Beware of the impact of land use legacy on genetic connectivity: A case study of the long-lived perennial *Primula veris*

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ABSTRACT

Land use change over the past century has drastically decreased the amount of semi-natural grasslands and jeopardises the functional connectivity, i.e., exchange of genes through pollen and seed dispersal, among populations of plants characteristic to these valuable habitats. Increasing evidence shows that landscape elements can influence gene flow between isolated plant populations. Still, the role of historical land use legacies in shaping the relationship between current landscape characteristics and gene flow in perennial grassland plants remains unclear. We studied the impact of landscape characteristics on the functional connectivity of 32 populations of the grassland plant *Primula veris* in two landscapes, both historically dominated by semi-natural grasslands. Nowadays, one landscape remains grassland-dominated, while forests and agricultural fields prevail in the other. We examined the genetic diversity and structure of these populations and assessed the role of landscape characteristics on gene flow using resistance- and corridor-based approaches. Two gene flow measures were calculated from >2300 SNP markers: pairwise genetic differentiation (F_{ST}) and pairwise mean assignment probability (MAP). The relative permeability of landscape elements for gene exchange depended on landscape context, likely mirroring opposite land use trajectories in these landscapes. Furthermore, the outcome was also affected by different gene flow indices, indicating their context-dependency. We conclude that applying connectivity measures based on findings from a landscape setting reflecting only one type of land use history should be avoided. Additionally, caution is needed when interpreting gene flow measures of long-lived plant species due to possible delays in their response to landscape change.

1. Introduction

Over the past hundred years, land use in Europe has changed drastically, causing the loss and fragmentation of natural and semi-natural habitats (Cousins et al., 2015; Haddad et al., 2015; Wilson et al., 2016). Notably, formerly abundant semi-natural grasslands - species-rich habitats created and maintained by moderate mowing or grazing - have overgrown with trees and bushes or were turned into intensively managed fields (Dengler et al., 2020). As a consequence, the remaining grasslands are often small and spatially isolated (Hooftman and Bullock, 2012). Plants growing in such habitat fragments are prone to lower within-population genetic diversity (Aguilar et al., 2008) and suffer from the loss of functional connectivity (i.e., exchange of genes through pollen and seed flow) because of reduced gene flow between isolated populations (Auffret et al., 2017). Gene flow between plant populations is essential for maintaining sufficient genetic diversity, ensuring high adaptive potential crucial in an era of ongoing environmental change (Young et al., 1996; Leimu et al., 2010). Therefore, achieving and maintaining intra-specific genetic diversity has been recognised as an important prerequisite for conserving and restoring self-sustaining ecosystems (Moreno-Mateos et al., 2020) and is increasingly mentioned as one of the goals of international biodiversity strategies (Krug et al., 2022). Nevertheless, reinstating gene flow between fragmented populations is rarely considered a specific target in practical conservation and restoration frameworks (Aavik and Helm, 2018), which is why the recovery of functional connectivity may remain

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unattainable (Aavik et al., 2013). Limited consideration of genetic diversity and gene flow in conservation actions is also mirrored in the Conservation Evidence database (https://www.conservationevidence.com/), which does not mention how to preserve the gene flow and genetic diversity of plants.

A baseline concept of population genetics suggests that there is generally less gene flow between populations geographically more distant from each other (isolation by distance; IBD; Wright, 1943). Often, however, IBD remains insufficient in explaining variation in gene flow (Jenkins et al., 2010; Sexton et al., 2014), since different environmental factors may considerably shape the patterns of gene exchange (isolation by environment; IBE; Wang and Bradburd, 2014). In particular, landscape characteristics between spatially separated populations may play a crucial role in assisting or hindering gene flow among those populations (e.g., Emel et al., 2020; Lehmair et al., 2020; Lobato-de Magalhaes et al., 2020). For example, woody landscape elements have been shown to impede the gene flow of grassland plants (Aavik et al., 2014; Hahn et al., 2013), whereas a higher proportion of grasslands might promote gene flow in these species (Reinula et al., 2021). Since plants are stationary, gene flow between their populations occurs mainly through wind or pollen and seed dispersal vectors (Holderegger et al., 2010). Hence, it is often seed and pollen vectors who perceive the characteristics of different landscape elements as a barrier or facilitator for movement. For instance, grazing networks have been found to promote higher functional connectivity in grassland plants by supporting the movement of seeds by grazing animals (DiLeo et al., 2017; Plue et al., 2019; Rico et al., 2014). In another study, DiLeo et al. (2018) observed more pollen deposition into insect-pollinated plant populations surrounded by a lower proportion of forest. These studies confirm that research and practice aiming at enhancing the functional connectivity of plants should consider not only the geographic distance between focal populations but must also assess the quality of landscapes enabling or hindering the movement of pollinators and seed-dispersing animals.

The impact of landscape characteristics on the genetic patterns of plant populations can be explored with the methods of landscape genetics - a discipline combining the theory and tools of population genetics and landscape ecology (Manel et al., 2003). It enables the evaluation of the response of gene flow, one of the main proxies for functional connectivity, in response to landscape composition and configuration (Keller et al., 2015). In light of the negative impacts of the human-induced loss and fragmentation of habitats on biodiversity, landscape genetics has a particularly high potential to propose and evaluate measures (e.g., the creation of stepping stones and dispersal corridors or the removal of barriers), which enable to restore functional connectivity between isolated populations during conservation actions (Holderegger et al., 2020; Keller et al., 2015). Rapid landscape change calls for an especially careful assessment of different methodological approaches to avoid misinterpretation of relationships between landscape characteristics and functional connectivity (Epps and Keyghobadi, 2015). This could lead to a distorted understanding of the situation of genetic diversity and gene flow of grassland plants, and a lack of crucial conservation actions. Furthermore, wrongly interpreting the influence of landscape elements may lead to misguided conservation activities.

Humans have shaped European landscapes over thousands of years. Moderate human intervention has also led to positive outcomes for biodiversity, exemplified by the creation and maintenance of semi-natural grassland habitats, holding the highest levels of biodiversity in temperate region (Dengler et al., 2020; Wilson et al., 2012). However, the last century has witnessed a rapid and severe loss of these valuable habitats (Fuchs et al., 2015; Henle et al., 2008; Lieskovský and Bürgi, 2018), predominantly due to land use intensification (Plieninger et al., 2016). In particular, in many European regions, the area of semi-natural grasslands reached its maximum about 100–150 years ago (Cousins and Eriksson, 2008; Eriksson et al., 2002; Helm et al., 2006; Hodgson et al., 2005). From this point onwards, the area and connectivity of grasslands have dramatically deteriorated, often with <10 % of the original area of grasslands left nowadays (e.g., Cousins et al., 2015; Dengler et al., 2020; Helm et al., 2006). However, many grassland plants are perennial, with life spans extending over several decades (Ehrlén and Lehtilä, 2002) and with relatively slow generation turnover. Hence, many grassland plant populations in current landscapes are most probably remnants of the time when grasslands were spatially and functionally connected (Auffret et al., 2018). Past legacies of land use (i.e., lagged responses of plant diversity to landscape change) are, therefore, most probably still present in patterns of plant species diversity in different European rural landscapes (Cousins, 2009; Helm et al., 2006). Yet, knowledge about lagged responses in the genetic patterns of plants is more scarce (Epps and Keyghobadi, 2015; Essl et al., 2015 but see e.g. Münzbergová et al., 2013; Reisch et al., 2017). A lack of knowledge about the possible delays in the response of genetic diversity to landscape change poses a challenge for conservation (Chen et al., 2023). First, unnoticed legacy effects may provide a distorted understanding of the impact of landscape characteristics on genetic diversity and can, therefore, lead to ineffective conservation actions or, in the worst case, to a lack of actions. Second, delays in the response of biodiversity may also offer a 'window of opportunity' for conservation (Essl et al., 2015), i.e., timely and well-planned conservation actions (e.g. restoration of habitats) can sustain the observed genetic patterns still corresponding to historical landscape and environment and prevent the predicted drop of genetic diversity in response to recent landscape change. Hence, accounting for delayed responses (e.g., by incorporating historical landscape data) in the genetic diversity and gene flow of long-lived plant species is crucial for designing effective conservation measures.

Genetic differentiation (F_{ST}) is one of the most commonly used indicators of gene flow. Even though this measure may demonstrate a more rapid response to landscape change than within-population measures of genetic diversity (e.g., heterozygosity; Epps and Keyghobadi, 2015), F_{ST} may be a problematic metric for assessing functional connectivity, i.e., gene flow, between populations in landscapes characterised by recent spatial changes. In the latter, assignment-test-based methods (i.e., methods that determine population membership of individuals or their probability to originate from a population based on genetic information) may be a much more suitable and informative approach as they capture more recent gene flow by using migration events (Manel et al., 2005) and the traces of these events would be erased in only a few generations (Holderegger and Wagner, 2008). Furthermore, assignment tests may also be more appropriate for detecting the effectiveness of recent restoration measures on genetic connectivity (Aavik et al., 2013). Even though there are some comparisons between different gene flow indices, they are usually on an individual level (Landguth et al., 2010) or between relatively recent and ancient gene flow (Rong et al., 2013). Nevertheless, there is a lack of knowledge about the performance of 'historical' and 'contemporary' or 'recent' (i.e., over the last few generations) population-based gene flow measures in rapidly changing landscapes. Without this understanding, the role of the landscape's structural connectivity on functional connectivity may be misinterpreted and, at worst, lead to legacy effects going unnoticed.

In this study, we explore the effect of different landscape elements (e.g., forests or grasslands) between spatially distinct plant populations on the functional connectivity of a relatively long-lived perennial grassland plant, *Primula veris,* in two historically analogous landscapes that used to have similar management, but have followed a different trajectory of land use change. In the past, both landscapes were dominated by semi-natural grasslands. In one of the landscapes, grasslands are still relatively widespread nowadays, while mainly forests and agricultural fields dominate the other landscape. The study species is an obligate outcrossing plant requiring pollinators to ensure effective pollen flow, and thus, represents the group of species most vulnerable to habitat fragmentation due to the parallel negative effects of land use change on pollinators (Aguilar et al., 2008). Furthermore, because of its relatively long life span (Ehrlén and Lehtilä, 2002) common to many grassland

plants, *P. veris* belongs to species that show delayed responses to landscape change (Aguilar et al., 2008). To clarify the dependence of the results on the measure of gene flow as a proxy for functional connectivity, we used two different approaches for assessing genetic connectivity between populations: genetic differentiation (F_{ST}) and pairwise mean assignment probability (MAP). We wanted to know whether there is an interacting effect of landscape elements and land use history on the gene flow of the study species. Due to possible legacy effects, we hypothesise that (1) the genetic diversity, structure and gene flow (i.e., functional connectivity) of *P. veris* in the two landscapes would be relatively similar regardless of the different present-day amounts and connectivity of grasslands in the study landscapes. However, we expect that (2) the choice of indicators used for genetic connectivity would influence whether the legacy effects are observed in patterns of gene flow in *P. veris*. In particular, we expect that since MAP is an assignment test-based indicator, it would be more informative in our changing study landscapes than F_{ST}, which is often described as a measure of 'historical' gene flow. Finally, we expect (3) that the amount of grasslands between the populations of *P. veris* would enhance the gene flow, and forests and shrubs would inhibit the gene flow between the populations of *P. veris*.
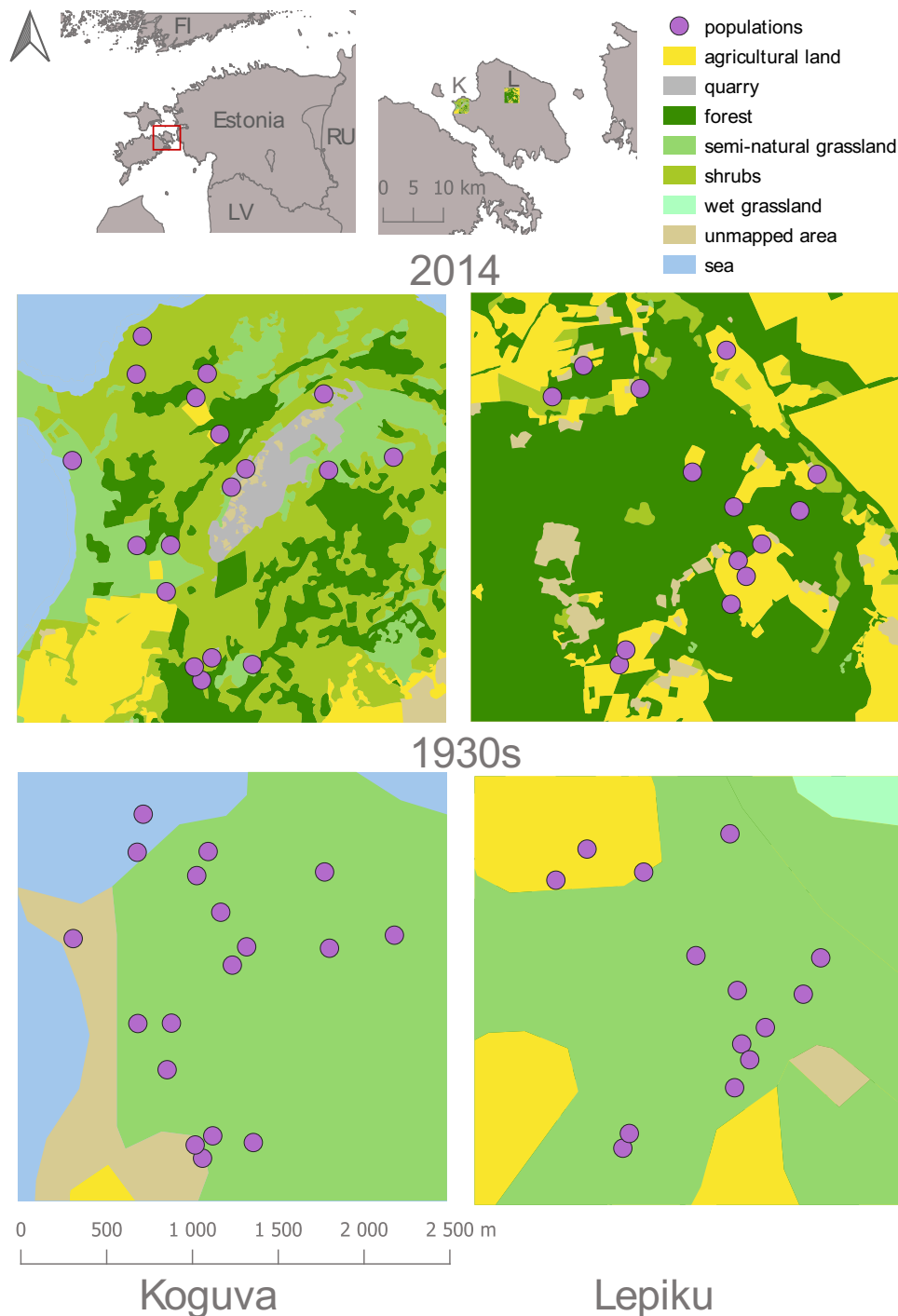
Fig. 1. Map of the study landscapes and populations of *Primula veris* with current (2014) and historical (1930s) land use types in Muhu, Estonia. Some areas in historical maps were not mapped, but most probably were also semi-natural grasslands. Due to some inaccurate mapping with historical data, it may seem like some populations in the northern part of the Koguva landscape were underwater during 1930s, but most likely, they were still on semi-natural grasslands.

An improved understanding of methods used for assessing functional connectivity is highly relevant for designing effective conservation and restoration activities, and for monitoring the success of connectivity measures. In our study system, it helps to clarify whether restoring grassland habitats would have positive consequences for the genetic diversity and gene flow of grassland plants as well as the existence and extent of the so-called genetic extinction debt (the future extinction of biodiversity due to events in the past). Therefore, we will discuss the results of this study in the light of conservation actions.

2. Methods

2.1. Study species

Primula veris L. (Primulaceae) is a perennial herb commonly occurring in calcareous grasslands but also road verges and other grassland-like habitats. In the current study region, Estonia, the species usually flowers in May. It is a heterostylous, obligate outcrossing plant depending on insect pollination (Deschepper et al., 2018) by mostly long-proboscid insects like Hymenoptera (mostly *Bombus* spp., also *Anthophora* spp. and *Halictus* spp.), but also Coleoptera (beetles; *Meligethes* spp.) and Lepidoptera (butterflies, moths; *Gonepteryx rhamni*, *Cucullia verbasci*) (Brys and Jacquemyn, 2009). Recent study in Estonia confirms that *Bombus* is the main pollinator group for *P. veris*, but also *Osmia* spp. and *Lasioglossum* spp. were frequent visitors on *P. veris* (unpublished results). Pollen dispersal is limited to a few meters, and most of the seed dispersal to a few centimeters from the maternal plant (Brys and Jacquemyn, 2009). The gene flow of insect-pollinated grasslands plants, such as *P. veris*, can mainly take place (1) via the movement of pollinators, (2) through seed dispersal, which can also be mediated by grazing animals (Kiviniemi and Eriksson, 1999; Plue et al., 2019; Rico et al., 2014) and wild animals (Auffret and Plue, 2014; Iravani et al., 2011), but also as a result of the movement of agricultural and other machinery (Auffret, 2011). Individual plants of the species can live up to several decades on average (Ehrlén and Lehtilä, 2002). The seed bank of *P. veris* is classified as short-term persistent, i.e., seeds persisting in soil 1–5 years (Thompson et al., 1997).

2.2. Study landscapes

Two study landscapes (Fig. 1) with the size of 2 × 2 km were chosen to represent contrasting landscape compositions in Western Estonia on Muhu island (58.613°, 23.094°; 58.629°, 23.235°). Landscapes were named after the settlements nearest to the landscapes – Koguva and Lepiku. The distance between the two landscapes was 6 km, i.e., higher than the maximum flight distance of potential pollinators of *P. veris* (Greenleaf et al., 2007; Walther-Hellwig and Frankl, 2000; Zurbuchen et al., 2010). According to the historical vegetation map from the 1930s (Laasimer, 1965), when semi-natural grasslands reached the peak of their total coverage in Estonia, calcareous semi-natural grassland was the main land cover type in both landscapes. In the 1930s, this was also a widespread land cover type in the rest of Western Estonia and Muhu island (Laasimer, 1965), where the study landscapes are located. Traditionally, these semi-natural grasslands were managed by grazing (e.g., sheep) (Pärtel et al., 1999). The area is located on Ordovician and Silurian bedrock, thus calcareous soils partly define the nature of habitats and plant communities in this region (Pärtel et al., 1999). In Koguva, 88 % of the area was covered by calcareous grasslands. In Lepiku, semi-natural grasslands occupied 82 % of the landscape. Nowadays, one of the landscapes (Koguva) is still in relatively good condition and open, with about 16 % of semi-natural grasslands covering the area. In addition, a large proportion of the area under shrubs represents former grasslands recently overgrown mainly by *Juniperus communis*. There is an active quarry covering about 4 % of the study area. According to historical map layers (Estonian Land Board) and communication with local municipality managers, small-scale

acquisition of dolomite in Koguva started about 100 years ago. However, a large-scale quarry was established in the 1960s, with substantial disturbance taking place in the 1990s. The other landscape (Lepiku), however, is covered primarily by forest or has been turned into intensively managed agricultural land, with only about 0.1 % of the area covered by semi-natural grasslands. The overgrowth of the grassland habitats and intensification of agriculture in the area has been rather gradual. By 1969, the contemporary layout of fields can be seen in Lepiku and by 1987, the overgrowth in the same landscape has reached similar extent to that of today (Estonian Land Board). Thus, the recent spatial dynamics of these landscapes represent two main trajectories of land use change common in many regions of Europe, where grasslands were once widespread - i.e., (1) abandonment of grassland management, which is followed by the overgrowth of grasslands with trees and bushes and (2) replacement of former grasslands by cultivated fields and forests (Habel et al., 2013).

In May 2014, all populations of *P. veris* in both landscapes were mapped and sampled for genetic analyses (18 populations in Koguva, 14 in Lepiku). A categorical estimation of the size of each population was carried out by separating populations into small (about 0–100 flowering individuals), average (100–500 individuals), large populations (500–1000 individuals) and very large populations (>1000 individuals). Most of the populations sampled in Koguva were located on patches of semi-natural grasslands. In Lepiku, some populations were located on small remnant grassland patches and road verges, while a few populations were sampled on abandoned fields in transition to grasslands. Most of the study locations were not actively managed at the time of the study. During flowering, 1–3 leaves from up to 20 random individuals in all populations were collected, with at least one meter between sampled individuals. Leaf samples were stored on silica gel until DNA extraction.

2.3. Genetic analyses

We weighed about 25 mg of the sample and ground it for 2 min with two 2.3 mm metal beads in a Mixer Mill 301 (Retsch GmbH). We extracted DNA using the LGC sbeadex plant maxi kit (LGC) with some modifications and a more detailed description specified in Träger et al. (2021). We did extraction steps of binding, washing and elution on a KingFisher Flex Purification System (Thermo Fisher Scientific).

After DNA extraction, we prepared a double-digest restriction site-associated DNA (ddRADseq; Peterson et al., 2012) library for sequencing with the extracted DNA. In the ddRADseq method, two restriction enzymes are used for cutting standardised DNA (concentration 120–250 ng/μl) in a two-step process (see Träger et al., 2021 for a thorough description of the preparation of the library). After purifying the DNA, the fragments were ligated to corresponding adapters (48 EcoRI adapters and 2 TaqI adapters). We pooled together the samples with different EcoRI adapters, but the same TaqI adapters and size-selected them for fragments with a length of 450 bp and with biotin-labelled TaqI adapters. After that, we did a polymerase chain reaction (PCR) and purified the PCR products (ddRAD libraries). Then, we calculated the molarity of the final ddRAD libraries according to their mean DNA fragment size. For the final library, we combined the libraries with unique multiplex indices that resulted in a library of 96 individuals with at least 5 nM. We prepared the pooled libraries according to the guidelines of the sequencing facility, and the libraries were sequenced on an Illumina HighSeq2500 (Illumina, Inc., San Diego, CA, USA) at the Functional Genomics Center Zurich (Switzerland), using one lane per library with 125 cycles in single-end read (125 bp), high-output mode. The sample set per library included negative (no sample DNA) and positive (sample replica) controls to exclude the possibility of contamination and to calculate the genotyping error of single nucleotide polymorphisms (SNPs).

We analysed and filtered the sequenced DNA fragments (reads) bioinformatically. First, we demultiplexed the reads with the process_radtags program of Stacks version 1.47 (Catchen et al., 2013;

Catchen et al., 2011). Secondly, we removed the PCR duplicates using the clone_filter program of STACKS. Then, we removed low-quality bases from sequences and filtered sequences depending on their length (minimum 50 bases) using TRIMMOMATIC v0.36 (Bolger et al., 2014). After that, we aligned and mapped the sequences against a draft reference genome of *P. veris* (Nowak et al., 2015) with BURROWS-WHEELER ALIGNER v0.7.17 (BWA; Li, 2013). We detected the SNPs with FREEBAYES v1.1.0-54-g49413aa (Garrison and Marth, 2012) and filtered the SNPs to exclude SNPs with low quality and individuals with too much missing data using VCFTOOLS v0.1.12b (Danecek et al., 2011) following DDOCENT SNP Filtering Tutorial (Puritz et al., 2014) with some adjustments (see Träger et al., 2021 for more information). We then removed indels (i.e., insertions or deletions of bases in the genome) and loci potentially in linkage disequilibrium with VCFTOOLS. We extracted the genotype information from the resulting VCF file using PGDSPIDER v2.1.1.3 (Lischer and Excoffier, 2012). We used putatively adaptive SNPs found in Träger et al. (2021) to filter for putatively neutral SNPs for further analysis as both study samples of *P. veris* were in the same region and laboratory, and bioinformatical treatments were the same. Träger et al. (2021) used environmental association analysis (EAA) to detect SNPs associated with environmental factors related to habitat overgrowth, and outlier tests to detect SNPs under potential diversifying or balancing selection.

We calculated population-based genetic diversity indices (unbiased expected and observed heterozygosity, uH_e and H_o, respectively) using GenAlEx version 6.503 (Peakall and Smouse, 2005, 2012) and mean nucleotide diversity (π) using vcftools within a window of 125 bp over all loci for each population. Unbiased expected heterozygosity (uH_e) accounts for differences in population sizes and is shortened as expected heterozygosity (H_e) below. We calculated the inbreeding coefficient (F_{IS}) in R version 3.4.2 (R Core Team, 2022) using the package *genepop* (Rousset, 2008).

Two different measures were used for assessing gene flow between the study populations: (1) pairwise genetic differentiation (F_{ST}) among populations as a measure of "historical" gene flow and (2) pairwise mean assignment probability (MAP) as a measure of "recent" gene flow (i.e., in about 1–2 generations). We calculated pairwise F_{ST} values in R version 3.4.2 (R Core Team, 2022) using the package *genepop* (Rousset, 2008) and MAP with the package *assignPOP* (Chen et al., 2018). For calculating MAP, we used assignment tests, which represent a way to evaluate population structure by assessing membership probabilities of individuals to each of the sampled study population. This is done by assigning or excluding populations as the origin of sampled individuals using multilocus genotype data (Manel et al., 2005). We performed assignment tests for which we filtered out loci with low variance (threshold at 0.95) and used Monte-Carlo cross-validation. All loci (100 %) were used as training data. The classification method for prediction was linear discriminant analysis. The resulting pairwise probabilities (membership accuracies across all individuals) were directional (e.g., 1 to 2, 2 to 1). We added these pairs together and divided them by two, resulting in one value per population pair (MAP; following van Strien et al., 2014). It should be noted for the interpretation of results that genetic distance grows with higher F_{ST} values and declines with higher MAP values.

2.4. Landscape data

While study populations were sampled at the scale of 2×2 km study landscapes, we added a 250 m buffer around the 2×2 km landscapes, resulting in two 2.5×2.5 km squares, which were used for characterising the structure of the study landscapes in order to take into account the surrounding landscape characteristics. We used five land use categories potentially affecting the gene flow in the study species: semi-natural grassland, agricultural land, shrubs, forest and quarry. These land use types also covered most of the area on both landscapes except for the quarry, which was present only in Koguva. We mapped land use types based on aerial photos of the study area from 2015 (Estonian Land

Board) and Estonian Basic Map (1:10,000, Estonian Land Board) from 2015. We transformed the resulting vector maps to 10×10 m raster data to compromise between the degree of reality and computational capabilities. Spatial analyses were done in QGIS version 3.16.15 (QGIS.org, 2023). Additionally, for the vector data, we made buffers (d = 100 m) around the direct routes between pairs of populations and assessed the proportion of the same five land use categories (excluding quarry in Lepiku) in the buffers. Specifically, we created a polyline layer of all possible direct linear connections between populations with the Line-String function and calculated the proportions of landscape elements in buffers around the direct lines (Fig. 2) using the Python console in QGIS with the native QGIS functions "buffer", "calculate area", "clip" and "field calculator". We used historical landscape data (Laasimer, 1965) for reference and to characterise the landscapes.

2.5. Data analysis

All statistical analyses were done in R version 4.2.1 (R Core Team, 2022). We performed Welch Two Sample *t*-tests to test for differences in genetic diversity indices (H_o, H_e, π and F_{IS}) between the two study landscapes. The genetic structure of populations was analysed with discriminant analysis of principle components (DAPC) in *adegenet* v2.1.1 (Jombart, 2008) package. DAPC uses uncorrelated principal component analysis (PCA) variables for discriminant analysis (Jombart et al., 2010). The optimal number of principal components was found using cross-validation, and populations were used as clusters.

To assess the permeability of different landscape elements for gene flow, we used the *ResistanceGA* package (Peterman, 2018) to generate resistance surfaces (Fig. 2). We assessed the influence of geographic distance between *P. veris* populations and raster data land cover of five land use categories (except quarry in Lepiku) on the two tested gene flow indices (F_{ST} and MAP) per landscape, resulting in two different resistance surfaces for each landscape. *ResistanceGA* uses a genetic algorithm (GA; Scrucca, 2013) to calculate resistance values using pairwise genetic data and resistance distances (for this study, using CIRCUITSCAPE ver 5.11.2 (Anantharaman et al., 2020) with Julia 1.8.3 (Bezanson et al., 2017)). Unlike other resistance surface calculation methods, *ResistanceGA* requires no a priori estimation of the permeability of different habitat types on gene flow, which is particularly suitable in the case of plants with multiple ways of gene flow (pollen flow by pollinators, seed dispersal). First, we made a set of resistance landscapes and calculated pairwise resistance distances for each landscape. Next, we fitted maximum likelihood population effect (MLPE) models with populations as a random variable for each landscape with pairwise genetic distance as the predictor and evaluated the models using log-likelihood as the objective function. These models account for the non-independence among the pairwise data (Clarke et al., 2002). The top 5 % of models were carried on to the next iteration where the landscapes could mix, and the same process was repeated until no improvement was made for 25 iterations. We used default parametrisations. In addition to the default models resulting from the analysis (null model, model with geographical distance, model with resistance distance), we made an additional model with both geographical distance and resistance distance. For each landscape and genetic distance metric, we evaluated these four models with the Akaike information criterion corrected for small sample sizes (AICc; Burnham and Anderson, 2002) with a modification in *ResistanceGA* that takes into account the number of land-use types in the resistance surfaces and treated models with ΔAICc <2 as equally good. Finally, we tested the effect of population size on the pairwise directional assignment probabilities with multivariate generalised mixed effect models (package *MCMCglmm*; Hadfield, 2010) to test whether the source population size affects the gene flow of *P. veris*.

We made multivariate generalised mixed-effect models (Hadfield, 2010) to assess the influence of land use categories in corridor buffers between pairs of populations on genetic distance measures (F_{ST} and MAP) (Fig. 2). Population identities of both populations in a pair were

resistance-based   corridor-based

resistance-based

- Whole landscape
- Relevance of all landscape elements together in models
- Ranks landscape elements by resistance
- Uses genetic algorithms and circuit theory for getting resistance values
- Model selection based on AICc

Similarities:
- Pairwise data
- Accounts for non-independence of the observations
- Model selection based on information criteria
- Mixed models

corridor-based

- Parts of landscape
- Relevance of various landscape element amounts separately in models
- No landscape element ranking
- Uses Markov chain Monte Carlo techniques for fitting MLPE models
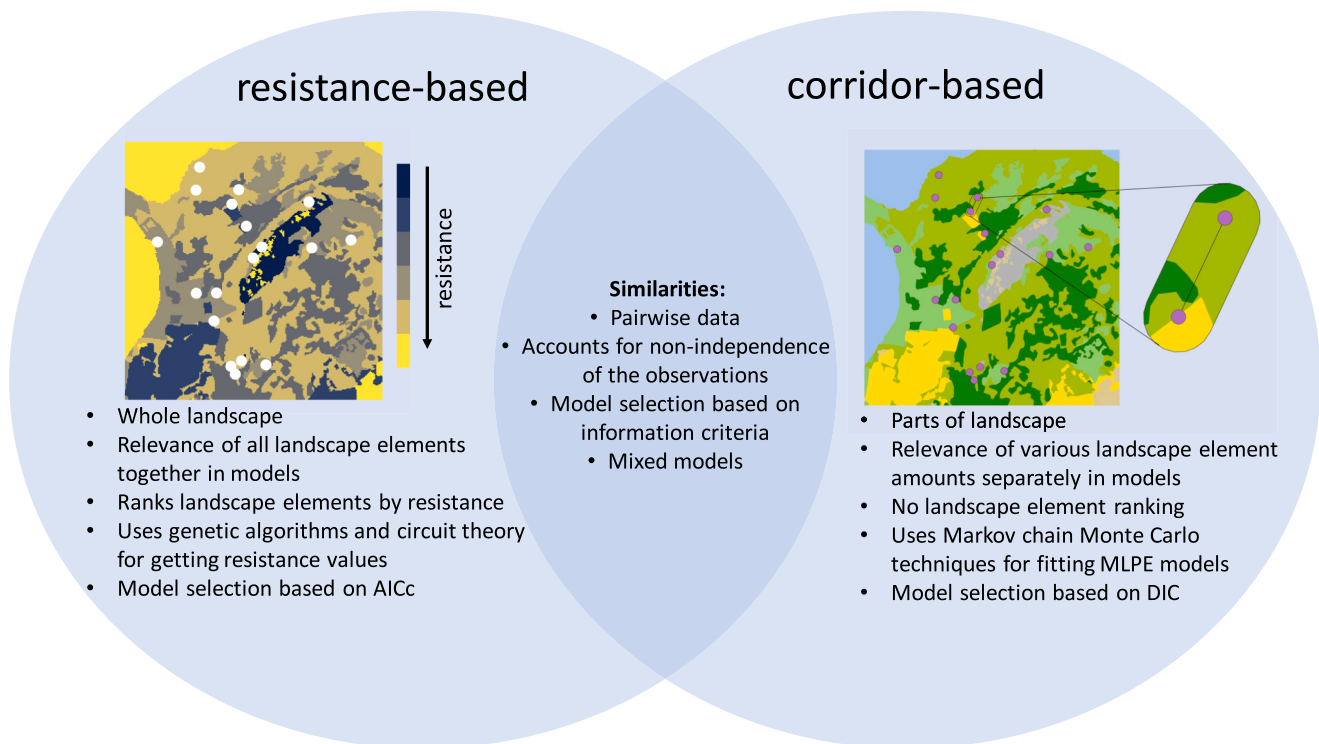- Model selection based on DIC

Fig. 2. Conceptual figure explaining the differences and similarities of the two analysis methods used in this study: resistance- and corridor-based approach. The general aim of both methods is to detect whether and how the landscape influences the gene flow between the study populations. Both methods use data on genetic distances between pairs of populations to assess gene flow (F_{ST} or MAP in the present study). Both methods take into account the fact that data is not completely independent due to using the same populations more than once. With both methods, information criteria have been used to select the best-supported mixed models (AICc in resistance-based method, DIC in corridor-based method). The resistance-based approach relies on whole map layers to create landscape resistance surfaces providing the best fit to the pairwise estimates of gene flow. In corridor-based methods, straight lines between pairs of populations are created around which the buffers are added to result in the so-called corridors, and the amount of different landscape features is calculated within each corridor with pre-defined width (in the current study the width of the corridor was 100 m). In the resistance-based method, the relevance of landscape elements was assessed all together, but in the corridor-based one, we assessed the importance of every landscape element separately in the models. Landscape elements are ranked by their resistance in the resistance-based method using genetic algorithms and circuit theory. With the corridor-based method, Markov chain Monte Carlo techniques are used for fitting the maximum likelihood population effects mixed effects MLPE models. In models relating landscape characteristics assessed in the corridors to the pairwise estimates of gene flow, regression parameter coefficients of different landscape features are interpreted as supporting or hindering gene flow within the corridors between the populations.

used as random variables in covariance matrices to account for potential non-independence of data points in distance matrices (maximum likelihood population effect (MLPE) models; Clarke et al., 2002; van Strien et al., 2012). We made all possible model combinations with geographical distance forced in, with some exceptions due to correlations between land use categories. Namely, we excluded semi-natural grasslands and shrubs in one model for Koguva ($r = 0.62$, $p < 0.001$), and forest and agricultural land for Lepiku ($r = 0.97$, p < 0.001). We also made null models with only random variables. We then assessed all models according to the deviance information criterion (DIC) and models with ΔDIC <2 were treated as equally good. We used DIC for multivariate generalised mixed effect models, as it is more suitable for Bayesian models than AIC (Meyer, 2016).

3. Results

Sequencing of ddRAD fragments resulted in about 150 million raw sequences per library, with about 1.2 million sequences per sample. SNP calling, quality filtering and exclusion of putatively adaptive loci resulted in 2328 putatively neutral SNPs in a total of 616 individuals (Koguva - 350, Lepiku - 266). The genetic diversity (Table 1) was significantly ($p < 0.05$) higher in Lepiku, the more fragmented landscape, than in Koguva (H_o: 0.319 ± 0.009 (mean ± SD) in Lepiku and 0.304 ± 0.017 in Koguva ($t = -4.35$, df = 29.93, $p < 0.001$); H_e: 0.323 ± 0.002 in Lepiku and 0.317 ± 0.003 in Koguva ($t = -6.61$, df = 29.93, $p < 0.001$)). Similarly, nuclear diversity (π) was slightly, but statistically

significantly higher in Lepiku (0.00300 ± 0.00005) than in Koguva (0.00289 ± 0.00002; $t = -6.87$, df = 17.27, $p < 0.001$). The inbreeding coefficient (F_{IS}) was significantly higher in Koguva (0.042 ± 0.033) than in Lepiku (0.015 ± 0.025; $t = 2.60$, df = 29.96, $p = 0.014$). DAPC revealed that Lepiku and Koguva populations separate into two genetically distinct clusters (Fig. 3), with Lepiku populations showing greater variance among populations compared to Koguva populations.

The average F_{ST} in Koguva was 0.0074 ± 0.0030 (Supplementary table A1) and in Lepiku 0.0103 ± 0.0045 (Supplementary table A2) being significantly higher in Lepiku ($t = -5.29$, df = 135.99, $p \leq 0.001$). The average MAP in Koguva was 0.0442 ± 0.0334 (Supplementary table A1) and in Lepiku 0.0501 ± 0.0441 (Supplementary table A2), and did not differ significantly between landscapes ($t = -1.11$, df = 151.32, $p = 0.3$). The correlation between F_{ST} and MAP was −0.68 (p < 0.001) in Koguva and − 0.73 (p < 0.001) in Lepiku. Population size did not have any effect on the directional assignment probabilities (Supplementary table B).

The resistance surface analysis (ResistanceGA) revealed that according to AICc (ΔAICc <2), the best-supported model for F_{ST} in both landscapes and MAP in Lepiku was the null model with only random variables (Table 2). For MAP in Koguva, there were two models with equal support: the null model and the one with geographical distance (Table 2). It should be noted that in all cases, ΔAICc for the geographical distance model was <3.

In contrast, corridor-based analysis revealed the influence of some landscape variables. For F_{ST} in Koguva, the best-supported models

Table 1

Geographic coordinates, the number of samples used in the analysis, observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}) and nucleotide diversity (π) in 32 study populations of *Primula veris* in the two study landscapes (Koguva and Lepiku) on the island of Muhu, Estonia.

Population ID	Region	Latitude	Longitude	Samples	H_o	H_e	F_{IS}	π
K1	Koguva	58.60487	23.09224	20	0.301	0.319	0.058	0.00289
K2	Koguva	58.60455	23.09632	20	0.293	0.316	0.075	0.00290
K3	Koguva	58.61382	23.09397	20	0.310	0.315	0.018	0.00286
K4	Koguva	58.61479	23.09539	20	0.292	0.315	0.075	0.00289
K5	Koguva	58.61876	23.1031	20	0.294	0.320	0.084	0.00291
K6	Koguva	58.61478	23.1037	17	0.297	0.318	0.071	0.00294
K7	Koguva	58.6155	23.11019	20	0.284	0.316	0.106	0.00291
K8	Koguva	58.60369	23.09125	20	0.301	0.314	0.045	0.00290
K9	Koguva	58.60438	23.09049	20	0.294	0.311	0.058	0.00285
K10	Koguva	58.6083	23.08758	20	0.300	0.317	0.054	0.00288
K11	Koguva	58.61074	23.08795	20	0.312	0.315	0.009	0.00285
K12	Koguva	58.6107	23.08458	20	0.316	0.322	0.021	0.00292
K13	Koguva	58.6151	23.07798	20	0.322	0.325	0.009	0.00294
K14	Koguva	58.61966	23.08428	20	0.308	0.317	0.029	0.00289
K15	Koguva	58.62166	23.08483	17	0.314	0.314	0.002	0.00291
K16	Koguva	58.61975	23.09139	19	0.304	0.315	0.036	0.00288
K17	Koguva	58.61848	23.09028	19	0.320	0.315	−0.018	0.00289
K18	Koguva	58.61658	23.09274	18	0.309	0.315	0.022	0.00290
L1	Lepiku	58.62131	23.23188	19	0.309	0.324	0.051	0.00298
L2	Lepiku	58.62209	23.23251	20	0.316	0.322	0.021	0.00298
L3	Lepiku	58.6353	23.22485	20	0.318	0.320	0.007	0.00291
L4	Lepiku	58.63695	23.22794	20	0.326	0.322	−0.010	0.00292
L5	Lepiku	58.63578	23.23365	19	0.315	0.322	0.022	0.00297
L6	Lepiku	58.62771	23.24604	19	0.326	0.326	0.003	0.00305
L7	Lepiku	58.62684	23.24369	19	0.311	0.322	0.035	0.00298
L8	Lepiku	58.62601	23.24451	16	0.312	0.323	0.034	0.00308
L9	Lepiku	58.62454	23.24302	20	0.330	0.322	−0.025	0.00303
L10	Lepiku	58.63143	23.23899	20	0.302	0.321	0.062	0.00298
L11	Lepiku	58.62964	23.24319	18	0.322	0.322	−0.001	0.00302
L12	Lepiku	58.63783	23.24227	20	0.321	0.326	0.017	0.00298
L13	Lepiku	58.63139	23.25152	19	0.333	0.329	−0.012	0.00304
L14	Lepiku	58.62948	23.24981	17	0.321	0.324	0.012	0.00308
Koguva average					0.304	0.317	0.042	0.00289
Lepiku average					0.319	0.323	0.015	0.00300
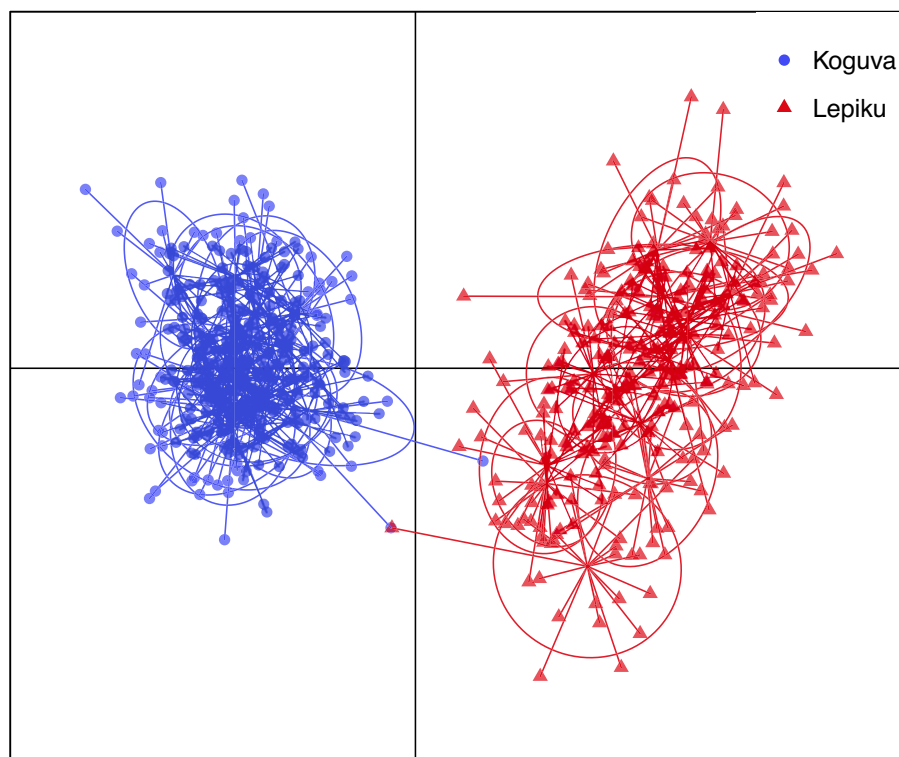
Fig. 3. Result of the discriminant analysis of principle components (DAPC) showing the genetic structure of *Primula veris* populations in the study landscapes of Koguva (blue dots) and Lepiku (red triangles), Estonia. The ellipses represent the populations and lines connect individuals to the populations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Model selection of the resistance surfaces for *Primula veris* populations in two landscapes (Koguva, Lepiku) and with two predictor variables (genetic differentiation 'F_{ST}', pairwise mean assignment probability 'MAP') with MLPE models and based on AICc. Resistance surfaces used were geographical distance ('dist') and categorical land use types (semi-natural grassland, shrubs, agricultural land, forest, quarry (for Koguva); 'landscape'). A null model (with only random variables) and a model with both resistance surfaces were added to the comparison. Models with ΔAICc < 2 were treated as equally good and are marked in bold. R^2m stands for marginal R^2, and R^2c stands for conditional R^2. LL stands for log-likelihood, and k stands for the number of parameters in the model.

Surface	AIC	AICc	ΔAICc	R^2m	R^2c	LL	k
Koguva, F_{ST}							
null	−1469.3	−1469.1	**0**	0	0.536	735.7	1
dist	−1467.6	−1466.8	2.2	0.001	0.534	735.8	2
landscape	−1460.2	−1444.2	24.8	0.187	0.581	738.1	8
dist + landscape	−1458.5	−1436.0	33.0	0.218	0.600	738.3	9
Koguva, MAP							
null	−636.0	−635.8	**0**	0	0.205	319.0	1
dist	−635.1	−634.2	**1.5**	0.007	0.208	319.5	2
landscape	−633.7	−617.7	18.1	0.203	0.253	324.8	8
dist + landscape	−637.1	−614.6	21.2	0.256	0.303	327.6	9
Lepiku, F_{ST}							
null	−789.3	−789.0	**0**	0	0.542	395.6	1
dist	−787.3	−786.2	2.7	0.000	0.539	395.7	2
landscape	−777.7	−759.0	29.9	0.021	0.548	395.9	7
dist + landscape	−776.0	−747.2	41.8	0.045	0.549	396.0	8
Lepiku, MAP							
null	−332.4	−332.0	**0**	0	0.272	167.2	1
dist	−330.8	−329.7	2.3	0.005	0.295	167.4	2
landscape	−320.4	−301.7	30.3	0.000	0.269	167.2	7
dist + landscape	−320.0	−291.2	40.9	0.056	0.336	168.0	8

included a significant positive effect of geographical distance and shrubs (Table 3). For F_{ST} in Lepiku, the best-supported models included a significant negative effect of geographical distance (Table 3). For MAP in Koguva, the best-supported models included a significant negative effect of geographical distance (Table 3; note that genetic distance grows with higher F_{ST} values and declines with higher MAP). For MAP in Lepiku, the best models included the significant positive effect of forest and the significant negative effect of agricultural land (Table 3).

4. Discussion

The past century has witnessed a drastic decline in the area and connectivity of semi-natural grasslands, which is one of the main drivers of biodiversity loss in non-forested habitats in Europe (Fischer et al., 2018). Furthermore, grasslands have continued to decline, with >50 % of the area of these valuable habitats having been lost over the past 30–40 years (Habel et al., 2013), mainly caused by the intensification of agriculture and overgrowth of habitats (Plieninger et al., 2016). In the ongoing 'Decade of Restoration' (United Nations General Assembly, 2019), conservation and restoration of semi-natural grasslands are therefore among the main activities for halting the further loss of species, and for maintaining related ecosystem functions and services (Bengtsson et al., 2019). In addition to habitat fragmentation, species are increasingly threatened by climate change and other environmental pressures. One of the core challenges of restoration initiatives is how to restore and maintain self-sustaining populations capable of overcoming these threats. Therefore, enhanced knowledge of factors affecting genetic diversity and gene flow of populations in fragmented habitats is highly relevant for achieving restoration goals in the long term (Moreno-Mateos et al., 2020).

In this study, our aim was to bring insight into the effect of landscape characteristics on the gene flow as a proxy for functional connectivity of the grassland plant *P. veris* in two landscapes, which were covered by grasslands historically, but have experienced contrasting change of land use in the last century (Fig. 1). First, we found that the inclusion of landscape characteristics in the models predicting gene flow in addition

Table 3

Model selection for the multivariate generalised mixed effect models for genetic connectivity (genetic differentiation 'F_{ST}', pairwise mean assignment probability 'MAP') and geographical distance ('dist') and land-use proportions (semi-natural grassland ('s-n grassland'), shrubs, agricultural land ('agric. land'), forest, quarry (for Koguva) in linear buffers (d = 100 m) in pairs of populations in the two study landscapes (Koguva, Lepiku) in Western Estonia. Model selection was based on deviance information criteria (DIC) and models with ΔDIC <2 were treated as equally good and are marked in bold. For these models, statistically significant variables are written out.

Model	F_{ST} ↑ values = ↓ gene flow		MAP ↑ values = ↑ gene flow	
	DIC	Significant variables	DIC	Significant variables
Koguva				
Null	−1358.17		−628.49	
dist	**−1362.12**	dist (+)	**−630.87**	dist (−)
dist + s-n grassland	−1361.51		**−629.32**	dist (−)
dist + forest	−1360.07		**−628.87**	dist (−)
dist + shrubs	**−1363.56**	shrubs (+)	**−629.00**	
dist + agric. land	−1360.89		**−629.01**	dist (−)
dist + quarry	−1360.66		**−630.55**	
dist + s-n grassland + agric. land	−1360.00		−627.52	
dist + s-n grassland + forest	−1359.57		−627.38	
dist + forest + shrubs	−1361.51		−626.99	
dist + agric. land + forest	−1358.91		−626.97	
dist + agric. land + shrubs	**−1363.18**	dist (+), shrubs (+)	−627.21	
dist + s-n grassland + quarry	−1360.21		**−628.90**	dist (−)
dist + forest + quarry	−1358.91		−628.67	
dist + shrubs + quarry	−1361.00		−628.52	
dist + agric. land + quarry	−1359.02		−628.48	
dist + s-n grassland + forest + agric. land	−1358.23		−625.54	
dist + agric. + forest + shrubs	−1361.03		−625.16	
dist + s-n grassland + forest + quarry	−1359.18		−626.93	
dist + s-n grassland + agric. land + quarry	−1358.26		−626.84	

Table 3 (*continued*)

| | F_{ST} ↑ values = ↓ gene flow | | MAP ↑ values = ↑ gene flow | |
Model	DIC	Significant variables	DIC	Significant variables
dist + shrubs + forest + quarry	−1358.90		−626.71	
dist + shrubs + agric. land + quarry	−1360.49		−626.42	
dist + forest + agric. land + quarry	−1357.40		−626.61	
dist + agric. land + forest + s-n grassland + quarry	−1357.43		−624.86	
dist + agric. land + forest + shrubs + quarry	−1358.45		−624.63	
Lepiku				
Null	−720.56		−307.26	
dist	**−728.14**	dist (−)	−307.08	
dist + s-n grassland	**−726.73**	dist (−)	−305.33	
dist + forest	**−726.25**	dist (−)	**−310.68**	forest (+)
dist + shrubs	**−727.15**	dist (−)	−307.00	
dist + agric. land	**−726.86**	dist (−)	**−311.66**	agric. land (−)
dist + s-n grassland + agric. land	−725.31		−309.60	
dist + s-n grassland + forest	−724.83		−308.81	
dist + s-n grassland + shrubs	−725.21		−305.06	
dist + forest + shrubs	−725.29		**−312.01**	forest (+)
dist + agric. land + shrubs	−725.65		**−311.29**	agric. land (−)
dist + s-n grassland + agric. land + shrubs	−723.69		−309.84	
dist + s-n grassland + forest + shrubs	−723.34		**−310.50**	forest (+)

to geographic distance can improve understanding of the factors affecting functional connectivity between fragmented populations. However, the effect of different landscape elements on the gene flow of *P. veris* was not similar in the two study landscapes, i.e., depended on landscape context, suggesting that different land use trajectories determine the current patterns of gene flow. Furthermore, different genetic connectivity indices (F_{ST} and MAP) revealed slightly different aspects influencing the gene flow of *P. veris* populations. Second, we did not find an effect of grassland amount, but a negative impact of shrubs and arable land, and a positive impact of forests on the gene flow of *P. veris*. Lastly, contrary to our expectations, we found that the genetic diversity of the more fragmented landscape (Lepiku) was higher than that of the less fragmented landscape (Koguva). In the following sections, we discuss the implications of these findings and provide recommendations for practice.

4.1. The permeability of landscape elements depends on the landscape context

We observed a negative impact of geographic distance on gene flow (i.e, isolation by distance, IBD) in Koguva, the less fragmented landscape. With both methods (resistance- and corridor-based), we found the negative influence of geographical distance on recent gene flow (MAP), whereas only the corridor-based method detected it for genetic differentiation F_{ST}, i.e., historical gene flow (Table 3). However, in Lepiku, the more fragmented landscape, we found a reverse result: populations that were geographically further from each other were genetically closer when using genetic differentiation F_{ST} as a measure of gene flow in a corridor-based approach. There was no influence of geographical distance on recent gene flow with any of the methods. One explanation for this unintuitive result might be that in Lepiku, some populations are probably newly established in road verges, former fields (fallows) and other novel habitats. The establishment of such novel populations would have occurred as a result of natural dispersal events or unintended human-mediated spread (e.g., by agricultural machinery) because wildflower seed mixes, which can significantly shape the genetic structure of wildflower plant populations (Aavik et al., 2012), had not been used before or during the sampling in this region. Mixed with some remnant populations, such novel patches of *P. veris* might result in geographical distance having no role in the more fragmented landscape, whereas in the less fragmented landscape, where historical population

structure is still somewhat preserved, geographical distance has an expected influence. In addition, the positive effect of herding on the genetic connectivity of grassland plants (Plue et al., 2019; Rico et al., 2014) may still be detectable in Koguva.

Surprisingly, with a resistance-based method, we did not find the geographic distance between populations to affect the patterns of gene flow (except MAP in Koguva). It is possible that at that scale, i.e., 2×2 km, the effect of geographical distance does not occur as clearly, particularly in outcrossing plants (Twyford et al., 2020). It should be noted, though, that the models with geographical distance stayed within the limits of $\Delta AIC < 3$, showing there might still be some support that there is an influence of geographical distance. Different drivers of gene flow may have a distinct effect depending on the considered spatial scale (Muños-Pajares et al., 2017). Furthermore, some pollinators are most probably able to fly these distances without restrictions (DiLeo et al., 2018; Lepais et al., 2010), and historical rotational herding occurred beyond our study scale (Plue et al., 2019; Rico et al., 2014). However, at the regional scale, the influence of geographical distance on the genetic differentiation of *P. veris* is clearly significant (Reinula et al., 2021). This calls for caution when interpreting results in conservation – different measures may be required at different spatial scales.

In addition, we found the variable effect of different landscape elements in the two landscapes. Landscape genetic studies generally focus on one study landscape due to limited resources, but sometimes also because of practitioners' interest in the conservation management of a specific region or a system of habitats. However, several syntheses have raised concerns about the lack of replication in landscape genetic studies (Holderegger and Wagner, 2008; Keller et al., 2015). Indeed, a few studies, where study landscapes have been replicated, demonstrated that the relative effect of landscape elements on gene flow may differ depending on the study region (Guiller et al., 2023; Schmidt et al., 2009). Our study confirms that interpretations based on only one study landscape must be treated with caution before further generalisation. In the less fragmented landscape (Koguva), shrubs had a negative influence on gene flow (Table 3), whereas, in the more fragmented landscape (Lepiku), forest had a positive influence and agricultural land a negative effect on gene flow (note that forest and agricultural land were correlated). We can thus assume that different processes operate in the two study landscapes. In Koguva, where no human disturbance occurred (with the exception of the quarry), gene flow by pollen movement has been prevalent in the recent past, while more stochastic processes have

determined the gene flow in Lepiku (i.e., the landscape with stronger human impact, such as agriculture and more intense road infrastructure).

We cannot distinguish whether grazing, the movement of pollinators, wild animals or machinery were responsible for the gene flow in our study system, but based on the results, we hypothesise that historically, grazing may have played a more important role, while more recently, i.e., after the abandonment of grasslands, gene flow by pollinators is predominating. Therefore, differences between the landscapes could partially be explained by the different behaviour of pollinators in distinct landscape settings (Viljur and Teder, 2016). When there are more grasslands, for example, pollinators may tend to avoid entering woody elements (like with shrubs in Koguva, the less fragmented landscape), whereas in landscapes with low amount of grasslands, pollinators may opt to choose the next best movement option, e.g., through forest (Zurbuchen et al., 2010), being the case in Lepiku, the more fragmented landscape. Furthermore, clear-cut corridors or clearings, which were not considered in the current study, may act as corridors or stepping stones for pollinators (Viljur and Teder, 2016). However, it is more common to find a negative effect of woody elements on the gene flow of grassland plants (Aavik et al., 2014, 2017; DiLeo et al., 2018). In addition, the proportion of forest and agricultural land were strongly correlated in Lepiku, making it hard to determine which of those elements is really influencing the gene flow. It is thus also possible that intensively managed agricultural land poses a barrier to pollinators of *P. veris* as the microclimate (such as a high wind velocity in larger fields) might be unsuitable for pollinators (Bergman et al., 1996). However, with the resistance-based analysis, we did not find support for landscape characteristics influencing the gene flow of *P. veris*. It has been previously suggested to analyse landscape genetic data sets with multiple methods to avoid method-dependent results (Balkenhol et al., 2009), and we conclude the same. As with corridor-based approach, we detected the influence of some landscape elements on the gene flow of *P. veris*, using only geographical distance as a proxy to gene flow is not advisable in this system.

4.2. Different gene flow indices

The use of two distinct measures of gene flow (F_{ST} and MAP) led to different results, most probably reflecting the contrasting trajectories of land use change in the two study landscapes (Table 3). In short, in the more stable and less fragmented landscape (Koguva), F_{ST} as a proxy for historical gene flow showed a response to landscape characteristics in addition to the effect of geographic distance, while in the more dynamic and fragmented landscape (Lepiku), mean pairwise assignment probabilities responded to the characteristics of the landscape between plant populations. A few previous studies have shown that the impact of a particular landscape element on gene flow may depend on the used index at both individual (Beninde et al., 2023; Shirk et al., 2017) and population level (van Strien et al., 2014). The inconsistent response of different indices to landscape variables in our study most probably reflects the distinct dynamics and age of *P. veris* populations in the two landscapes. In the less fragmented landscape (Koguva), the populations are mostly old, growing on the remnants of semi-natural grasslands, and may have acted as a large (meta-)population in the past. The once large grassland has gradually started to overgrow with shrubs and bushes, causing a sub-structuring of populations reflected in the patterns of genetic differentiation F_{ST}. In the more fragmented landscape (Lepiku), some populations most likely established only fairly recently, e.g., at road verges and previously cultivated patches of field, which is why estimates of contemporary or recent gene flow, and not the measures of genetic differentiation F_{ST}, are more sensitive to landscape parameters. Thus, the age of populations itself might play a role (Jacquemyn et al., 2006) in whether there is a lagged response. *Primula veris* is a relatively long-lived grassland plant (Ehrlén and Lehtilä, 2002), which is why such legacy effects in patterns of genetic differentiation are especially likely.

4.3. Patterns of genetic diversity in landscapes with contrasting land use change

In addition to the relative permeability of landscape elements for the gene flow of *P. veris* in landscapes with distinct land use change, we were interested in the structuring and diversity of genetic variation of the species in these contrasting landscape settings. Surprisingly, the genetic diversity in the more fragmented landscape (Lepiku) was slightly but significantly higher than in Koguva, which still harbours a relatively well-connected grassland system. In addition, both genetic differentiation as well as average pairwise mean assignment probability (MAP) among the study populations of Lepiku were higher. In line with that, the analysis of the genetic structure revealed a more spread out clustering of populations in Lepiku than in Koguva (Fig. 3). It is generally expected that populations in more connected landscapes are genetically more diverse due to gene flow facilitating the exchange of genetic material and at the same time, genetically more similar to each other (Leimu et al., 2010). Our study confirms the latter with relatively lower genetic differences between the populations in Koguva. There has been little disturbance in this landscape (with the exception of the creation of the quarry), which is why most of the populations in Koguva are very likely the remnants of former times when grasslands dominated the landscape. Populations in Lepiku, however, may at least partially be novel (e.g., at road verges or abandoned fields) and hence represent more distinct clusters of genetic variation. This is also in line with the results of the gene flow analyses. This result shows that smaller and newer populations should not be underestimated when looking at the genetic diversity and gene flow of populations in a landscape.

The unexpectedly lower genetic diversity in the well-connected landscape may partly be explained by pollinator behaviour. In larger and more connected grasslands and plant populations, pollinators might not have to move as much to collect a sufficient amount of food compared to fragmented landscapes (Westphal et al., 2006). The latter would force pollinators to move across a more inhospitable matrix between fragmented habitats, thereby supporting the influx of genetic variation into fragmented populations. For conservation, this means that higher genetic diversity does not always equal higher gene flow between populations or connectivity. Alternatively, temporarily higher genetic diversity may also represent the adaptive transition of populations from open environments to more closed conditions. In the populations of *P. veris* in Western Estonia, Träger et al. (2021) found that the adaptive genetic diversity of *P. veris* populations was higher in more overgrown sites, indicating possible adaptation in process. In this study, we did not focus on adaptive genetic variation, but diversity at adaptive loci in landscapes with different trajectories of land use change certainly deserves more attention. In particular, knowledge of the movement of adaptive genes in landscapes with different structural connectivity would be highly important for conservation planning in an era of ongoing climate change.

4.4. Conservation implications

Our landscape genetic study in two spatially close landscapes - both having been historically covered by semi-natural grasslands but having followed a dissimilar land use trajectory - shows that the findings about the effects of landscape characteristics on the gene flow of a grassland plant are context-dependent and even the commonly expected IBD may not hold true in changing landscapes. While semi-natural grasslands have experienced a dramatic fragmentation over a relatively short period, many of the grassland plant species are long-lived perennials, sometimes having even longer life spans than the time windows when substantial turnover of landscapes took place (Ehrlén and Lehtilä, 2002). Hence, it can be expected that many of these populations are not at equilibrium with landscape change and that a mixture of responses in the genetic diversity, structure and gene flow may be observed depending on the characteristics, speed and magnitude of landscape

change. This possible extinction debt (i.e., delayed response) may give an opportunity for nature conservationists to restore the habitats before the negative consequences of habitat loss are manifested in the first place. Nevertheless, as the length of these time lags may differ in different landscapes even within the same species, generalisations should not be made, and landscapes should be seen as unique units, where conservation decisions are made based on the properties of the landscape and target species or habitat. Furthermore, our study shows that both the landscape context and the longevity of the species need to be taken into account when choosing the most appropriate index for assessing gene flow between populations. In particular, the interpretation of results based on genetic differentiation F_{ST} - currently still the most widely-used proxy for gene flow - in spatio-temporally dynamic landscapes should be done with high caution as the role of some landscape elements hindering contemporary gene flow may not be revealed at all and might therefore remain neglected in conservation planning. Similarly, the positive effects of connectivity measures, which have often been carried out in the recent past, may remain unseen, which, in turn, may influence the design of future conservation and restoration actions. Hence, in dynamic landscapes, it is advisable to use an assignment test-based index revealing more recent gene flow to avoid these consequences or, ideally, use more than one index. Lastly, the landscape should be not only suitable for plants, but also for their pollinators and seed vectors.

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CRediT authorship contribution statement

Iris Reinula: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sabrina Träger:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Hanna-Triinu Järvine:** Writing – review & editing, Investigation. **Vete-Mari Kuningas:** Writing – review & editing, Formal analysis. **Marianne Kaldra:** Writing – review & editing. **Tsipe Aavik:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Data availability

Sequence data used in this study is available at the European Nucleotide Archive (ENA; study accession no. PRJEB73475). Genetic diversity and differentiation, and landscape data are available at the Dryad Digital Repository (DOI https://doi.org/10.5061/dryad.ns1rn8q1f).

Acknowledgements

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