum & Hardy 2022 and references cited therein; Dillon *et al.* 2023). For instance, a successful increase in genetic diversity has been found in genetically depauperate populations after reinforcement by sowing seeds or planting young plants of local or non-local provenance, for *Arnica montana*, an insect-pollinated self-incompatible clonal herb (Van Rossum *et al.* 2020), and for *Pulsatilla vulgaris*, an insect-pollinated predominantly outcrossing herb (Gargiulo *et al.* 2019). Using multiple seed sources in mixture for plant translocation could provide new populations with high genetic diversity and genetic composition representative of the wild genetic pool, as reported for several insect-pollinated herb species, such as the self-compatible *Arenaria grandiflora* (Zavodna *et al.* 2015) and *Dianthus deltoides* (Van Rossum & Le Pajolec 2021), and the self-incompatible *Campanula glomerata* (Van Rossum *et al.* 2023a), and for bird-, mammal- and/or insect-pollinated self-compatible small tree or shrub species, such as *Banksia brownii* (Dillon *et al.* 2023) and *Lambertia orbifolia* (Monks *et al.* 2021). However, new cohorts in translocated populations of *B. brownii*, *D. deltoides* and *L. orbifolia* were characterized by high selfing rates, while restricted seed and pollen dispersal distances, already from the first recruited generation, were found in translocated populations of *C. glomerata*. Those mating patterns might be related to insufficient availability of dispersal vectors, pollinator behaviour related to local floral abundance and plant density, and/or translocation site environment (Monks *et al.* 2021; Dillon *et al.* 2023; Van Rossum 2023; Van Rossum *et al.* 2023a). When seed source populations are of small size or with high clonal extent, only a small number of potential parents may contribute to the seeds used for propagating transplants. As a result, many transplants can be related (i.e. full sibs or half sibs), so that biparental inbreeding may also arise in the newly established cohorts (Aguilar *et al.* 2019; Doyle *et al.* 2023), as reported for *D. deltoides* (Van Rossum & Le Pajolec 2021), for *Primula vulgaris*, a wild bee-pollinated heterostylous herb (Barmentlo *et al.* 2018), and for *Hakea nitida*, a bird- and insect-pollinated shrub (Millar *et al.* 2021).

Small populations may show inbreeding depression, i.e. reduced fitness of inbred seed progeny, but also drift load, that is reduced fitness of outbred offspring due to the accumulation and fixation of recessive deleterious mutations over time through genetic drift (Paland & Schmid 2003; Willi *et al.* 2005). In the case of reinforcement of declining populations suffering from inbreeding depression and/or drift load, whether there is genetic rescue has to be evaluated, so whether introducing new genetic variation and/or heterosis (higher fitness of cross progeny) have counteracted inbreeding and drift issues (Bell *et al.* 2019). Genetic rescue cannot be evaluated based on neutral molecular markers only. Fitness-related quantitative traits are required (Willi *et al.* 2006; Nicotra *et al.* 2015). Moreover, inbreeding (or outbreeding) depression, and local adaptation issues, with maladaptation possibly related to maternal effects in case of differences between source and translocation environments, may arise in translocated populations (Vergeer *et al.* 2004; Edmands 2007; Schuler & Orrock 2012; Zavodna *et al.* 2015). For instance, despite evidence from neutral molecular markers of global increase in genetic diversity and of genetic mixing (admixture) in the newly established post-translocation generations, reduced plant size, floral display or reproductive performance in inbred offspring, indicating inbreeding depression, was found in translocated populations of *B. brownii* (Rodger *et al.* 2023), *D. deltoides* (Van Rossum & Le Pajolec 2021), *A. grandiflora* (Zavodna *et al.* 2015),

and *P. vulgaris* (Barmentlo *et al.* 2018). For the last two species, reduced fitness in admixed offspring (so with parents from different source origins), indicating outbreeding depression and local adaptation as well as heterosis for within-population outcrossed offspring, were detected. Maternal effects at early growth progeny stages as well as high phenotypic plasticity at later stages was reported for *A. montana* (Van Rossum *et al.* 2020) and *D. deltoides* (Van Rossum & Le Pajolec 2021). Local adaptation through increased biomass and flowering of local transplants has been reported in *Succisa pratensis*, an insect-pollinated self-compatible herb (Vergeer *et al.* 2004). Maladaptation of non-local transplants indicated by lower reproductive success was found for *Silene douglasii* (Lofflin & Kephart 2005). Therefore, investigating fitness-related quantitative traits combined with molecular markers may contribute to a finer assessment of inbreeding, selective or rescue processes occurring in translocated populations, which may have long-term consequences for population dynamics and viability. It can provide information for proposing adaptive-oriented management of the populations (Willi *et al.* 2006; Van Rossum & Hardy 2022; Dillon *et al.* 2023; Rodger *et al.* 2023).

In this paper, we investigated fitness-related quantitative traits (seed germination, seedling viability, juvenile growth and mortality) combined with molecular markers (microsatellites) of the first post-translocation seed progeny produced by the transplants, in translocated populations of the insect-pollinated, self-incompatible, *Campanula glomerata* L. (Campanulaceae). Local small populations were used as seed sources for plant translocations in southern Belgium (Godefroid *et al.* 2016). Previous genetic studies (Van Rossum *et al.* 2022, 2023a) based on neutral nuclear microsatellite markers showed that the adult generation of these small populations still retained high, likely old (pre-fragmentation) genetic diversity. For the offspring generation (used for transplant propagation in controlled cultivation conditions), there was evidence of extensive contemporary pollen dispersal within populations. However, demographic bottlenecks, genetic erosion (due to genetic drift effects) and biparental inbreeding were found in some of the source populations, as well as inbreeding depression, and differences in plant size among seed source populations (Van Rossum *et al.* 2022). Given the small effective population sizes (Van Rossum *et al.* 2022), drift load might also be expected (Paland & Schmid 2003; Willi *et al.* 2005, 2013). In the translocated populations, genetic admixture between seed source origins was favoured by the mixed spatial arrangement of the transplants, but seed and pollen dispersal at short distances has led to spatial structuring of the genetic variation of the newly established recruits (Van Rossum *et al.* 2023a). Mating among relatives might be expected in the subsequent post-translocation generations, which might lead to biparental inbreeding but also to reduced seed set (Aguilar *et al.* 2019; Doyle *et al.* 2023). Therefore, examining whether inbreeding depression and heterosis in admixed offspring (indicating drift load; Willi *et al.* 2013) might occur, and whether translocation site environment, and maternal transplant seed source origin and lineage (and so maternal genetic and environmental effects), might affect seed quality and progeny growth of the first post-translocation generation, can provide some additional insights on possible outcomes for the next generations. More precisely, we addressed the following questions: (1) Does transplant seed quality (seed germination, seedling viability, and juvenile mortality) and seed progeny fitness (rosette diameter and number of leaves) differ between translocation sites, reflecting environmental effects, and thus possible future selective processes? (2) Does progeny fitness differ among maternal transplant seed source origins and transplant site lineages, reflecting possible maternal genetic and environmental effects, and are the differences in progeny fitness maintained with time or is there evidence of phenotypic plasticity? (3) Is there evidence of (biparental) inbreeding / outbreeding depression (lower fitness of inbred / admixed offspring) and of heterosis (higher fitness of the between-source progeny) in the seed progeny?

MATERIALS AND METHODS

Study species

Campanula glomerata L. (Campanulaceae) has a wide continental Eurasian distribution range, and typically occurs in calcareous grasslands, scrub, open woodlands and sand dunes (habitat code 6210 according to EU 92/42/EEC Habitats Directive) (Klotz *et al.* 2002; Denisow & Wrzesień 2015). Those habitats, and their associated species, have strongly declined in northwestern Europe, with plant populations becoming small and fragmented (Hill *et al.* 2004; Bachmann & Hensen 2007; Godefroid *et al.* 2016). In Belgium, *C. glomerata* is critically endangered, with a few small remaining wild populations, occurring in degraded calcareous habitats (evolving into more competing, nutrient-richer hay meadow vegetation) (Godefroid *et al.* 2016; Godefroid, unpublished data).

This herbaceous perennial species is characterized by a gametophytic self-incompatible breeding system, and can be considered as an obligate outcrosser (Gadella 1964). Plants flower in June-August (Klotz *et al.* 2002; Bachmann & Hensen 2007). Flowers produce nectar and are pollinated by insects, mainly bumble bees, solitary bees, honey bees and hoverflies (Albrecht *et al.* 2007; Denisow & Wrzesień 2015). The fruit is a capsule containing numerous and tiny seeds (up to 100), which are dispersed over short distances by capsule shaking but also possibly over longer distances through zoochory by grazing sheep. Seeds do not form a persistent seed bank in the soil, but plants can live for 25-30 years and subsist as non-sprouting rhizomes (Klotz *et al.* 2002; Bachmann & Hensen 2006, 2007).

Study populations and sampling

Plant translocations (500 transplants in each site propagated from mixed seed sources) were conducted in four sites (restored calcareous grasslands, planting on scraped, bare soil) from Belgian Lorraine (southern Belgium) in September 2015 (Fig. 1; Godefroid *et al.* 2016; Van Rossum *et al.* 2022). Fitness measures and seed collection for the present study were conducted in two translocated populations, Ficherulle (FICH; 49°42'54"N, 5°13'31"E) and Sainte-Cécile2 (CEC2; 49°43'40"N, 5°14'00"E), located at 1.53 km from each other. The two translocation sites differed in soil and vegetation cover and composition: FICH was characterized by a sandy-marly soil and a dense (80% cover on average) grassland vegetation (dominated by tall herb species, in particular *Tanacetum vulgare* L., *Leucanthemum ircutianum* DC., *Rubus* sp., *Rhinanthus angustifolius* C.C. Gmel., *Silene latifolia* Poir., *Centaurea scabiosa* L. and *Dactylis glomerata* L.), and CEC2 by a marly soil and sparse (50% cover on average) grassland vegetation (dominated by *Holcus lanatus* L., *Bromopsis erecta* (Huds.) Fourr. and by short forb species, in particular *Ranunculus repens* L., *Trifolium repens* L., *Taraxacum* F.H. Wigg., and *Geranium columbinum* L.) (Fig. S1).

The transplants of the translocated populations originated from seeds collected in five small wild populations (flowering population size ranging 7-28 flowering individuals): Chassepierre (CHA), Emond (EMO), Fontenoille (FON), Lambermont (LAM) and Watrinsart (WAT) (Van Rossum *et al.* 2022, 2023a). Based on sibship assignment analyses (Van Rossum *et al*. 2022), but considering *C. glomerata* as self-compatible in the parameters, no evidence of breakdown of the self-incompatibility system leading to selfing was found in the small seed source populations (Van Rossum, unpublished results). The percentage of offspring (used as transplants for plant translocation) sharing at least one parent (full sibs and half sibs) ranged 9.0-54.6% (Figure 1), and biparental inbreeding was detected in CHA and LAM (significantly positive F_{IS} values after correction for null allele frequency = 0.016 and 0.043, respectively) (Van Rossum *et al.* 2022).

Seeds (F1 generation) were collected in 2016 on 188 transplants (95 and 93 in CEC2 and FICH translocation sites, respectively, and 16 to 20 per seed source origin; Fig. 1). They were stored in a dry-cold environment (15°C, 15% relative moisture), until the germination experiment.

Maternal effects were taken into account by seed source origin of the maternal transplants, by maternal lineage, and by two quantitative variables. First, the number of flowering stems and of flowers per stem (for max. five flowering stems) was counted in 2016 for the 188 transplants, and used to estimate floral display size as the total number of flowers per transplant (Godefroid & Van Rossum, unpubl. data). Second, rosette diameter (in cm) measured 2 months after germination (just before translocation) was used as an estimator of vegetative maternal transplant size (data from Le Pajolec *et al.* 2021).

Quantitative trait measures of seed progeny

At the beginning of April 2017 (after 1 month at 5°C to break the dormancy), 100 seeds for each of the 188 transplants were germinated in Petri dishes on 1% agar (10 g/L) in incubators (20°C, 8/16 light/dark photoperiods). Seed germination (the proportion of germinated seeds) and the proportion of viable seedlings (i.e. fully developed green seedlings over the total -chlorotic and green- number of seedlings, excluding rotten seedlings) were recorded after three weeks. A total of 1,215 viable seedlings (up to 12 per transplant, for 146 maternal transplants) were transferred to an unheated greenhouse in pots of 3 cm diameter, and repotted four weeks later in 9 cm pots with (pH 6.5) extra fine potting soil (Godefroid *et al.* 2016). The pots were randomized every two weeks. When the plants were 6-weeks old, we started to measure plant size, i.e. rosette diameter (in cm) every two weeks (measurement repeated four times). The number of leaves was counted at the end of the experiment (12 weeks after germination). We also recorded mortality (the proportion of dead plants) at the end of the experiment (for fitness datasets, see Van Rossum *et al.* 2023b). The 12 week-old plants were delivered to practitioners (Natagora) for translocation in two additional sites in Belgian Lorraine.

Individual inbreeding and parental seed source origin assessed from multilocus genotypic data

In order to test for heterosis (higher fitness of the heterozygotes) and (biparental) inbreeding / outbreeding depression (lower fitness of inbred / admixed offspring) in the seed progeny, fitness quantitative traits were analysed in relation to multilocus genotypic data based on 15 microsatellite loci obtained in a previous study (Van Rossum *et al.* 2023a) for 166 and 136 transplants and for 122 and 125 seed progenies (1-3 offspring per maternal transplant) in CEC2 and FICH translocation sites, respectively (for datasets, see Van Rossum & Godé 2021, 2023). For this purpose, Homozygosity by Loci (HL), as an indicator of individual inbreeding level, was computed for each sampled transplant and seed progeny, separately in each population, using the software GENHET (Coulon 2010). HL can vary from 0 (all loci are heterozygous) to 1 (all loci are homozygous; Aparicio *et al.* 2006). One microsatellite locus (CAM27) was excluded from the analyses, because its high frequency of null allele (ranging 28-38%; Van Rossum & Godé 2022) might falsely inflate HL.

The five seed source populations were found to be genetically differentiated from each other based on 15 microsatellite loci (F_{ST} values between populations ranging 0.089-0.159), and corresponded to distinct clusters in STRUCTURE (Pritchard *et al.* 2000) Bayesian clustering analysis (Van Rossum *et al.* 2023a). Transplants could be assigned to their seed source origin (CHA, EMO, FON, LAM or WAT) with high cluster membership values ($Q \geq 80\%$). Therefore, it was possible to identify the seed source origin of the parental transplants of the seed progeny from the STRUCTURE analysis, and so whether both parents belonged to the same or different

source origins (Van Rossum *et al.* 2023a). Two cross categories (within or between seed sources) were therefore defined based on cluster membership (*Q)* values inferred from STRUCTURE analysis, i.e. whether seed progeny descended from natural crosses between parental transplants of the same or different source origins, respectively. The threshold was set at $Q = 80\%$, with within-source crosses assumed when maximum Q value for the best cluster was ≥ 80% and between-source crosses (admixed individuals) when Q value was < 80% in all clusters (Van Rossum *et al.* 2023a).

Data analysis

We conducted two-way analyses of covariance (ANCOVA) together with pairwise Tukey HSD post-hoc tests for testing for differences in fitness variables in relation to translocation site, seed source origin and lineage of the maternal transplants and/or maternal (transplant) fitness (Table S1, Fig. 1). We used a Generalized Linear Model (logit function) for the total number of flowers (negative binomial distribution) and for proportion variables (seed germination, viable seedlings, juvenile mortality; binomial distribution, taking overdispersion into account by dividing the deviance by its degrees of freedom to estimate the dispersion parameter; McCullagh & Nelder 1989). The transplant variables were tested in relation to translocation site and seed source origin of the maternal transplants, with rosette diameter, total number of flowers (log-transformed) and HL (Box-Cox-transformed) of the maternal transplants as covariates (Table S1). Differences in transplant HL between the five source origins were tested by performing an one-way Analysis of Variance (ANOVA) together with pairwise Tukey tests.

For seed progeny, leaf number (Box-Cox-transformed) and rosette diameter (as repeated measures over time: t1 to t4; with Greenhouse-Geisser adjusted probabilities) were tested using a linear model in relation to translocation site, seed source origin of the maternal transplants, and maternal transplant lineage (nested in source origin), with rosette diameter, total number of flowers and HL of the maternal transplants as covariates (Table S1). The effects of categorical variables and covariates were tested against the variation among maternal transplant lineages. The effect of maternal transplant lineage was tested against the residual (error) variation among individuals. The analyses were also conducted on the data subset analysed with molecular markers, in relation to translocation site, seed source origin of the maternal transplants, and progeny HL as covariate. As only 31 out of 247 seed progenies were identified as within-source cross category, Mann-Whitney *U* tests were performed for testing for differences in rosette diameter, leaf number and progeny HL between the two cross categories. We performed Pearson's or Gamma correlation analyses (for normally- and non-normally-distributed variables, respectively) between progeny or transplants fitness variables and HL. All analyses were performed using STATISTICA version 12 (Dell Inc., Tulsa, Oklahoma, USA).

RESULTS

Translocation site environmental effect

There was a translocation site effect on maternal transplant fitness variables (Table S2): the total number of flowers and seed germination were significantly higher in CEC2 than in FICH translocation site (Fig. 2ab, Table S3). Seed germination was low (≤ 0.49 at individual maternal transplant level; Fig. 2), but most seedlings were viable (Table S3), as only 22 out of the 2,038 germinated seedlings were chlorotic (12 in CEC2 and 10 in FICH translocation site). Therefore, the proportion of viable seedlings was not further considered in the analyses. There was no effect of translocation site on juvenile mortality and on seed progeny fitness variables (Fig. 2cf and S2, Tables S2, S5 and S6).

Maternal seed source origin and lineage effects

There was a seed source origin effect on maternal transplant fitness variables. The transplants of CHA seed source origin produced on average more flowers than the transplants originating from EMO, FON and WAT (Tukey tests, $P = 0.003$-0.014; Fig. 2a, Tables S2 and S3). Juvenile mortality was higher for maternal transplants originating from FON than from LAM and from WAT (Tukey tests, $P = 0.020$ and 0.006, respectively; Tables S2 and S3; Fig. 2c). The other pairwise Tukey tests testing for differences in total number of flowers, seed germination or juvenile mortality between seed source origins were not significant (P ranging from 0.059 to 0.998).

For seed progeny, we found a significant effect of seed source origin of the maternal transplants on progeny rosette diameter for the four measured periods of growth, with WAT and LAM seed source origins growing larger seed progeny plants (Fig. 2de and S2, Tables S4 and S5). Progeny of WAT transplants produced less leaves than those of CHA and EMO, but only in CEC2 translocation site (Fig. 2f). We found significant differences in progeny plant size (rosette diameter, leaf number) among maternal transplants (Tables S5 and S6). Progeny rosette diameter increased with maternal rosette diameter at t1, t2 and t3 (Pearson's correlation coefficient between maternal rosette diameter and average progeny rosette diameter per maternal transplant $r = 0.319$, 0.309, and 0.245, respectively, $P \leq 0.003$; Fig. 3), while at t4 the positive correlation was only a trend ($r = 0.155$, $P = 0.064$). Progeny rosette diameter and leaf number were not significantly ($P > 0.05$) related to maternal floral display size, except at t4 (β estimate of the ANCOVA = -0.176, $P = 0.022$, but Pearson's correlation coefficient between maternal floral display size and average progeny rosette diameter per maternal transplant $r = -0.080$, $P = 0.338$) and to maternal HL (Tables S5 and S6). Maternal rosette diameter maternal and floral display size were not correlated ($r = 0.086$, $P = 0.239$).

Testing for biparental inbreeding / outbreeding depression and heterosis

There was no significant correlation between maternal HL and the total number of flowers of the transplants (Pearson's correlation coefficient $r = 0.082$, $P = 0.315$), seed germination and juvenile mortality (Gamma correlation coefficient $\Gamma = 0.061$ and 0.031, $P = 0.271$ and 0.613, respectively). The ANOVA on maternal HL showed a significant effect of seed source origin ($F_{(4,148)} = 3.04$, $P = 0.019$); this was only due to significantly higher HL values for transplants originating from FON than for WAT seed source (mean value ± SE = 0.26 ± 0.03 and 0.15 ± 0.02, respectively; Tukey test, $P = 0.010$). The other pairwise Tukey tests testing for differences in transplant HL between seed source origins were not significant ($P = 0.112$-0.994).

Progeny HL was negatively correlated with progeny rosette diameter at the first measure (t1) ($\Gamma = -0.090$, $P = 0.038$), but it was not correlated with older stages of rosette diameter and with leaf number (Γ ranging from -0.075 to 0.012, $P \geq 0.082$). Rosette diameter at t1 was significantly higher by 10% on average for between-source cross category than for within-source cross category (mean value ± SE = 11.3 ± 0.2 cm and 10.0 ± 0.5 cm, respectively; Mann-Whitney U tests, $Z = 2.30$, $P = 0.021$; Fig. 4, Table S4), but the differences were not significant neither for the next measures of rosette diameter (Z values ranging with time from 0.80 to 0.67, $P > 0.421$) nor for leaf number ($Z = -0.31$, $P = 0.754$). Progeny HL was significantly higher for within-source cross category than for between-source category (HL ranging from 0.060 to 0.505 and from 0 to 0.365, respectively; $Z = -4.79$, $P < 0.001$). When performing a Mann-Whitney U test for the same range of progeny HL (HL = 0.060-0.302), the test was not significant anymore ($Z = 1.25$, $P = 0.210$), indicating that the difference in rosette diameter at t1 could be rather ascribed to HL (inbreeding depression) than to a between-source cross effect (heterosis).

DISCUSSION

Differences in transplant reproductive fitness between translocation sites

Seed germination in controlled conditions (after three weeks) was low, but similar to values reported in other studies (Bachmann *et al.* 2005; Koutsovoulou *et al.* 2014). The high number of seeds per capsule (up to 100 seeds; Bachmann & Hansen 2007) and per plant (94.5 and 48.9 flowers on average in CEC2 and FICH translocation sites, respectively; Table S3) can however compensate for this low germination rate. Reproductive fitness of the transplants was higher in CEC2 than in FICH translocation site, with transplants producing more flowers and higher seed germination, and so possibly better seed quality, despite similar global genetic diversity (based on neutral molecular markers) and inbreeding levels between sites (Van Rossum *et al.* 2023a). The two translocation sites differ in environmental conditions, in particular in soil and vegetation cover and composition, with a more sandy (vs. a more marly) soil and a denser vegetation in FICH than in CEC2 (Fig. S1), so that there may be differences in resource and water availability, shading and competition, but also in pollinator services between sites. Differences in abundance and composition of simultaneously flowering nectar-producing species might increase competition or facilitation (through attractiveness) for pollinators, reducing or increasing pollen transfer efficiency, which may in turn affect seed quality (Mitchell *et al.* 2009). Flowering of *C. glomerata* has been reported to be reduced by shading from neighbour plants of competing species (Bachmann *et al.* 2005), and seed quality in *C. glomerata* was found to be related to pollination services, through seed mass increasing with pollinator species richness and abundance (Albrecht *et al.* 2007). Increasing maternal nutrient resources and light intensity were associated with increasing seed mass and decreasing seed germination in *Campanula americana* (Galloway 2001, 2005).

Differences in seed progeny growth according to maternal transplant seed source origin and lineage

No effect of translocation site origin was detected on further life stages, but there were differences in progeny growth according to maternal transplant seed source origin and lineage. These differences in progeny growth were coherent with differences in plant size found among transplants before planting (Van Rossum *et al.* 2022): large transplants, one year after translocation, also produced large progenies in controlled cultivation conditions (Fig. 3), suggesting that there might be a genetic basis of the maternal effects on early growth stage (Roach & Wulff 1987). Moreover, the differences in rosette diameter found in controlled conditions between some maternal transplant seed source origins and lineages were maintained across the whole growth period measured, suggesting strong maternal genetic and/or environmental effects. Strong maternal environmental effects can be expected in the case of very restricted seed dispersal (as found for *C. glomerata*), as seeds will germinate at proximity of the maternal plants, and so in the mother's environment (Galloway 2005). Our findings on *C. glomerata* contrast thus with similar experimental studies on *A. montana* and *D. deltoides*, which had found evidence of considerable phenotypic plasticity. Indeed, for these species, differences in plant size of the F1 generation found during the first weeks of growth had already disappeared after eight weeks of cultivation (Van Rossum *et al.* 2020; Van Rossum & Le Pajolec 2021). Progeny rosette diameter at t4 tended to decrease with increasing maternal floral display size. However, the correlation was not significant with average values, suggesting that this finding was not indicative of maternal effects, but rather reflected processes related to pollination and to resource limitation. For instance, competition for resources among fruits or developing seeds, possibly combined with pollination (and so seed development) timing, might affect individual seed quality, and consequently offspring size, and might be higher in large floral displays (Fenner & Thompson 2005).

Phenotypic plasticity can provide ability to adapt to changing or new environments, while strong maternal environmental and genetic effects can reflect local adaptation (Galloway 2005; Schuler & Orrock 2012; Nicotra *et al.* 2015). If this large range of variation among maternal lineages suggests high –possibly adaptive– genetic variability in the translocated populations (Hamilton *et al.* 2017), we may wonder whether such fitness differences might be maintained or exacerbated under field conditions (expected to be more stressful –especially drier– than cultivation conditions). The maintenance of fitness differences might result in differential transplant survival and reproductive success in translocation sites. In particular, the adults of small populations used as seed sources might consist of old, resilient genotypes, which had survived in unfavourable conditions, in particular, degraded habitat quality due to grassland recolonisation by dense, competing, cover vegetation resulting in nutrient-richer soils due to litter accumulation, and increased edge effects due to population occurrence in fragmented remnants (Jacquemyn *et al.* 2003; Oostermeijer *et al.* 2003; Maurice *et al.* 2012; this study). Plants of *C. glomerata* have been reported to increase in height in dense cover vegetation (Bachmann *et al.* 2005). In translocation sites, nutrient-poor, open grassland conditions were restored by scraping soil, usually followed by livestock grazing, aiming at being optimal for grassland specialist species (Goret *et al.* 2021). The maternal environment of the transplants (in seed source populations) may thus differ from the post-translocation progeny environment. Strong maternal effects still related to marginal ecological conditions might produce maladapted progenies in restored grasslands (Reckinger *et al.* 2010; Schuler & Orrock 2012).

Inbreeding depression at early progeny growth stage

Maternal effects can also be associated with maternal inbreeding levels, as in *Campanula rapunculoides*, which were reported to affect seed set and seed germination timing, while inbreeding depression was found at all plant life stages (Vogler *et al.* 1999). Inbreeding depression is an important issue for translocated population viability in the long term, as reduced fitness expressed through reduced plant vigour in inbred progeny may result in lower reproductive success and plant survival (Edmands 2007; Aguilar *et al.* 2019). Despite relatively low homozygosity by loci (HL) values (≤ 0.505), which might be expected for self-incompatible species, we found evidence of inbreeding depression (rather than heterosis) for the post-translocation generation of *C. glomerata*, at very early stages of the life cycle. Indeed, rosette diameter at the beginning of growth was smaller for plants showing the highest homozygosity levels. Similarly, two-month old transplants with higher HL values were also smaller (Van Rossum *et al.* 2022). Also, juvenile mortality was the highest for maternal transplants originating from FON, the seed source population showing on average the highest HL values. The similar fitness values found for the two cross categories for the same range of HL values suggest that inbreeding was the main component involved in fitness reduction. In the case of drift load, we might have expected heterosis, i.e. a higher fitness for seed progeny obtained from crosses between seed source origins than from crosses within seed source origins (Paland & Schmid 2003). Drift load might also have been expressed at very early stages of development, e.g. through reduced seed germination ability, while inbreeding through juvenile mortality might have contributed to the purging of deleterious recessive alleles (Byers & Waller 1999; Willi *et al.* 2013). Given plant longevity, the high (pre-fragmentation) genetic diversity still kept in the old adult generation, and the early stage of genetic erosion in the offspring generation (Van Rossum *et al.* 2022, 2023a), there might also not have been enough generations yet for accumulation and fixation of deleterious mutations due to drift, and so for drift load to be expressed (Willi *et al.* 2013).

Because of self-incompatibility, inbreeding depression may be related to biparental inbreeding among relatives: higher HL values for progeny obtained from crosses within than between seed

sources suggest biparental inbreeding between transplants that may be full sibs or half sibs. Indeed, given the very small size of some seed source populations (flowering population sizes ranging 7 to 28), and evidence of restricted gene flow among populations (Van Rossum *et al.* 2022), the number of potential mates for producing the seeds used for propagating transplants is limited, so that we may expect some relatedness between a number of transplants despite extensive within-population pollen dispersal (Barmentlo *et al.* 2018; Aguilar *et al.* 2019; Millar *et al.* 2021). As parentage analyses revealed short-distance seed and pollen dispersal in the two translocated populations of *C. glomerata* (with most dispersal events within a few meters), leading to spatial genetic structure in the newly established generation at a fine scale (Van Rossum *et al.* 2023a), biparental inbreeding and so inbreeding depression issues might still be expected in the next generations. No effect of inbreeding could be detected at later growth stages, but measures were made in good cultivation conditions (especially watering). More stressful hydric conditions on the field (dry grassland habitats) might negatively impact inbred juvenile survival (Willi *et al.* 2006; Edmands 2007).

Conclusion

In conclusion, our findings highlighted that maternal transplant origin, especially when seed sources consisted of small fragmented remnants, might possibly have a selective value on fitness at seedling establishment in the post-translocation generations. How maternal effects and inbreeding depression might persist and affect global genetic diversity patterns in the long term might be investigated by comparing the genetic composition of seed progeny with the newly established generation once it has reached the adult stage. Moreover, whether further admixture in the next generations might buffer maternal and inbreeding effects or on the contrary lead to outbreeding depression (Edmands 2007; Barmentlo *et al.* 2018), certainly merits to be investigated, e.g. by comparing fitness of seed progenies identified for their parental seed source origins from successive generations.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting information section at the end of the article.

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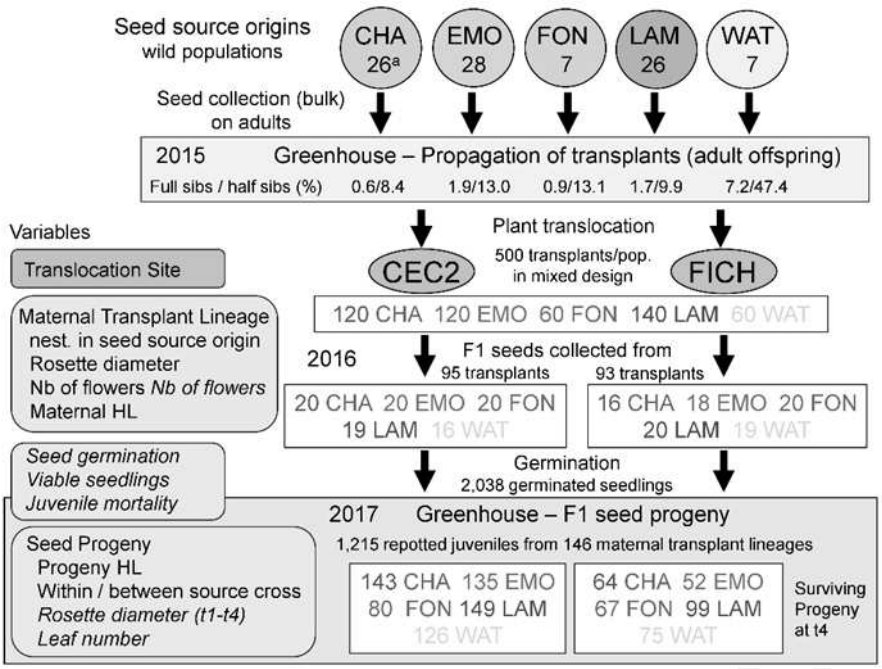

Fig. 1. Summary of sampling and translocation design and of the greenhouse experiment on F1 seed progeny, with the tested response (in italics) and predictor variables in this study (number of flowers of maternal transplants was both a response and a predictor variable; see Table S1). Percentage of full sibs and half sibs in transplants for each seed source population from Van Rossum *et al.* (2022). [a]Number of flowering adults.

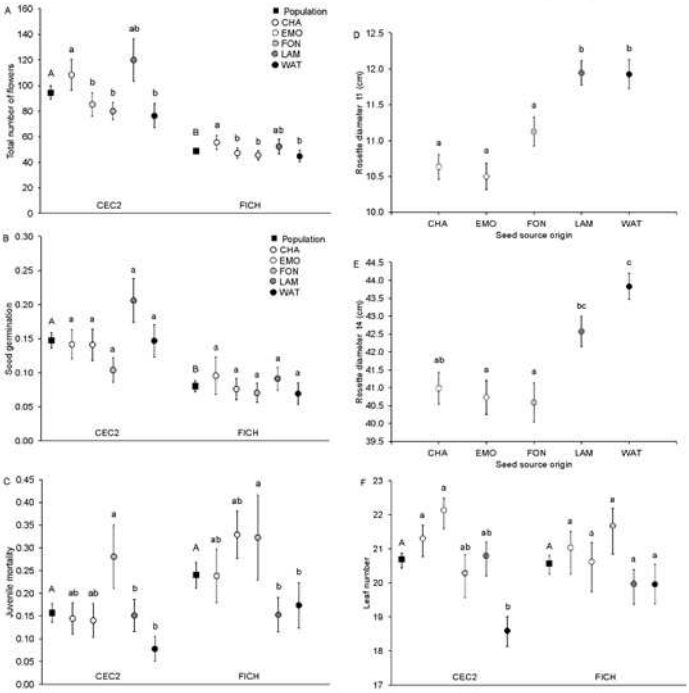

Fig. 2. Fitness variables (mean value ± SE) of the maternal transplants (a-b) or seed progeny (c-f) according to translocation site (CEC2, FICH), and maternal seed source origin (CHA, EMO, FON, LAM and WAT): (a) total number of flowers; (b) seed germination; (c) juvenile mortality; (d) rosette diameter (cm) at t1; (e) rosette diameter (cm) at t4; and (f) leaf number. Different symbols indicate significant ($P < 0.05$) differences between sites (capital letters) based on ANCOVA (F tests) or Generalized Linear Model (Wald tests), and among source origins (small letters) based on Tukey tests.

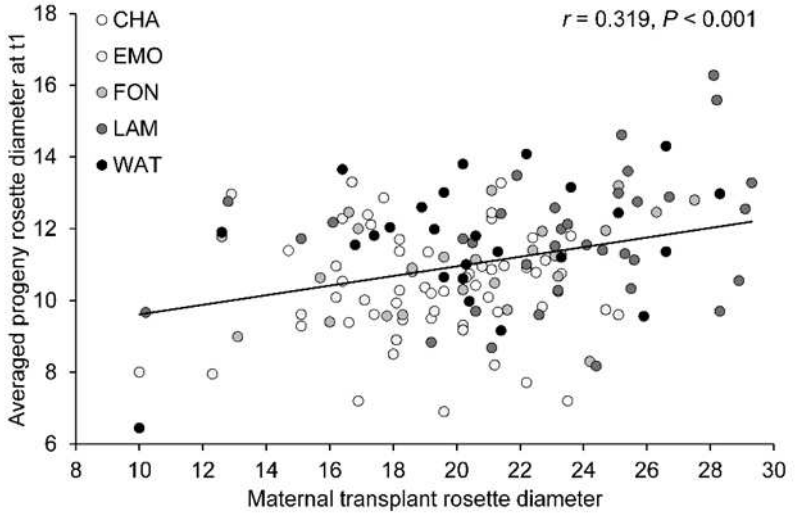

Fig. 3. Rosette diameter (mean value per maternal transplant in cm) of seed progeny at t1 as a function of rosette diameter of maternal transplants (in cm). *r*, Pearson's correlation coefficient.

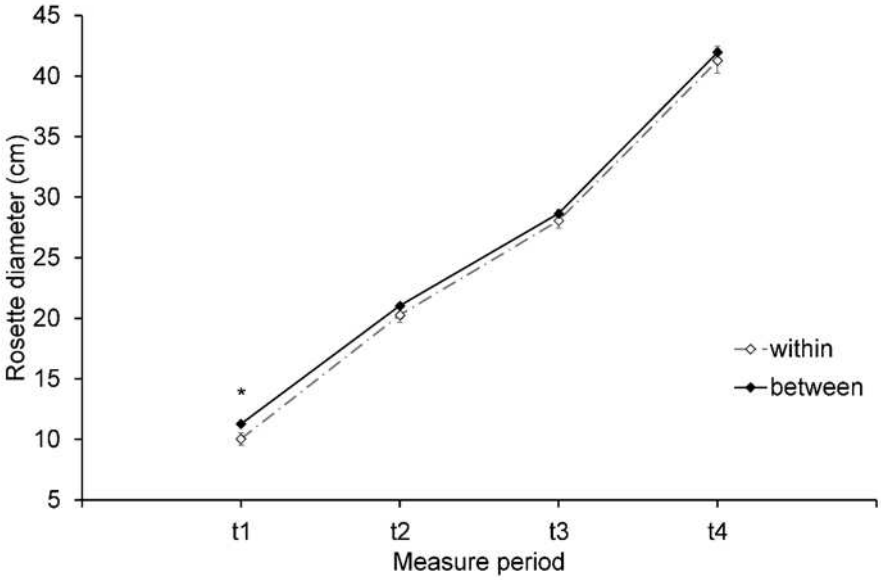

Fig. 4. Rosette diameter (mean ± SE) of seed progeny according to cross category (within/between seed source origins). * $P < 0.05$ according to Mann-Whitney *U* test between cross categories.