Biological Conservation 305 (2025) 111044

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Small populations, big challenges: Genetic, demographic, and landscape context collectively shape population performance of a perennial herb

Sina Bohm [a,*], Niamh Kelly [a], Maarten Postuma [a], Niels C.A.M. Wagemaker [b], Sharon ter Haar [b], Jeroen Scheper [a], Philippine Vergeer [a,b]

[a] *Plant Ecology and Nature Conservation Group (PEN), Wageningen University & Research, Droevendaalsesteeg 3a, 6708PB, Wageningen, the Netherlands*
[b] *Radboud Institute for Biological and Environmental Sciences (RIBES), University of Nijmegen, Heyendaalseweg 135, 6525AJ, Nijmegen, the Netherlands*

ARTICLE INFO

Keywords:
Genetic diversity
Population differentiation
Plant-pollinator interactions
Population performance
Landscape context
Primula elatior

ABSTRACT

Habitat loss and fragmentation have led to smaller and more isolated plant populations, impacting population performance through changes in genetic processes, demographic structure, and pollinator availability. Understanding the interactive effects of these factors is crucial for sustaining and restoring viable populations. This study analysed the genetic structure of natural populations of the long-lived herb *Primula elatior* and investigated plant and population reproductive performance in relation to population size, floral morph type ratios, genetic diversity, pollinator abundance, and landscape context. Plant reproductive performance was measured in 33 natural populations in the Netherlands and related to population size and genetic diversity. Additionally, the landscape context, *i.e.,* surface cover estimates of different land-use types, was assessed. Pollinator surveys were performed in a subset of 15 populations. Genetic divergence increased with geographic distance between populations. Structure analysis identified five genetic clusters corresponding to geographic regions. Genetic diversity was strongly positively correlated with population size but was not significantly associated with plant reproductive performance measures. Plant reproductive performance was however affected by floral morph ratio, pollinator abundance, and forest cover within 1000 m. Seed production increased with a more balanced floral morph ratio and higher pollinator abundance, and showed a unimodal relationship with the percentage of forest cover within 1000 m around the survey site. This study demonstrates that both floral morph ratio and landscape context simultaneously influence population performance and impose pressures particularly on small populations. This study underscores the need to adopt a landscape-oriented perspective to fully comprehend population performance.

1. Introduction

Land use change and the intensification of agriculture has resulted in loss, fragmentation, and degradation of natural habitat (Foley et al., 2005; Green et al., 2005). As a consequence, many plant populations have become increasingly small and isolated (Leimu et al., 2006), often surrounded by hostile environments. A decline in population size and land use change can limit plant reproductive success (Aguilar et al., 2006; Angeloni et al., 2011), leading to small populations that rejuvenate poorly and mostly contain old individuals. Various factors may contribute to these limitations, including genetic processes (Angeloni et al., 2011) and plant-pollinator interactions (Vanbergen, 2014), that are often influenced by a larger spatial context (Avon et al., 2015). Studying the different processes and their interactive effects increases our understanding of what limits population performance.

When populations become small and isolated, random genetic drift and inbreeding can lead to genetic erosion and fixation of deleterious alleles (Grueber et al., 2013; Leimu et al., 2010), which eventually may lower a population's ability to adapt to environmental change (García-Dorado and Caballero, 2021). Genetic drift enhances genetic differentiation between populations (Huang et al., 2009), in particular in small and isolated populations (Méndez et al., 2014). Inbreeding results in an increased frequency of homozygotes (Wright et al., 2008), possibly leading to reduced plant fitness when recessive deleterious alleles become expressed in the homozygous state (*i.e.,* inbreeding depression). Similarly, plant fitness can be reduced by the loss of favourable heterozygote combinations (Angeloni et al., 2011; Wright et al., 2008).

Self-incompatibility, the inability of a plant to produce viable seeds

---

[*] Corresponding author.
E-mail address: sina.bohm@wur.nl (S. Bohm).

https://doi.org/10.1016/j.biocon.2025.111044
Received 8 August 2024; Received in revised form 19 February 2025; Accepted 22 February 2025
Available online 3 March 2025

upon self-pollination, is a common mechanism in flowering plants that prevents or lowers the risk of inbreeding. Self-incompatible species are able to identify and reject their own pollen as well as pollen from a similar genotype. While in most species, the genotypes are morphologically indistinguishable from another (homomorphic plants), in some species, they can be distinguished based on differences in flower morphology (heteromorphic plants) (Charlesworth, 2010). In distylous species, short-styled and long-styled morph types coexist in a population with their stigmas and anthers reciprocally placed. This self-incompatibility system ensures that short- and long-styled plants are each compatible only with the other morph (Charlesworth, 2010). Distyly works beneficially in populations in which both morph types are present in balanced and large numbers. Skewed morph ratios can, however, complicate matching receptive morphs, leading to a limitation of suitable mates. Mate limitation can thus affect a population's genetic structure (Luque et al., 2016) and result in a decrease in individual plant fitness (Endels et al., 2002; Meeus et al., 2012). In small populations, skewed morph ratios are more common as a result of stochastic processes (Aavik et al., 2020; Kéry et al., 2003), resulting in mate limitation being one among several processes through which population size can affect plant fitness (Kéry et al., 2000).

Another way population size can influence plant fitness is *via* pollinator attraction. When small populations are less visited by pollinators, which are essential for the seed production in distylous species (Wilcock and Neiland, 2002), plant seed production can be reduced. Reported declines in wild pollinators (Bartomeus et al., 2019; Biesmeijer et al., 2006; Powney et al., 2019) may result in plant populations becoming increasingly pollination-limited (Clough et al., 2014; Dainese et al., 2019; Ricketts et al., 2008), resulting in a reduced reproductive capacity. Pollinator abundance is known to be strongly affected by the landscape context, *i.e.,* the proportions of land-use types such as forests, grasslands or arable fields (Steffan-Dewenter et al., 2002; Viana et al., 2012). In fragmented landscapes, this is exacerbated by reduced connectivity and pollinator movement (Aguilar et al., 2006; Reinula et al., 2024). Particularly in small populations of self-incompatible species, this can significantly impact reproductive capacity and, consequently, population fitness (Aguilar et al., 2006).

Genetic variation, population size, pollinator abundance, and the landscape context can thus simultaneously affect an individual's performance and, consequently, the overall performance of a population. Understanding the interactive effects on plant and population performance is therefore critical for choosing effective conservation strategies, yet only few studies have investigated these interactions (*e.g.,* Busch and Reisch, 2016; Peterson et al., 2008). We used *Primula elatior,* a distylous long-lived perennial, as a model species. *P. elatior* is targeted in local conservation efforts due to recent population declines. We address the following research questions: (1) Is population genetic structure related to geographic distance and/or habitat type? (2) How are population size, genetic diversity, and floral morph ratios linked to plant and population reproductive performance? (3) How is population reproductive performance linked to population demographic structure? (4) Is seed production related to pollinator abundance and/or the landscape context?

We expect geographic distance to be strongly associated with genetic distance due to the low dispersal ability of *P. elatior* (Fayard et al., 2009; Szövényi et al., 2012). As forest *versus* grassland populations might be adapted to the different habitat types, habitat type may explain genetic divergence as well. We expect population size to be positively associated with genetic diversity and (more) balanced floral morph ratios (Kaldra et al., 2023) (Supplemental Fig. A1). We also expect a positive relationship between genetic diversity and plant reproductive performance (Jacquemyn et al., 2001; Kéry et al., 2000; Van Rossum et al., 2006), and assume that populations consisting of many well-performing plants show greater overall performance (Supplemental Fig. A1). Population performance is expected to affect population demographic structure, with greater population performance being associated with higher proportions of young individuals and lower proportions of old ones. As *P. elatior* is an insect-pollinated, obligate outcrossing species, we expect seed production to increase with more balanced floral morph ratios (Brys et al., 2008) and higher pollinator abundance. Finally, we expect seed production to be negatively affected by arable field cover (Senapathi et al., 2017) and to exhibit a positive or a unimodal relationship with forest and grassland cover (Valdes and García, 2011). This expectation arises primarily because many pollinators, which are essential for seed production in *P. elatior*, require woody habitat for nesting and benefit from both forest and grassland as foraging areas, while also potentially benefiting from higher landscape heterogeneity (Senapathi et al., 2017).

2. Methods

2.1. Study species

We studied 33 natural *P. elatior* populations across the Netherlands that varied largely in population size and surrounding landscape. *Primula elatior* is a herbaceous perennial plant native to western and central Europe (De Keersmaeker et al., 2015; Taylor and Woodell, 2008). The hemicryptophyte, rosette-forming species typically grows in two distinct habitat types: well-buffered wet forests (*Primulo elatioris-Carpinetum* and multiple associations of the *Alno-Padion*) and wet grasslands (*Angelico-Cirsietum oleracei*) (Weeda et al., 2005). Both habitat types have moderately nutrient-rich and moderately acidic soils. Buffering is achieved through groundwater influence or fractions of loam in the soil. Only in the southern region of the country buffering is caused by limestone. The plant emerges in late February and flowers from early March to mid-May (Taylor and Woodell, 2008). Seeds disperse in early August, followed by a period of dormancy until the next spring (Taylor and Woodell, 2008). It takes several years for individuals to mature from small flowering adults to plants with multiple rosettes, as plants produce one rosette per year under benign conditions (Jacquemyn et al., 2009). Individuals can survive for many years, and evidence points to a half-life of approximately 50 years (Jacquemyn et al., 2009).

P. elatior is an insect-pollinated, outcrossing species. Its floral dimorphism largely prevents self-fertilization (Taylor and Woodell, 2008): the species has two distinctive flower morphologies, referred to as pin and thrum morphs, that coexist within populations (Keller et al., 2016). Inter-morph fertilization is the norm; intra-morph pollination rarely takes place and yields very low seed production (Keller et al., 2014; Vaerbak and Andersen, 1997). Pollination is mediated by a variety of insects (mainly bees and flies), although pollinator visitation rates are generally low (Farwig et al., 2009).

2.2. Population selection and sampling

The 33 natural focal populations were selected across the Netherlands with varying population sizes in forest and grassland habitats as well as in mixed grassland-forest habitats. The focal grasslands and forests, and small (<500 individuals) and large (≥500 individuals) populations were fairly evenly spread over different regions in the Netherlands (Fig. 1A). Since the Dutch landscape has been highly influenced by human activities, causing habitat changes in many areas over the past century, we used historical maps to select populations that have experienced the same habitat type since at least 1950.

Data was collected in two rounds in 2020: the first round during peak flowering from the end of March until the end of April, the second round during the period of seed formation from the end of June until the beginning of July. During the first round, population characteristics and plant reproductive performance were measured, and leaf material was collected for genetic analysis. In each population, ten plots of 9 m^2 each were selected with the aim to capture as much of the variation in plant vitality as possible across the population site. One plot was placed where

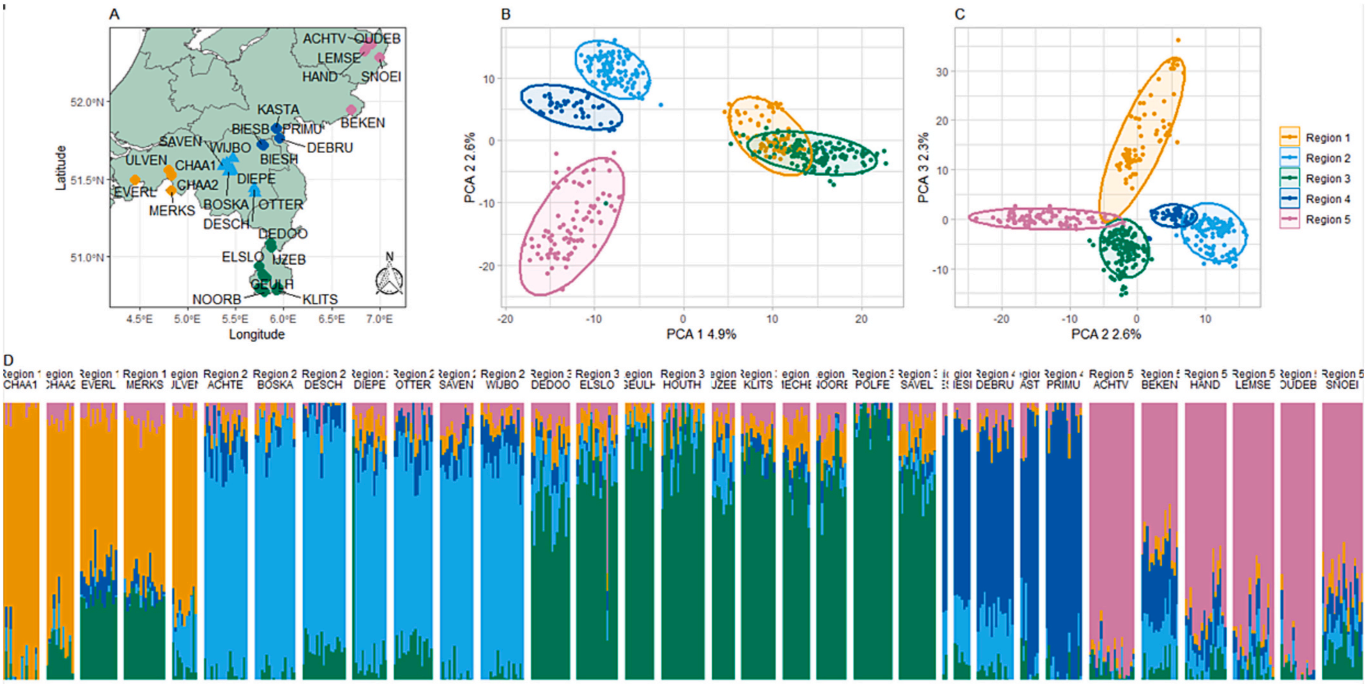

Fig. 1. A) Map showing parts of the Netherlands with 33 *Primula elatior* populations that were analysed. B) Plot of principal component axes 1 and 2 and C) plot of principal component axes 2 and 3 based on genetic distance among 33 populations of *P. elatior*. PC axis 1 explained 4.9 % of the variation in the data, PC axis 2 explained additional 2.6 %, and PC axis 3 explained additional 2.3 %. D) Visualization of sparse non-negative matrix factorization analysis with K = 5 clusters among the 33 analysed populations ($n = 507$) based on 44,969 SNPs. Colours represent the different genetic regions based on an optimal number of K = 5 clusters: orange for region 1, light blue for region 2, green for region 3, dark blue for region 4 and pink for region 5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

plant were visually the most vital in the population (high plant and flower density, large plant size, many seedlings present = ‘optimal plot’). Four plots were placed at suboptimal conditions, containing the visually least vital plants (low plant and flower density, small plants, plants with deviant phenotype implying stress such as yellowish leaves, no seedlings present = ‘suboptimal plots’). The other five plots were placed at random growing sites (‘random plots’). In small populations where fewer than ten plots could be laid out, at least the most vital and the least vital plants were measured. Population size estimates were based on mature plants only; seedlings were not included. Population size was estimated by counting the number of individuals in populations with fewer than 500 plants. In populations with ≥500 individuals, populations were categorized in one of four classes 500–1000, 1000–2500, 2500–5000, or > 5000 individuals by extrapolating plant density and population surface based on observations in the field and observation data retrieved from the National Databank Flora en Fauna (NDFF, 2020). When population size was used as a continuous variable for modelling, we used the mean values for the first three size categories or the value 5000 for the last one.

In each plot, performance traits related to plant age and flowering capacity were measured. The number of individuals were counted in four age classes: old individuals (more than five rosettes), intermediate-aged individuals (two to five rosettes), young individuals (one rosette), and seedlings. Since the rosettes of an individual are tightly clustered, individuals can usually be clearly distinguished. In cases where the distinction was unclear, we inspected whether two rosettes were physically connected by temporarily displacing a small amount of surface soil to uncover the roots. The number of rosettes, inflorescence number, the number of flowers of the highest inflorescence, and floral morph type were measured per individual and plot for a maximum of 20 individuals. Since the measurements inflorescence number, flower number of the highest inflorescence, and adult number per plot were not correlated, we used these variables to estimate a proxy for flower density (*i.e.,* flower number per m^2). Flower density was calculated as the mean flower number per plant per plot, where the flower number per plant was estimated by multiplying the flower number of the highest inflorescence per plant by the plants' total inflorescence number. This value was then multiplied by the number of adults per plot and divided by plot size. Seedling counts were used to derive seedling density (*i.e.*, seedlings per m^2).

For each population, leaf material (approximately 2 cm^2) from ten randomly selected young and ten randomly selected old individuals was collected for genetic analysis. Genetic samples were generally from the same individuals as the plant reproductive performance measurements. Leaf material was dried with silica gel immediately after collection and stored at −20 °C until further processing.

In the second sampling round, we revisited populations to collect seeds from ten random plants per population by sampling multiple open fruiting bodies per mother plant. Those seeds were used to estimate seed germinability. In ten populations, seeds from fewer than ten mother plants were collected due to few individuals with ripe seeds present, and in two of those ten populations, no seeds were found at all (Supplemental Table A1). Reasons for low seed production in some populations included herbivory by deer or isopoda. Additionally, one closed fruiting body for five individuals per population was collected to estimate seed number per fruit and seed weight, although in eleven populations, fewer than five closed fruiting bodies could be found (Supplemental Table A1). This small sample size of closed fruiting bodies per population was because most fruiting bodies were already open, in addition to the above mentioned reasons for low seed production. Closed instead of open fruiting bodies were chosen to ensure that no seeds had been released prior to sampling. Seeds were dried at room temperature and stored with silica gel at 4 °C. Ripe seeds from open fruiting bodies were used to test for germinability, whereas seeds from closed fruits were used to estimate seed number per fruit and seed weight.

2.3. Pollinator survey

Pollinator abundances were surveyed in a subset of 15 populations. This subset included a selection of populations from forest, grassland, and mixed habitat populations in three of the five geographic regions of the study (Supplemental Fig. A2). Care was taken to ensure that the subset covered different population sizes as well. At each site, a 4 m^2 plot was laid out containing the highest density of *P. elatior* plants ('optimal plot'). The number of *P. elatior* plants, inflorescences, and flowers were counted. Conform to the minimum observation duration for estimating pollinator flower visitation (Fijen and Kleijn, 2017), insect visitors were counted during 30 min at each location between 11:00 and 17:00 at temperatures ranging from 16 to 26 °C on sunny days with low wind speeds ($<$ 4 Beaufort). In addition, flower cover of all insect pollinated flowers was estimated in a 20 m × 20 m square around the plot to account for potential effects of flower cover on pollinator abundance. Each location was visited once in March and April 2020 at the peak bloom of *P. elatior*.

2.4. Landscape context

Cover percentages of the different land-use types - forest, grassland, and agricultural fields - were retrieved in the surroundings of all 33 populations (circles with radii of 250 m, 500 m, 750 m, and 1000 m around each population) by using data from the Dutch national spatial data infrastructure (BRT TOP10NL, 2021). These land use types together made up the large majority of the landscape composition around our study sites, and are known to either positively (grassland, forest) or negatively (arable land) affect pollinator populations and their pollination services through the availability of floral and/or nesting resources (Ammann et al., 2024; Dainese et al., 2019; Sõber et al., 2020). The forest category consisted of (combined) broadleaf, coniferous, and mixed forests, as well as willow coppices. Generally, collinearity among land use cover variables was low (Supplemental Fig. A3).

2.5. Genetic sampling and library preparation

Leaf samples of ten young and ten old individuals per population were collected from March to April. When populations contained fewer than ten young or old individuals, fewer individuals were sampled (median: a total of 16 plants per population). Leaf samples were immediately dried with silica gel and stored at −20 °C until further processing. A total of 620 plants from 33 populations were sampled.

Genomic DNA extraction was done by using a Nucleospin 96 plant II Kit (Marchery Nagel, Germany), following the manufacturer's instructions. Genetic variation was measured by using Genotyping by Sequencing (GBS). First, 14 to 297 ng of genomic DNA (gDNA) of each of the 620 samples was digested by two restriction enzymes (*Ase*I and *Nsi*I, Marchery Nagel, Germany), after which two indexed adapters were ligated to the DNA fragments. Per adapter, three Random Unique Molecule Identifier (UMI) nucleotides were incorporated to identify PCR duplicates within each amplified GBS library. After ligation, individual samples were cleaned by two subsequent Nucleomag (Marchery Nagel, Germany) clean-up steps using 1× beads and 0.8× beads, respectively. A small volume test PCR was performed by using KAPA HiFi HotStart readyMix (Roche Diagnostics, Switzerland) (15 cycles). The resulting product was diluted 10,000 times prior to qPCR quantification (KAPA Library Quantification Kit for HTS, Roche Diagnostics, Switzerland). The result of the qPCR was subsequently used to equimolarly pool the original cleaned digestion/ligation products per 129–130 individuals. These pooled products were concentrated by using a column-based PCR clean-up (Marchery Nagel, Germany) and nick repaired by using DNA polymerase I (25 microL reaction). The nick repaired products were amplified in five reactions per pool of 10 microL each and cleaned by two subsequent Nucleomag (Marchery Nagel, Germany) clean-up steps; 1× beads and 0.8× beads, respectively. The final four pooled GBS libraries were quantified by qPCR. The average library size were 514, 517, 515, and 572 bp, respectively. The final GBS libraries were spiked with 10 % PhiX DNA to increase the DNA complexity of the library, aiming to improve the HiSeq colour matrix estimation. For this, the first 10 sequencing cycles are used, which overlap with our index region. Sequencing was performed by Novogene (Hong Kong) on an Illumina (USA) HiSeq X-Ten sequencer; 2x150bp Paired-End (PE) sequencing reads.

2.6. Germination test

Seed germinability was assessed six months after seed collection. For each population, up to 30 seeds from up to 10 mother plants were sown on moist filter paper in one petri dish per mother plant. Occasionally, fewer seeds or mother plants were available because of little seed production. In total, 7884 seeds of 270 mother plants were sown. After undergoing a cold stratification for six weeks at 4 °C, the petri dishes were placed in a climate chamber with a cyclic temperature regime of 10/25 °C for 10/14 h and a light/dark regime for 10/14 h per night/day. Germinated seeds were counted weekly over a total period of 8 months. Seed germinability was defined as the proportion of germinated seeds.

2.7. Data analyses

Demultiplexing, *de novo* reference construction, mapping, and SNP calling of the DNA sequences were conducted by using Stacks version 2.4 (Catchen et al., 2013). PCR duplicates were removed using clone_filter based on 3 random oligo (UMI) nucleotides, followed by demultiplexing using process_radtags. Subsequently, denovo_map.pl was run on a subset of 40 random samples to determine the optimal parameter combination, which was found to be $M = 4$ (Paris et al., 2017). Denovo_map.pl was then rerun on all individuals. Mapping data was filtered by using the population program in Stacks. Individuals with >30 % missing data were removed, *i.e.*, individuals with relatively low coverage distributed evenly across populations. This yielded a total amount of 499 individuals out of 599. Next, we excluded all SNPs that were not present in at least 80 % of individuals, and that had a max observed heterozygosity over 50 % across all individuals, and a minor allele frequency < 0.05 (*i.e.,* a minor allele has to be present in 25 out of the 499 individuals). Nucleotide diversity (Pi), proportion of polymorphic loci (PP), and fixation index (F_{ST}) values were calculated by using the population program in Stacks. Isolation-by-distance of Euclidean geographic and genetic distances between populations was tested by using the Mantel test with Pearson's correlation coefficient in the *vegan* package (Dixon, 2003). Principle component analyses (PCA) for genetic data were performed by using the *adegenet* package (Jombart, 2008). Structure analysis was performed by using the snmf() function of the *LEA* package (Frichot and François, 2015), which estimates admixture coefficients by using sparse non-negative matrix factorization (sNMF) algorithms.

We employed (generalized) linear models and an information theoretic approach (Burnham et al., 2011) to examine the effects of different explanatory variables on our response variables of interest. In principle, we aimed to construct a model set that contained all possible combinations of the different predictors, including an intercept-only model, in each analysis. We deviated from this approach, when two variables were highly collinear (Pearson $r > 0.7$), which was the case for PP and Pi (Pearson $r = 0.86$) as well as population size and PP (Pearson $r = 0.72$; Supplemental Fig. A4), or when the sample sizes were too small to include all possible combinations of the different predictors, which applied to the model sets that investigated the effect of land use cover on seed number per fruit and pollinator abundance. We ranked all models based on AICc and restricted our candidate model set to models within ΔAICc <2. We calculated the model weight (ω) for each model in the candidate set. For every predictor in the candidate model set, we calculated a full-model averaged parameter estimate (β) and confidence interval (including zero when predictors were not included in a particular model). Furthermore, we calculated the variable weights (vw) for all variables, which are the sum of the Akaike weights of all models in the candidate set that include the variable of interest. We compared likelihoods of models by calculating evidence weight ratios, *i.e.*, ω of the model of interest divided by ω of the model to compare to. Prior to modelling, we estimated Pearson's correlation coefficients among explanatory variables (Supplemental Figs. A2 and A3).

In all models with multiple continuous predictors, all continuous explanatory variables were centred around the mean and divided by one standard deviation to aid comparison of effect sizes. To improve normality and homoscedasticity of residuals, the variables population size, flower density, seedling density, flower cover m^{-2}, and pollinator visitation rate were ln-transformed in all models prior to scaling. For variables with a minimum value of zero, a small constant was added before ln-transformation. Model assumptions were verified by inspecting diagnostic plots for normality and homogeneity of residuals, and for overdispersion and underdispersion. All analyses were performed in R version 4.4.1 (R Core Team, 2021) by using the packages 'lme4' (Bates et al., 2015) and 'MuMIn' version 1.46.0 (Barton, 2009).

We assessed the effect of geographic distance, population size, and habitat type on pairwise-F_{ST} values (Supplemental Table A2). To do so, we assigned each pair of populations to one of three categories for pairwise population size (small-small, small-large and large-large) and to one of six categories for pairwise habitat type (forest-forest, forest-grassland, grassland-grassland, forest-mixed, grassland-mixed, mixed-mixed). We accounted for pairwise dependencies among values by using Clarke's maximum likelihood population effects model (Clarke et al., 2002) with the package '*corMLPE*'. We fitted a generalized linear least squares (GLS) model with pairwise-F_{ST} as a function of pairwise geographic distance (ln-transformed), pairwise population size category, and pairwise habitat type category, and included terms for both population labels to define the pairwise correlation structure of the data.

We used linear models, with variables averaged at population-level, to examine the effects of different explanatory variables on performance measures (Supplemental Table A2). We investigated the effect of population size on measures of population genetic diversity (proportion of polymorphic loci, PP, and nucleotide diversity, Pi) and on floral morph ratio (Supplemental Fig. A1), because we expected population size to positively affect both genetic diversity and floral morph balance. Floral morph ratio was calculated as the total count of individuals with one morph type divided by the total count of individuals with the other morph type, with the higher count number as the denominator.

Next, we analysed the effects of population size, genetic diversity, and floral morph ratio on plant reproductive performance, measured as seed number per fruit, germinability, seed weight, and flower number of the highest inflorescence (hereafter collectively referred to as plant reproductive performance), by using linear models (Supplemental Fig. A1). Model sets were defined with population size, PP, and floral morph ratio as predictor variables and the different measures for plant reproductive performance as response variables (Supplemental Table A2). Models that included both PP and population size were omitted from the model sets because the two variables were highly collinear. In the model set for seed germinability, three populations were excluded from the analysis because the mean estimate of germinability was not considered reliable enough: for these three populations, seed germinability tests had been performed with seeds from only three or fewer mother plants instead of ten because populations had very little seed production.

We investigated the effect of plant reproductive performance on population-level reproductive performance, measured as flower and seedling density (hereafter referred to as population reproductive performance), by using linear models (Supplemental Fig. A1). We modelled flower density as a linear function of flower number of the highest inflorescence (Supplemental Table A2), expecting a positive relationship. We modelled seedling density (*i.e.*, the number of seedlings per m^2) as a linear function of seed germinability, seed weight, and the number of seeds per fruit (see Supplemental Table A2), expecting positive associations between these variables.

Furthermore, we investigated the relationship between population reproductive performance and population demographic structure, measured as the percentages of young and old individuals of a population, by using linear models (Supplemental Fig. A1). Both percentages were modelled as linear functions of seedling density (Supplemental Table A2), because we expected populations with higher seedling densities consisting of relatively more young (1-rosette) individuals and fewer old (>5-rosette) ones.

We modelled the effect of the landscape context, *i.e.*, the proportion of forest, grassland, and agricultural fields surrounding the population, on seed number per fruit for all study populations. As effects of landscape context on pollination, and thus seed formation, may be scale-dependent (Steffan-Dewenter et al., 2002), the effect of each land-use type was examined within radii of 250 m, 500 m, 750 m, and 1000 m around the populations. The variable population size was used as a binary variable for small (<500 individuals) *versus* large (≥500 individuals) populations to decrease the degrees of freedom needed. We included population size in this analysis because, from a pollinators' perspective, large populations may be more attractive than small populations (Blaauw and Isaacs, 2014; Mustajärvi et al., 2001). The cut-off of 500 individuals was chosen to divide the study populations in two almost equally sized groups (17 small and 16 large populations). We constructed a model set consisting of an intercept-only model, a model including population size, univariate models for each land-use type for each radius, and multivariate models with the additive effect of population size and each of the land-use types for each radius. For the land-use types forest and grassland, we additionally included models with a quadratic term for each radius because we expected that population seed number per fruit may show an optimum response curve to the surrounding coverage of forest or grassland, whereas the cover of agricultural fields was expected to negatively affect seed number per fruit in a linear relationship. This resulted in a full model set of 34 models (Supplemental Table A3).

To substantiate the modelling results on seed number per fruit and landscape context, we examined the relationship between seed number per fruit and pollinator services as well as the effect of the landscape context on pollinator abundance for a subset of 15 populations. We built a model set consisting of four models with seed number per fruit as the response variable (Supplemental Table A3). Because the previous multi-model inference identified a model including population size and a quadratic effect of forest cover within a 1000 m radius as the best candidate model (see Results), these explanatory variables were included in the null model. Three other models were built that each included one of the following variables: flower cover m^{-2}, pollinator abundance, and pollinator visitation rate. Correlation coefficients among explanatory variables were below 0.7 (Supplemental Fig. A5). Furthermore, we defined a model set for pollinator abundance that included linear and quadratic effects of forest cover within the radii 250 m, 500 m, 750 m, and 1000 m to test whether pollinator abundance responded similarly to forest cover as seed number per fruit (Supplemental Table A3). The model set also contained an intercept-only model. The count data of pollinator abundance were modelled by using generalized linear models with Poisson error distribution and log link function.

3. Results

3.1. Genetic structure

After filtering, 44,969 SNPs were discovered in 499 plants for analyses of genetic diversity metrics and population genetic structure. We observed strong population differentiation based on geographic region (Fig. 1). SNMF analysis revealed two clearly defined main clusters with a maximum of five clusters. The most likely number of clusters was K = 5. The minimum number of K = 2 clusters separated the genetic regions 1 (North-Brabant West) and 3 (South-Limburg) from the genetic regions 2 (North-Brabant Centre), 4 (North-Brabant East), and 5 (Overijssel). PCA visualized that individuals from the different populations clustered by region and not by habitat type (Supplemental Fig. A6). In line with these results, a significant positive correlation between genetic distance and geographic distance was observed (Mantel statistics $r = 0.467$, $p = 0.001$), indicating that population genetic similarity decreases with geographic distance. Pairwise-F_{ST} values ranged from 0.025 to 0.146, with the lowest F_{ST} values in populations within the same geographic region, showing substantial genetic divergence between different geographic regions. Pairwise-F_{ST} values were best explained by a model containing geographic distance only (ω = 1.0, vw = 1.0; Supplemental Table A4). This was the only model in the candidate model set; both the variables pairwise population size and habitat type were not selected. Hence, population size and habitat type do not add substantial information for explaining variation in population genetic differentiation. This was also visualized by the lack of genetic clustering per habitat type by PCA (Supplemental Fig. A6).

3.2. *Plant and population performance*

Population size ranged from 12 to >5000 individuals (Supplemental Table A1). Genetic diversity increased with population size, as reflected in the results for both PP (β = 0.046, 95 % CI: 0.030–0.062, ω = 1.00; Fig. 2A) and Pi (β = 0.005, 95 % CI: 0.002–0.007, ω = 1.00; Fig. 2B) (Supplemental Table A5). The ratio of floral morphs was more balanced with increasing population size (β = 0.071, 95 % CI: 0.027–0.116, ω = 0.98; Fig. 2C; Supplemental Table A5).

We found a positive effect of floral morph ratio on seed number per fruit, whereas the other measures for plant reproductive performance were not significantly associated with population size, PP, or floral morph ratio. Seed number per fruit was best explained by a model with floral morph ratio as the only explanatory variable (vw = 0.79, ω = 0.47; Supplemental Table A6). According to this model, seed number per fruit increased with increasing evenness in morph ratio (β = 4.037, CI: 0.517–9.658; Fig. 2D). No association between seed number per fruit and population size or PP was found. Seed germinability, seed weight, and flower number of the highest inflorescence were not related to floral morph ratio, population size, or PP, illustrated by the intercept-only models being the highest ranked models in all three cases (Supplemental Table A6).

We found a positive relationship between plant reproductive performance and population reproductive performance for flower density, but not for seedling density. Flower density was best explained by a model with flower number of the highest inflorescence as the only explanatory variable (vw = 1.0, ω = 1.0; Supplemental Table A7), with the two variables being positively associated (β = 0.2, CI: 0.096–0.297; Fig. 2E). For seedling density, the intercept-only model was the highest ranked model (ω = 0.69), showing that germinability, seed weight, and seed number per fruit were not associated with seedling density (Supplemental Table A7). The percentage of young individuals increased (β =16.22, CI: 5.678–26.767, vw = 1.00, ω = 1.0; Fig. 2F) and the percentage of old individuals decreased with increasing seedling density (β = −11.72, CI: −19.083 to −4.352, vw = 1.00, ω = 1.0; Supplemental Table A8).

Forest cover in a 1000 m radius around the population was related to seed number per fruit. This was indicated by seed number per fruit modelled as a quadratic function of forest in a 1000 m radius (vw = 1.00) and population size (vw = 1.00; ω = 1.0; Supplemental Table A9) being the only model within ΔAICc <2. Seed number per fruit showed an optimum response curve with regard to forest cover, with smaller populations producing fewer seeds per fruit than larger populations

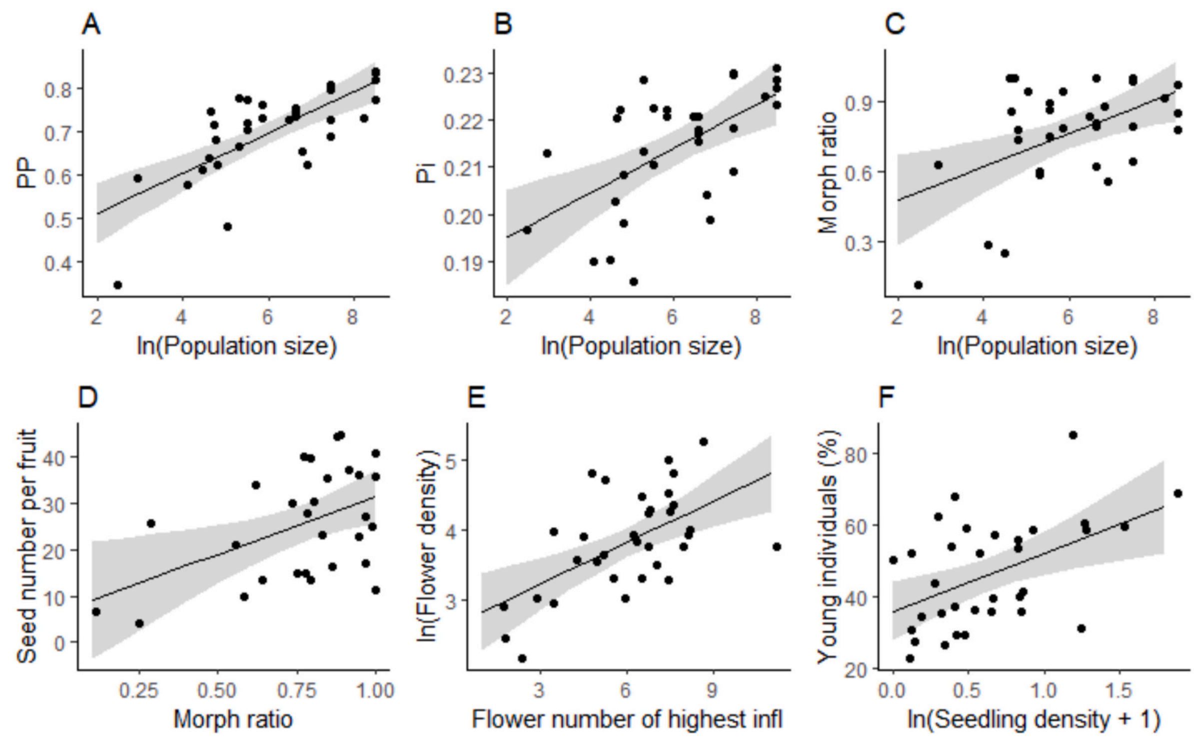

Fig. 2. Relationships between population size (ln-transformed) and A) proportion of polymorphic loci (PP), B) nucleotide diversity (Pi), C) floral morph ratio, and D) floral morph ratio and seed number per fruit, E) flower number of the highest inflorescence and population flower density (flower number per m^2), and F) percentage of young individuals and seedling density in *Primula elatior*. Lines represent predicted values from the Gaussian linear models with 95 % confidence intervals (A-C: M1-models in Supplemental Table A5; D: model LM3 for seed number per fruit in Supplemental Table A6, E: model LM1 for flower density in Supplemental Table A7, F: model LM1 for percentage of young individuals in Supplemental Table A8).

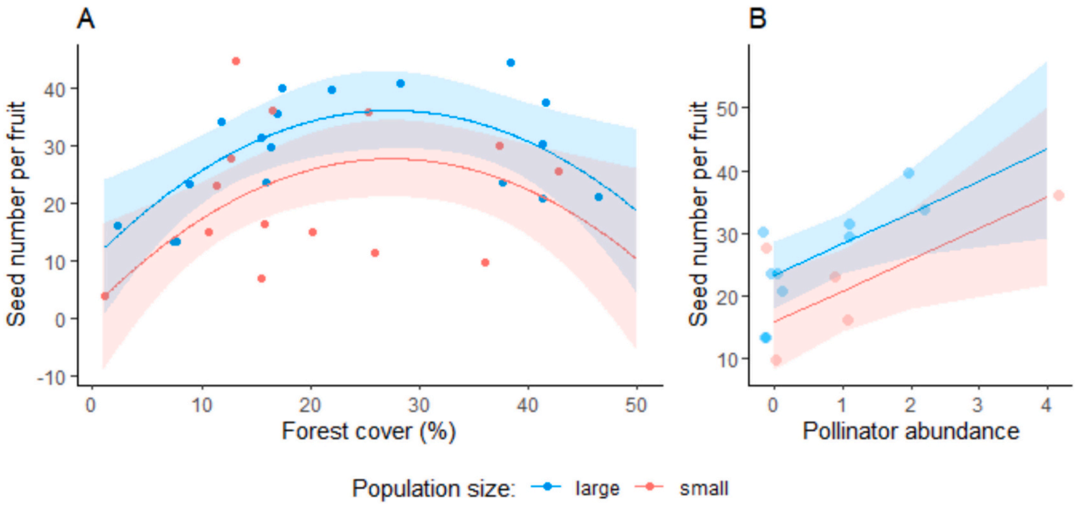

Fig. 3. A) Seed number per fruit in *Primula elatior* as a function of percentage of forest cover within a radius of 1000 m around the survey site. B) Seed number per fruit in *Primula elatior* as a linear function of pollinator abundance. Data points are jittered to make overlapping points visible. In both plots, observations from small populations (<500 individuals) are depicted in red and observations from large populations (≥500 individuals) in blue. Lines represent predicted values from Gaussian linear models based on the F12-model in Supplemental Table A9 for plot A and the M2-model in Supplemental Table A11 for plot B. Coloured bands represent 95 % confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 3A). The highest value for seed number per fruit was predicted for approximately 25 % forest cover. Neither grassland cover nor agricultural field cover surrounding the population had an effect on seed number per fruit (Supplemental Table A9).

The pollinator surveys in the subset of 15 populations revealed that pollinator abundance was generally low, ranging from 0 to 4 individuals with a mean of 0.8 individuals per site (Supplemental Table A10). The model best explaining seed number per fruit included pollinator abundance as explanatory variable (ω = 1.00; vw = 1.00; Supplemental Table A11), with seed number per fruit being positively correlated with pollinator abundance (Fig. 3B). This model was 3.5 times more likely to be the best approximating model than the second best model (ΔAICc = 2.50) that only included population size and the quadratic effect of forest within 1000 m (null model). For pollinator abundance, the two models in the candidate set both indicated a quadratic effect of forest cover, with more support for the model including forest cover within a 750 m radius (ω = 0.72; ΔAICc = 0; Supplemental Fig. A7) than the model including forest cover within a 1000 m radius (ω = 0.28; ΔAICc = 1.88; Supplemental Table A12). Both models were 46 and 18 times more likely than the intercept-only model, respectively.

4. Discussion

We show that plant and population performance in *P. elatior* is affected by multiple factors, acting across various spatial scales. Seed production, defined as seed number per fruit, is positively associated with a balanced floral morph ratio. Skewed floral morph ratios can limit seed production, particularly in small populations. Moreover, seed production is affected by the surrounding landscape context, likely through its impact on pollinator abundance. Furthermore, our study demonstrates that small populations are genetically less diverse than large populations. Considering human-induced habitat loss and fragmentation leading to increasingly small and isolated populations, we argue that skewed morph ratios, and reduced pollinator abundance are likely to restrict plant performance, with cascading effects on population reproductive performance and demographic structure.

4.1. Population differentiation

Our study populations of *P. elatior* showed a pattern of geographic isolation and population differentiation between the different regions. These isolation-by-distance effects are most likely explained by a limited gene flow in combination with stochastic genetic processes such as drift. Studies on other poorly dispersing plant species show similarly strong isolation-by-distance effects (*e.g.,* Capria et al., 2023; Suárez et al., 2022; Van Rossum and Triest, 2003). Furthermore, we found little support for the hypothesis that grassland and forest populations are genetically differentiated. This contrasts with a study on a related species, *Primula veris*, which suggested genetic differentiation between open grasslands and old forests (Deschepper et al., 2017). A possible explanation is the landscape history: the Dutch landscape has been greatly influenced by human activities, causing frequent transformations from one habitat type to another within the last centuries (*i.e.*, grassland afforestation and clearcut of forests). Therefore, populations of long-lived *P. elatior* may have experienced changes in habitat type within a few generations, likely preventing the population to adapt to a specific habitat type. Indeed, field observations showed that the ranges of many populations expanded over forests and grasslands and also other landscape elements like hedges and ditches. This implies that *P. elatior* plants are probably able to thrive under a variety of habitat conditions.

4.2. Plant and population performance

We found a positive effect of population size on genetic diversity and floral morph balance. Our findings align with previous research demonstrating a positive association between population size and genetic diversity (Honnay and Jacquemyn, 2007; Leimu et al., 2006), as well as population size and floral morph balance (Aavik et al., 2020). The strong genetic erosion in small populations is concerning. *P. elatior* has a long generation time and it is likely that habitat loss and fragmentation have resulted in population decline only a few generations ago. The fact that small populations are genetically less diverse, suggests that genetic diversity is declining rapidly.

Seed production, measured as seed number per fruit, was positively associated with more even floral morph ratios. In case of strongly skewed morph ratios, compatible mates become limited resulting in few seeds per fruit and subsequently lower seedling densities. This is more likely to happen in small populations, as our and studies on other heterostylous species such as *Primula vulgaris* (Endels et al., 2002) or *Pulmonaria officinalis* (Brys et al., 2008) demonstrate. According to Endels et al. (2002), skewed morph ratios can reduce population persistence on the long term.

No relationship between genetic diversity and plant reproductive performance was found. For seed production, measured as seed number per fruit, our results suggest that floral morph ratio and pollinator abundance are more important direct drivers than genetic diversity. These findings suggest that while the smaller populations are genetically less diverse, they do not (yet) experience negative effects from inbreeding (*i.e.*, inbreeding depression). In long-lived species, such as *P. elatior*, the negative effects of inbreeding may, however, become less apparent due to overlapping generations (Li et al., 2012; Taylor et al., 2017). Although there is no evidence of inbreeding depression, the discovery of smaller populations showing lower genetic diversity, uneven floral morph ratios, reduced rejuvenation, and a higher proportion of older individuals is alarming.

Plant and population reproductive performance were linked through a positive effect of flower number of the highest inflorescence on flower density. As flower number of the highest inflorescence was not associated with population size, genetic diversity, or floral morph ratio, it is possible that factors beyond the scope of our study, such as habitat quality, may have played a role in this relationship. Benign habitat quality can support flower formation as well as seedling emergence (Adriaens et al., 2009). This may also explain the relatively strong collinearity between flower density and seedling density (Supplemental Fig. A4). Population reproductive performance and demographic structure were connected through a positive relationship between seedling density and the percentage of young individuals, and a negative relationship between seedling density and the percentage of old individuals. Additionally, the percentage of old individuals showed a relatively strong negative correlation with population size (Supplemental Fig. A4). These findings suggest that smaller populations tend to have a higher percentage of old individuals, potentially due to limited rejuvenation.

4.3. Pollinator abundance

Our results suggest that pollinator abundance is one of the limiting factors for seed production. However, this result should be interpreted with caution given the generally low pollinator counts during our surveys. Our findings also imply that this limitation may be, at least partly, driven by the surrounding landscape context, particularly the percentage of forest cover. Especially in obligate outcrossing species, pollination can limit seed production (*e.g.*, Agren, 1996; Sih and Baltus, 1987). Pollen limitation has been reported in other *Primula* species, especially in small and isolated populations (Washitani et al., 1994). Our finding that seed production is limited by pollinator abundance is particularly concerning in the light of recent declines in pollinator abundances (Bartomeus et al., 2019; Biesmeijer et al., 2006; Powney et al., 2019), which are mainly driven by a lack of suitable nesting and foraging habitat in contemporary simplified landscapes (Scheper et al., 2014; Steffan-Dewenter and Schiele, 2008). Low pollinator abundances limiting seed production could be one driving factor for recent disappearance of insect-pollinated plants in plant communities (Sõber et al., 2024).

Interestingly, the analysis of the landscape context (Supplemental Table A9) revealed a significant effect of population size category on seed production. This contrasts with earlier models, where no significant effect was observed, although population size was included among the candidate models with ΔAICc <2 (Supplemental Table A6). Our decision to include population size to the landscape context analysis was driven by the hypothesis that larger populations would attract more pollinators (Mustajärvi et al., 2001). However, we also found a significant positive correlation between population size and floral morph ratio (Supplemental Table A5) and, subsequently, between floral morph ratio and seed production (Supplemental Table A6). This suggests that the observed effect of population size category on seed production in the landscape context analysis may be linked to the influence of floral morph ratio.

Both seed production and pollinator abundance displayed a unimodal response to the percentage of forest cover, peaking at approximately 25 % of forest cover in the landscape. To successfully complete their life cycle, pollinators often require different habitats (*i.e.*, partial habitats *sensu* Westrich, 1996) throughout the season to meet their requirements for foraging, nesting, and overwintering (Proesmans et al., 2019). Forests and other woody habitats support pollinator populations by providing nesting sites (Svensson et al., 2000) and abundant floral resources in spring (Ammann et al., 2024; Kämper et al., 2016), which explains why pollinator abundance and seed production initially increase with increasing forest cover in the landscape. Additionally, in landscapes with too little forest cover, forest fragments may be too small and isolated to attract or support pollinators (Honnay et al., 2005; Sih and Baltus, 1987) with negative consequences for *P. elatior* pollination and seed production. However, later in the season, food supplies for pollinators in forests are limited (Mola et al., 2021; Timberlake et al., 2021). Therefore, as the colonies and floral resource requirements of eusocial pollinator species such as bumblebees grow throughout the season, they become increasingly dependent on habitats other than forests (Inari et al., 2012; Mola et al., 2021). This could explain why the initially positive effect of forest cover becomes negative at high forest cover percentage, suggesting that resource complementation between forest and the other habitats in the landscape becomes less than optimal.

4.4. Implications for conservation

Our study emphasizes the importance of maintaining large populations for the conservation of intraspecific genetic diversity and the performance of plants and populations. Many small populations of *P. elatior* are currently characterized by a low genetic diversity, skewed floral morph ratios, reduced seed production, and a high percentage of old individuals. As a result, the long-term persistence of these small populations is at risk, and additional conservation measures are needed. To prevent further loss of intraspecific genetic diversity, *ex situ* seed storage, followed by (re)introduction into suitable or restored habitats, can be effective - especially where small populations remain in isolated habitat patches. We recommend maintaining the original spatial genetic clusters of populations during (re)introduction efforts. Furthermore, our study provides an example of how the landscape context can restrict pollination and plant performance in populations of self-incompatible species (Aguilar et al., 2006). Thereby, it highlights the importance of adopting a landscape approach for effective conservation of plant populations.

CRediT authorship contribution statement

Sina Bohm: Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Niamh Kelly:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maarten Postuma:** Writing – review & editing, Visualization, Resources, Methodology, Formal analysis. **Niels C.A.M. Wagemaker:** Writing – review & editing, Supervision, Resources, Methodology, Formal analysis. **Sharon ter Haar:** Writing – review & editing, Investigation, Data curation. **Jeroen Scheper:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Philippine Vergeer:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve readability and language quality. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Funding

This research was financed by the province of Noord-Brabant, the Netherlands. MP was funded by the NWO Open Competition ENW-M program.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the Dutch province of Noord-Brabant and the NWO Open Competition ENW-M program for funding this research, and local authorities for giving permission for data collection at field locations. The authors acknowledge the support of Bosgroepen Zuid Nederland for the selection of field sites. The authors thank Joop Schaminée, Leon van den Berg, Nika van den Meiracker, Wiene Bakker, and Dieke de Boer for helping with the field work, and the anonymous reviewers for their helpful comments on the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2025.111044.

Data availability

The genetic data was deposited in the National Library of Medicine – National Center for Biotechnology Information under the reference number PRJNA837403. Remaining datasets and R code used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

Aavik, T., et al., 2020. Landscape context and plant population size affect morph frequencies in heterostylous *Primula veris*—results of a nationwide citizen-science campaign. J. Ecol. 108, 2169–2183. https://doi.org/10.1111/1365-2745.13488.

Adriaens, D., Jacquemyn, H., Honnay, O., Hermy, M., 2009. Conservation of remnant populations of *Colchicum autumnale* – the relative importance of local habitat quality and habitat fragmentation. Acta Oecol. 35, 69–82. https://doi.org/10.1016/j.actao.2008.08.003.

Agren, J., 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. Ecology 77, 1779–1790. https://doi.org/10.2307/2265783.

Aguilar, R., Ashworth, L., Galetto, L., Aizen, M.A., 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecol. Lett. 9, 968–980. https://doi.org/10.1111/j.1461-0248.2006.00927.x.

Ammann, L., Bosem-Baillod, A., Herzog, F., Frey, D., Entling, M.H., Albrecht, M., 2024. Spatio-temporal complementarity of floral resources sustains wild bee pollinators in agricultural landscapes. Agr. Ecosyst. Environ. 359, 108754. https://doi.org/10.1016/j.agee.2023.108754.

Angeloni, F., Ouborg, N.J., Leimu, R., 2011. Meta-analysis on the association of population size and life history with inbreeding depression in plants. Biol. Conserv. 144, 35–43. https://doi.org/10.1016/j.biocon.2010.08.016.

Avon, C., Bergès, L., Dupouey, J.-L., 2015. Landscape effects on plants in forests: large-scale context determines local plant response. Landsc. Urban Plan. 144, 65–73. https://doi.org/10.1016/j.landurbplan.2015.07.016.

Bartomeus, I., Stavert, J., Ward, D., Aguado, O., 2019. Historical collections as a tool for assessing the global pollination crisis. Philos. Trans. R. Soc. B 374, 20170389. https://doi.org/10.1098/rstb.2017.0389.

Barton, K., 2009. MuMIn: multi-model inference. http://r-forge/. R-project. Org/projects/mumin/.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Biesmeijer, J.C., et al., 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313, 351–354. https://doi.org/10.1126/science.1127863.

Blaauw, B.R., Isaacs, R., 2014. Larger patches of diverse floral resources increase insect pollinator density, diversity, and their pollination of native wildflowers. Basic Appl. Ecol. 15, 701–711. https://doi.org/10.1016/j.baae.2014.10.001.

BRT TOP10NL (2021). In: PDOK (ed), 2021 edn.

Brys, R., Jacquemyn, H., Beeckman, T., 2008. Morph-ratio variation, population size and female reproductive success in distylous *Pulmonaria officinalis* (Boraginaceae). J. Evol. Biol. 21, 1281–1289. https://doi.org/10.1111/j.1420-9101.2008.01569.x.

Burnham, K.P., Anderson, D.R., Huyvaert, K.P., 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behav. Ecol. Sociobiol. 65, 23–35. https://doi.org/10.1007/s00265-010-1029-6.

Busch, V., Reisch, C., 2016. Population size and land use affect the genetic variation and performance of the endangered plant species *Dianthus seguieri* ssp. *glaber*. Conserv. Genet. 17, 425–436. https://doi.org/10.1007/s10592-015-0794-1.

Capria, L., Liepelt, S., Eimert, K., Leyer, I., Mosner, E., 2023. Neutral genetic diversity follows a latitudinal gradient in the endangered plant *Arnica montana* L.: a range-wide study. Conserv. Genet. 1–14. https://doi.org/10.1007/s10592-023-01559-6.

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22, 3124–3140. https://doi.org/10.1111/mec.12354.

Charlesworth, D., 2010. Self-incompatibility. F1000 Biol. Rep. 2.

Clarke, R.T., Rothery, P., Raybould, A.F., 2002. Confidence limits for regression relationships between distance matrices: estimating gene flow with distance. J. Agric. Biol. Environ. Stat. 7, 361–372. https://doi.org/10.1198/108571102320.

Clough, Y., et al., 2014. Density of insect-pollinated grassland plants decreases with increasing surrounding land-use intensity. Ecol. Lett. 17, 1168–1177. https://doi.org/10.1111/ele.12325.

Dainese, M., et al., 2019. A global synthesis reveals biodiversity-mediated benefits for crop production. Sci. Adv. 5, eaax0121. https://doi.org/10.1126/sciadv.aax0121.

De Keersmaeker, L., et al., 2015. The analysis of spatio-temporal forest changes (1775–2000) in Flanders (northern Belgium) indicates habitat-specific levels of fragmentation and area loss. Landsc. Ecol. 30, 247–259. https://doi.org/10.1007/s10980-014-0119-7.

Deschepper, P., Brys, R., Fortuna, M.A., Jacquemyn, H., 2017. Analysis of spatial genetic variation reveals genetic divergence among populations of *Primula veris* associated to contrasting habitats. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-09154-9.

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x.

Endels, P., Jacquemyn, H., Brys, R., Hermy, M., 2002. Changes in pin-thrum ratios in populations of the heterostyle *Primula vulgaris* Huds.: does imbalance affect population persistence? Flora: Morphol. Distrib. Funct. Ecol. Plants 197, 326–331. https://doi.org/10.1078/0367-2530-00048.

Farwig, N., et al., 2009. Isolation from forest reduces pollination, seed predation and insect scavenging in Swiss farmland. Landsc. Ecol. 24, 919–927. https://doi.org/10.1007/s10980-009-9376-2.

Fayard, J., Klein, E.K., Lefèvre, F., 2009. Long distance dispersal and the fate of a gene from the colonization front. J. Evol. Biol. 22, 2171–2182. https://doi.org/10.1111/j.1420-9101.2009.01832.x.

Fijen, T.P., Kleijn, D., 2017. How to efficiently obtain accurate estimates of flower visitation rates by pollinators. Basic Appl. Ecol. 19, 11–18. https://doi.org/10.1016/j.baae.2017.01.004.

Foley, J.A., et al., 2005. Global consequences of land use. Science 309, 570–574. https://doi.org/10.1126/science.1111772.

Frichot, E., François, O., 2015. LEA: an R package for landscape and ecological association studies. Methods Ecol. Evol. 6, 925–929. https://doi.org/10.1111/2041-210X.12382.

García-Dorado, A., Caballero, A., 2021. Neutral genetic diversity as a useful tool for conservation biology. Conserv. Genet. 22, 541–545. https://doi.org/10.1007/s10592-021-01384-9.

Green, R.E., Cornell, S.J., Scharlemann, J.P., Balmford, A., 2005. Farming and the fate of wild nature. Science 307, 550–555. https://doi.org/10.1126/science.1106049.

Grueber, C.E., Wallis, G.P., Jamieson, I.G., 2013. Genetic drift outweighs natural selection at toll-like receptor (TLR) immunity loci in a re-introduced population of a threatened species. Mol. Ecol. 22, 4470–4482. https://doi.org/10.1111/mec.12404.

Honnay, O., Jacquemyn, H., 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. Conserv. Biol. 21, 823–831. https://doi.org/10.1111/j.1523-1739.2006.00646.x.

Honnay, O., Jacquemyn, H., Bossuyt, B., Hermy, M., 2005. Forest fragmentation effects on patch occupancy and population viability of herbaceous plant species. New Phytol. 166, 723–736. https://doi.org/10.1111/j.1469-8137.2005.01352.x.

Huang, Y., Zhang, C.Q., Li, D.Z., 2009. Low genetic diversity and high genetic differentiation in the critically endangered *Omphalogramma souliei* (Primulaceae): implications for its conservation. J. Syst. Evol. 47, 103–109. https://doi.org/10.1111/j.1759-6831.2009.00008.x.

Inari, N., Hiura, T., Toda, M.J., Kudo, G., 2012. Pollination linkage between canopy flowering, bumble bee abundance and seed production of understorey plants in a cool temperate forest. J. Ecol. 100, 1534–1543. https://doi.org/10.1111/j.1365-2745.2012.02021.x.

Jacquemyn, H., Brys, R., Hermy, M., 2001. Within and between plant variation in seed number, seed mass and germinability of *Primula elatior*: effect of population size. Plant Biol. 3, 561–568. https://doi.org/10.1055/s-2001-17728.

Jacquemyn, H., Vandepitte, K., Roldán-Ruiz, I., Honnay, O., 2009. Rapid loss of genetic variation in a founding population of *Primula elatior* (Primulaceae) after colonization. Ann. Bot. 103, 777–783. https://doi.org/10.1093/aob/mcn253.

Jombart, T., 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129.

Kaldra, M., Träger, S., Reinula, I., Keller, B., Conti, E., Aavik, T., 2023. Skewed morph ratios lead to lower genetic diversity of the heterostylous *Primula veris* in fragmented grasslands. Plant Biol. 25, 703–714. https://doi.org/10.1111/plb.13531.

Kämper, W., et al., 2016. How landscape, pollen intake and pollen quality affect colony growth in *Bombus terrestris*. Landsc. Ecol. 31, 2245–2258. https://doi.org/10.1007/s10980-016-0395-5.

Keller, B., Thomson, J.D., Conti, E., 2014. Heterostyly promotes disassortative pollination and reduces sexual interference in Darwin's primroses: evidence from experimental studies. Funct. Ecol. 28, 1413–1425. https://doi.org/10.1111/1365-2435.12274.

Keller, B., de Vos, J.M., Schmidt-Lebuhn, A.N., Thomson, J.D., Conti, E., 2016. Both morph-and species-dependent asymmetries affect reproductive barriers between heterostylous species. Ecol. Evol. 6, 6223–6244. https://doi.org/10.1007/s10980-016-0395-5.

Kéry, M., Matthies, D., Spillmann, H.H., 2000. Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. J. Ecol. 88, 17–30. https://doi.org/10.1046/j.1365-2745.2000.00422.x.

Kéry, M., Matthies, D., Schmid, B., 2003. Demographic stochasticity in population fragments of the declining distylous perennial *Primula veris* (Primulaceae). Basic Appl. Ecol. 4, 197–206. https://doi.org/10.1078/1439-1791-00142.

Leimu, R., Mutikainen, P., Koricheva, J., Fischer, M., 2006. How general are positive relationships between plant population size, fitness and genetic variation? J. Ecol. 94, 942–952. https://doi.org/10.1111/j.1365-2745.2006.01150.x.

Leimu, R., Vergeer, P., Angeloni, F., Ouborg, N.J., 2010. Habitat fragmentation, climate change, and inbreeding in plants. Ann. N. Y. Acad. Sci. 1195, 84–98. https://doi.org/10.1111/j.1749-6632.2010.05450.x.

Li, Y.-Y., Tsang, E.P.K., Cui, M.-Y., Chen, X.-Y., 2012. Too early to call it success: an evaluation of the natural regeneration of the endangered *Metasequoia glyptostroboides*. Biol. Conserv. 150, 1–4. https://doi.org/10.1016/j.biocon.2012.02.020.

Luque, G.M., Vayssade, C., Facon, B., Guillemaud, T., Courchamp, F., Fauvergue, X., 2016. The genetic Allee effect: a unified framework for the genetics and demography of small populations. Ecosphere 7, e01413. https://doi.org/10.1002/ecs2.1413.

Meeus, S., Honnay, O., Brys, R., Jacquemyn, H., 2012. Biased morph ratios and skewed mating success contribute to loss of genetic diversity in the distylous *Pulmonaria officinalis*. Ann. Bot. 109, 227–235. https://doi.org/10.1093/aob/mcr272.

Méndez, M., Vögeli, M., Tella, J.L., Godoy, J.A., 2014. Joint effects of population size and isolation on genetic erosion in fragmented populations: finding fragmentation thresholds for management. Evol. Appl. 7, 506–518. https://doi.org/10.1111/eva.12154.

Mola, J.M., Richardson, L.L., Spyreas, G., Zaya, D.N., Pearse, I.S., 2021. Long-term surveys support declines in early season forest plants used by bumblebees. J. Appl. Ecol. 58, 1431–1441. https://doi.org/10.1111/1365-2664.13886.

Mustajärvi, K., Siikamäki, P., Rytkönen, S., Lammi, A., 2001. Consequences of plant population size and density for plant-pollinator interactions and plant performance. J. Ecol. 80–87. https://doi.org/10.1046/j.1365-2745.2001.00521.x.

NDFF, 2020. Primula elatior. In: Ecogrid Nationale Databank Flora en Fauna.

Paris, J.R., Stevens, J.R., Catchen, J.M., 2017. Lost in parameter space: a road map for stacks. Methods Ecol. Evol. 8, 1360–1373. https://doi.org/10.1111/2041-210X.12775.

Peterson, A., Bartish, I.V., Peterson, J., 2008. Effects of population size on genetic diversity, fitness and pollinator community composition in fragmented populations of *Anthericum liliago* L. Plant Ecol. 198, 101–110. https://doi.org/10.1007/s11258-007-9388-4.

Powney, G.D., et al., 2019. Widespread losses of pollinating insects in Britain. Nat. Commun. 10, 1–6. https://doi.org/10.1038/s41467-019-08974-9.

Proesmans, W., et al., 2019. Small forest patches as pollinator habitat: oases in an agricultural desert? Landsc. Ecol. 34, 487–501. https://doi.org/10.1007/s10980-019-00782-2.

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reinula, I., Träger, S., Järvine, H.-T., Kuningas, V.-M., Kaldra, M., Aavik, T., 2024. Beware of the impact of land use legacy on genetic connectivity: a case study of the long-lived perennial *Primula veris*. Biol. Conserv. 292, 110518. https://doi.org/10.1016/j.biocon.2024.110518.

Ricketts, T.H., et al., 2008. Landscape effects on crop pollination services: are there general patterns? Ecol. Lett. 11, 499–515. https://doi.org/10.1111/j.1461-0248.2008.01157.x.

Scheper, J., et al., 2014. Museum specimens reveal loss of pollen host plants as key factor driving wild bee decline in the Netherlands. Proc. Natl. Acad. Sci. 111, 17552–17557. https://doi.org/10.1073/pnas.1412973111.

Senapathi, D., Goddard, M.A., Kunin, W.E., Baldock, K.C., 2017. Landscape impacts on pollinator communities in temperate systems: evidence and knowledge gaps. Funct. Ecol. 31, 26–37. https://doi.org/10.1111/1365-2435.12809.

Sih, A., Baltus, M.-S., 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. Ecology 68, 1679–1690. https://doi.org/10.2307/1939860.

Sõber, V., Leps, M., Kaasik, A., Mänd, M., Teder, T., 2020. Forest proximity supports bumblebee species richness and abundance in hemi-boreal agricultural landscape. Agr Ecosyst Environ 298, 106961. https://doi.org/10.1016/j.agee.2020.106961.

Sõber, V., Aavik, T., Kaasik, A., Mesipuu, M., Teder, T., 2024. Insect-pollinated plants are first to disappear from overgrowing grasslands: implications for restoring functional ecosystems. Biol. Conserv. 291, 110457. https://doi.org/10.1016/j.biocon.2024.110457.

Steffan-Dewenter, I., Schiele, S., 2008. Do resources or natural enemies drive bee population dynamics in fragmented habitats. Ecology 89, 1375–1387. https://doi.org/10.1890/06-1323.1.

Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C., Tscharntke, T., 2002. Scale-dependent effects of landscape context on three pollinator guilds. Ecology 83, 1421–1432. https://doi.org/10.1890/0012-9658(2002)083%5B1421:SDEOLC%5D2.0.CO;2.

Suárez, D., Arribas, P., Jiménez-García, E., Emerson, B.C., 2022. Dispersal ability and its consequences for population genetic differentiation and diversification. Proc. R. Soc. B 289, 20220489. https://doi.org/10.1098/rspb.2022.0489.

Svensson, B., Lagerlöf, J., Svensson, B.G., 2000. Habitat preferences of nest-seeking bumble bees (Hymenoptera: Apidae) in an agricultural landscape. Agr. Ecosyst. Environ. 77, 247–255. https://doi.org/10.1016/S0167-8809(99)00106-1.

Szövényi, P., Sundberg, S., Shaw, A.J., 2012. Long-distance dispersal and genetic structure of natural populations: an assessment of the inverse isolation hypothesis in peat mosses. Mol. Ecol. 21, 5461–5472. https://doi.org/10.1111/mec.12055.

Taylor, H.R., Colbourne, R.M., Robertson, H.A., Nelson, N.J., Allendorf, F.W., Ramstad, K.M., 2017. Cryptic inbreeding depression in a growing population of a long-lived species. Mol. Ecol. 26, 799–813. https://doi.org/10.1111/mec.13977.

Taylor, K., Woodell, S.R., 2008. Biological flora of the British isles: *Primula elatior* (L.) hill. J. Ecol. 96, 1098–1116. https://doi.org/10.1111/j.1365-2745.2008.01418.x.

Timberlake, T.P., Vaughan, I.P., Baude, M., Memmott, J., 2021. Bumblebee colony density on farmland is influenced by late-summer nectar supply and garden cover. J. Appl. Ecol. 58, 1006–1016. https://doi.org/10.1111/1365-2664.13826.

Vaerbak, S., Andersen, S., 1997. Genetic control of seed set linked and unlinked to flower heteromorphism in inbred lines of *Primula vulgaris* Hudson. Euphytica 93, 55–62.

Valdes, A., García, D., 2011. Direct and indirect effects of landscape change on the reproduction of a temperate perennial herb. J. Appl. Ecol. 48, 1422–1431. https://doi.org/10.1111/j.1365-2664.2011.02047.x.

Van Rossum, F., Triest, L., 2003. Spatial genetic structure and reproductive success in fragmented and continuous populations of *Primula vulgaris*. Folia Geobot. 38, 239–254.

Van Rossum, F., De Sousa, S.C., Triest, L., 2006. Morph-specific differences in reproductive success in the distylous *Primula veris* in a context of habitat fragmentation. Acta Oecol. 30, 426–433. https://doi.org/10.1016/j.actao.2006.06.005.

Vanbergen, A.J., 2014. Landscape alteration and habitat modification: impacts on plant–pollinator systems. Curr. Opin. Insect Sci. 5, 44–49. https://doi.org/10.1016/j.cois.2014.09.004.

Viana, B.F., et al., 2012. How well do we understand landscape effects on pollinators and pollination services? J. Pollinat. Ecol. 7. https://doi.org/10.26786/1920-7603(2012)2.

Washitani, I., Osawa, R., Namai, H., Niwa, M., 1994. Patterns of female fertility in heterostylous *Primula sieboldii* under severe pollinator limitation. J. Ecol. 571–579. https://doi.org/10.2307/2261265.

Weeda, E.J., Schaminée, J.H.J., van Duuren, L., 2005. Atlas van plantengemeenschappen in Nederland; deel 4 bossen, struwelen en ruigten. KNNV.

Westrich, P., 1996. Habitat Requirements of Central European Bees and the Problems of Partial Habitats Linnean Society Symposium Series, vol. 18. Academic Press Limited, pp. 1–16.

Wilcock, C., Neiland, R., 2002. Pollination failure in plants: why it happens and when it matters. Trends Plant Sci. 7, 270–277. https://doi.org/10.1016/S1360-1385(02)02258-6.

Wright, L.I., Tregenza, T., Hosken, D.J., 2008. Inbreeding, inbreeding depression and extinction. Conserv. Genet. 9, 833–843. https://doi.org/10.1007/s10592-007-9405-0.