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Research article

Genetic structure and diversity of the declining orchid *Gymnadenia conopsea* in Scandinavia: implications for conservation and management

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Understanding how historical versus contemporary processes shape population genetic structure and diversity is important to design effective management actions for threatened species. We genotyped 1834 SNPs in 1120 individuals from 110 Scandinavian populations of the declining orchid *Gymnadenia conopsea*, in three different habitat types, to examine whether genetic structure was related to wind speed, terrain ruggedness, forest cover, and seasonality at the landscape scale, and whether genetic diversity increases with census population size and is higher in core habitats (fen and meadow) than in marginal, coastal habitats. We identified three genetic clusters and pronounced isolation by distance, consistent with two independent colonization routes after the last glacial maximum, followed by admixture. Effective population size was highest in the admixed cluster. Estimates of effective migration indicated reduced gene flow along the Atlantic coast, between coastal and inland populations, and among southern meadow populations. High landscape resistance to gene flow was associated with complex topography and pronounced seasonality. Genetic diversity increased with population size but did not vary among habitat types. Genetic diversity peaked in core habitats, i.e. southern meadows and inland fens along the Scandes Mountains. The lowest genetic diversity was found along the Atlantic coast and in a few scattered populations. Current genetic structure suggests a strong legacy of historical events, and the high genetic diversity documented in the main Scandinavian range indicates that current viability and future adaptation potential are high. To maintain genetic diversity and connectivity between genetic groups, it is particularly important to preserve southern meadow populations, which are currently in strong decline. Overall, our results illustrate how a declining species can help us understand the impact of historical and current processes, how landscape genetic data can inform proactive conservation,

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and how a slow genetic response to fragmentation can allow time to maintain genetic diversity through habitat restoration and management.

Keywords: connectivity, grassland, landscape genetics, landscape resistance, population size, proactive conservation

Introduction

Anthropogenic changes in land use and climate are driving habitat degradation and fragmentation, resulting in the loss of biodiversity worldwide (Turvey and Crees 2019, Román-Palacios and Wiens 2020). Proactive management of declining species is often the best approach to counteract the expected negative effects of ongoing habitat loss and change (Drechsler et al. 2011). This includes efforts to preserve genetic diversity, which are recommended by the IUCN and explicitly targeted by the CBD, but still rarely incorporated into management policies (Laikre et al. 2010, Love Stowell et al. 2017, Ralls et al. 2018, Hoban et al. 2021). To create effective management that promotes long-time species persistence, it is crucial to understand historical and ongoing processes that govern population genetic structure and variation.

Historical events, such as colonizations or the presence of glacial refugia, can result in strong population genetic structure. A well-studied example is the postglacial colonization of the Scandinavian Peninsula, where southern and northern colonization routes are evident in the contemporary genetic structure of many plants and animals (Taberlet et al. 1998, Kullman 2008, Knopp and Merilä 2009, Nota et al. 2022). With range expansion, step-by-step founder events can lead to a gradual increase in genetic drift and loss of genetic diversity along the axis of expansion (Austerlitz et al. 1997, Hewitt 2000, Slatkin and Excoffier 2012), and in Scandinavia, increased differentiation and a decline in diversity with latitude is common (Seljestad et al. 2020, Thörn et al. 2021). In addition, gene flow typically decreases over distance in species with a limited dispersal range. This leads to increasing genetic differentiation with geographic distance, a pattern known as isolation by distance (IBD) (Wright 1943). Landscape features affecting gene flow also influence the pattern of IBD. Strong barriers or corridors to gene flow – either natural (e.g. mountains, forests or lakes) or human-caused (e.g. land use) – could isolate populations that are geographically close or link populations located far apart (McRae 2006). By examining population genetic structure across large scales, we can begin to tease apart historical and current processes.

Whether genetic structure mainly reflects historical events or ongoing processes will influence which conservation actions are appropriate. Strong genetic differentiation due to historical events or adaptation to differing local conditions might imply dividing populations into different management groups (Moritz 2002, Luck et al. 2003). Management plans should then aim to safeguard viable populations in each group while also minimizing negative or unintended outcomes, such as outbreeding depression in genetic rescue attempts when mixing groups (Frankham et al. 2011). On the other hand, if genetic differentiation is due to genetic drift caused by habitat fragmentation, management actions should facilitate gene flow where possible in order to reconnect populations (Willi et al. 2022). Additionally, information about genetic variation can help detect populations at risk due to low genetic diversity and prioritize populations with high genetic diversity.

A group of species in strong decline are those connected to grasslands and semi-open habitats favoured by traditional human land use. One example is the outcrossing, terrestrial orchid *Gymnadenia conopsea*, which is distributed across Eurasia (Hultén and Fries 1986; see the Supporting information for a detailed species description). The main habitat types of *G. conopsea* in Scandinavia are calcareous grasslands and fens, with likely later migration into coastal habitats at the range margin. *Gymnadenia conopsea* is currently classified as a 'Least concern' species in Sweden and Norway, but abandoned or intensified management is driving regional decreases and extirpations, especially of meadow populations (Tyler 2007, Johansson et al. 2016, Ståhl and Berglund 2016) that are highly dependent on traditional land use. The species is in decline in large parts of northern Europe and is red-listed as 'Vulnerable' in Finland (Hyvärinen et al. 2019), 'Near threatened' in Germany (Metzing et al. 2018), 'Endangered' in the Czech Republic (Grulich 2012), and 'Critically endangered' in Denmark (Moeslund et al. 2023) and Belgium (Maes et al. 2020). The main pollinators of *G. conopsea* in Scandinavia are hawkmoths and noctuids (Sletvold and Ågren 2010, Chapurlat et al. 2015) that can transport pollen over long distances (Skogen et al. 2019, Lewis et al. 2023). Like other orchids, *G. conopsea* produces dust-like seeds that are dispersed by wind, and although most orchid seed dispersal is local (Jersáková and Malinová 2007), the fraction that disperses out of the population is expected to allow efficient gene flow (Vanden Broeck et al. 2014, Kotilínek et al. 2020, Evans et al. 2023). Still, seed germination tests conducted in unoccupied versus occupied sites suggest no difference in the ability to establish, indicating that dispersal limitation is important in determining orchid distribution at the landscape scale (reviewed by McCormick and Jacquemyn 2014). Both pollen- and seed-mediated gene flow are thus likely to influence landscape-scale genetic structure. The reliance on wind as the main seed dispersal agent suggests that gene flow is mediated by large-scale wind patterns (Kling and Ackerly 2021), and that landscape resistance to gene flow may increase with terrain ruggedness and forest cover, which both influence wind patterns and speed. In addition, marked differences in seasonality associated with the coastal-inland gradient across the Scandinavian peninsula could reduce gene flow via pollen

dispersal. Populations located in inland, high-altitude regions with strong seasonality have a short growing season and a late flowering period and can thus be expected to be more isolated genetically (Gustafsson and Lönn 2003). In addition, within a given region, meadow populations usually start flowering earlier than fen populations (Sletvold unpubl.), and divergence in phenology could contribute to isolation by habitat.

Here, we examine spatial population genetic structure, genetic diversity, and connectivity in *G. conopsea* across the entire Scandinavian peninsula to clarify the role of historical and current processes in shaping these patterns and the potential implications for conservation efforts. To this end, we genotyped 1834 SNPs from 110 populations that vary widely in contemporary population census size and are found in three main habitat types: meadow, fen and coastal habitats. We specifically ask if: 1) there are indications of several independent postglacial colonization events, 2) genetic differentiation increases with geographical distance and is stronger between population pairs in different habitats than in the same habitat, 3) variations in landscape features explain more of the genetic differentiation than geographic distance alone, and 4) estimates of genetic diversity increase with population size and are higher in core habitat types (meadow and fen) than in the more marginal coastal habitat.

Material and methods

Sample collections in the field

We included a total of 1120 individuals of *G. conopsea* from 110 populations across Norway and Sweden (Fig. 1, Supporting information), where each population was sampled once between 2012 and 2017. The populations were separated by a range of 1.8 to 1735.0 km, spanning 14° in latitude and 25° in longitude. We sampled 11 individuals per population (n = 86), but only 1–10 individuals in the 24 smallest populations. Sample sizes were based on simulations and empirical studies that indicate that this was sufficient to obtain accurate estimates of intra- and interpopulation genetic diversity with large SNP arrays (Jeffries et al. 2016, Nazareno et al. 2017). Sampling was always conducted across the population. At each sampling location, we estimated the census population size as the number of flowering individuals. We counted all flowering individuals in populations with less than approximately 1000 individuals. In larger populations, we extrapolated a count of the first thousand to the total area. There was no correlation between census population size and latitude ($r_s = -0.06$, p = 0.55, n = 110) or longitude ($r_s = 0.001$, p = 0.95, n = 110). We classified the local habitat as fen, meadow or coastal. Fen populations are found throughout the Scandinavian peninsula, whereas meadow populations are mainly located in the south and coastal populations along the Atlantic coast in the north (Fig. 1). Census population size did not vary among habitat types (one-way ANOVA; $F_{2,107} = 0.45$, p = 0.64). We also included 4–5 individuals from each of the four meadow populations from the Czech Republic as an outgroup for assessing genetic structure in Scandinavia (Supporting information).

Sequencing and genotyping

From each plant, we collected leaf tissue on silica gel for DNA extraction with the Qiagen Plant DNeasy kit following standard protocol (Qiagen). We genotyped all DNA samples (n = 1139) using a custom-designed Illumina iSelect chip with a 2803 single nucleotide polymorphism (SNP) assay (hereafter referred to as the Orchid3K chip). To construct the Orchid3K chip, we initially used double-digest RAD sequencing to generate genome-wide polymorphism data (detailed methods description in the Supporting information).

Spatial structure

To examine population genetic structure, we performed a PCA in R, using the package 'ade4' ver. 1.7.18 (Dray and Dufour 2007), and visualized results using the package 'PCAviz' ver. 0.3.37 (Novembre et al. 2019). To infer individual ancestry components we conducted unsupervised clustering using the software 'Admixture' ver. 1.3.0 (Alexander et al. 2009) and R-package 'conStruct' ver. 1.0.4 (Bradburd 2019), and visualized results using QGIS ver. 3.16.1 (QGIS Development Team 2022). See detailed methods in the Supporting information. We estimated effective population size for all supported clusters by grouping individuals from populations with ≥ 75% of shared ancestry component in Admixture analysis using $K = 3$. Effective population size was quantified from linkage disequilibrium based on Pearson correlation approximation (Waples et al. 2016), using the function *ldNe* from the 'strataG' R-package (Archer et al. 2017), with minimum allele frequency at 0.05 and excluding loci with missing genotypes.

Population differentiation

We estimated genetic differentiation between population pairs using F_{ST} averaged over all SNPs (Weir and Cockerham 1984). We tested the significance of isolation by distance (IBD) across the study area with a Mantel test with 1 000 000 replicates, in the R package 'vegan' ver. 2.5.7 (Oksanen et al. 2020), including all populations pairs. To investigate IBD in relation to habitat type, we split the dataset into a southern (S: latitude < 65°N) and a northern (N: latitude > 65°N) group (Fig. 1, Supporting information). This was done because the distribution of habitat type is correlated with latitude – coastal habitats are limited to the north and meadows to the south. We then tested for IBD within each habitat type in N and S, using separate Mantel tests with 1 000 000 replicates.

To test whether genetic differentiation depended on habitat, we limited comparisons to fen and meadow in the southern group, as strong covariation between habitat type and geography precludes the separation of any habitat effect in the north (Fig. 1). We used two approaches to test if

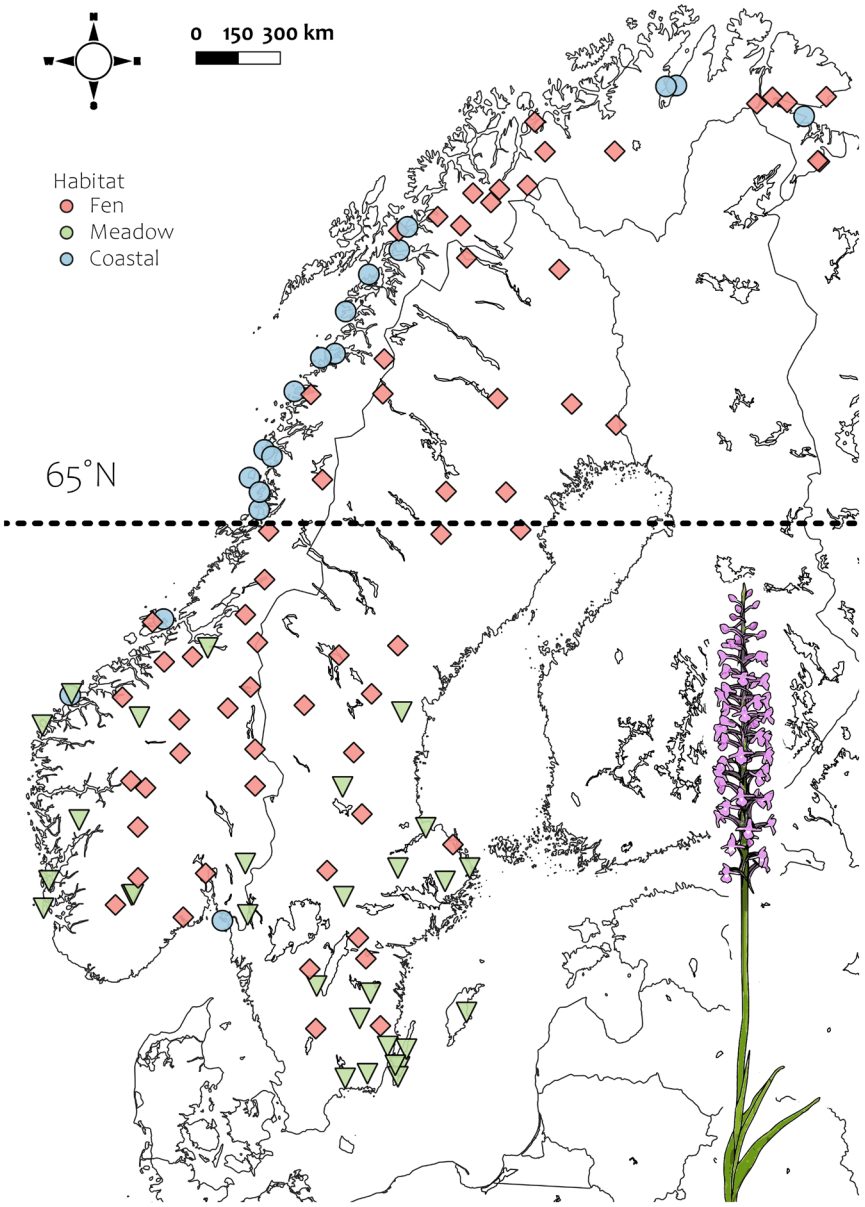


Figure 1. Overview of the 110 *Gymnadenia conopsea* populations sampled in Scandinavia. Dotted line marks 65°N, used as the division between a northern group with fen and coastal habitats, and a southern group with meadow and fen habitats. For population names see the Supporting information.

differentiation between populations located in different habitats is stronger than between populations within the same habitat, which could be caused by earlier flowering phenology in meadows, compared with fens. First, we created a pairwise matrix for S in which we assigned the distance of within-habitat comparisons to '0' (within fen and meadow), and between-habitat comparisons to '1' (between fen and meadow), and ran a Mantel test with 1 000 000 replicates (see details in the Supporting information). Second, because the influence of habitat type on gene flow is mainly expected to occur on a local scale, we selected closely situated (< 180 km separation) population pairs in meadow, fen, and contrasting habitats (one meadow and one fen population). We included eight unique population pairs of each type (Supporting information). The average distance between pairs was 66 km and did not differ between habitat types (one-way ANOVA; $F_{2,21} = 0.62$, $p = 0.55$, $n = 24$). We used a one-way ANOVA to test whether pairwise genetic differentiation (F_{ST}) depended on habitat (meadow, fen, contrasting).

EEMS and landscape resistance analyses

To visualise how gene flow (effective migration processes) could have given rise to the observed spatial variation in allele frequency, we used the software EEMS (Estimated effective migration surfaces, ver. 20160605; Petkova et al.

2016). EEMS can highlight deviations from isolation by distance and thus illustrate potential corridors and barriers to gene flow (details in the Supporting information). To identify landscape features that could cause variation in gene flow by creating barriers or corridors, we used the R package 'ResistanceGA' ver. 4.2–10 (Peterman 2018). It calculates a resistance distance between pairs of populations by optimizing resistance surfaces based on genetic distance data (here, the matrix of F_{ST} values between all pairs of populations). We created four resistance surfaces based on landscape factors expected to influence gene flow – terrain ruggedness index (TRI), forest land cover, precipitation seasonality, and annual mean wind speed at 10 m above ground level (details in the Supporting information). We expected landscape resistance to gene flow to increase with ruggedness, forest cover and seasonality, and decrease with wind speed.

Genetic diversity in relation to population size and habitat

All summary statistics were calculated in R ver. 4.1.2 (www.r-project.org) on the filtered dataset (detailed description in the Supporting information). We focus on the proportion of polymorphic loci (P) and expected heterozygosity (H_E) in the following statistical tests. We used a linear regression to determine if estimates of genetic diversity (P and H_E) increased with census population size. Furthermore, we used an ANCOVA to test if estimates of genetic diversity were higher in core habitat types (meadow and fen) compared with the marginal coastal habitat, including census population size as a covariate using the same subgroups, S and N, as described above (Fig. 1, Supporting information). We ran a separate ANCOVA for each geographic group, contrasting fen (n = 14) and meadow (n = 24) populations in S (where no difference is expected), and fen (n = 21) and coastal (n = 11) populations in N (expecting fen > coastal, due to fen being a core habitat). One population, Ski, was an outlier with low estimates for P and H_E, and we reran the analysis of global genetic diversity without it.

Results

Spatial structure and effective population size

Analysis of genetic structure using PCA indicated clustering based on geographic location, with the first principal component (PC1) being strongly related to latitude (linear regression, $F_{1,1118} = 2050$, $p < 0.001$, $R^2 = 0.65$; Fig. 2a, Supporting information). PC1 represented 6.2% of the variance, and PC2–4, 1.3–0.6% (Supporting information). Analysis using both Admixture and 'conStruct' resulted in genetic clustering of northwestern versus southeastern populations (Fig. 2b, Supporting information). Using two ancestral populations, $K = 2$, a gradual division between the clusters occurred in mid-Scandinavia along the mountain range at the Swedish–Norwegian national border. At $K = 3$ the southern cluster split roughly into a northern and a southern cluster. Finally, at $K = 4$ a few populations spread across Scandinavia clustered together. Across all values of K, the southeastern Scandinavian populations consistently clustered with the Czech populations. Admixture did not reveal any clear optimal value of K, as the cross-validation error decreased with increasing K for all tested values (Supporting information). The analyses gave the same overall clustering for both the spatial and the non-spatial 'conStruct' models using $K = 2$–3, and the cross-validation suggested that the spatial model using three clusters

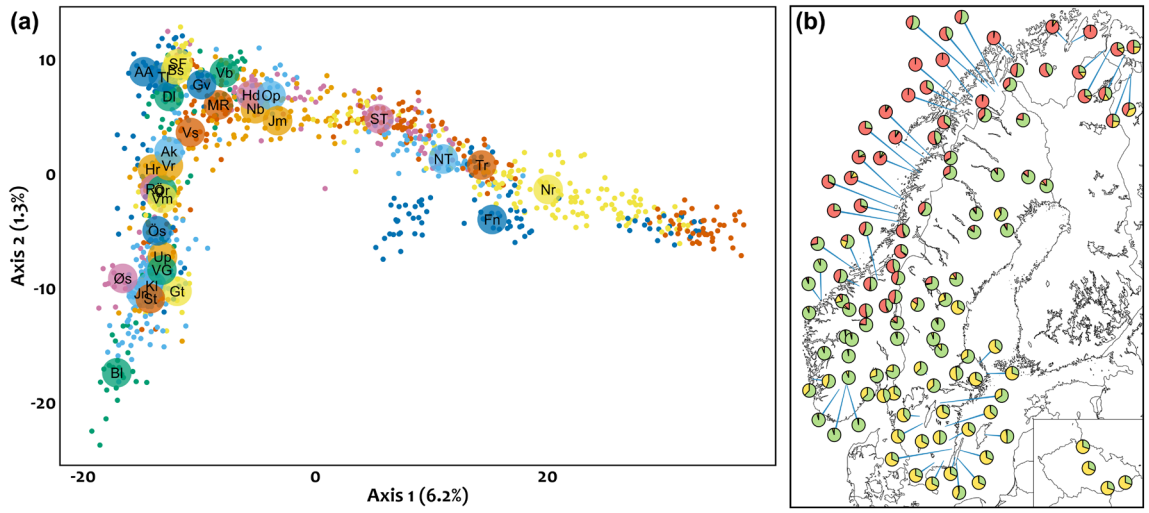


Figure 2. (a) Principal component analysis (PCA) plot for axis 1 and 2. Points indicate individuals, colors indicate geographical grouping of populations (by county), and larger semi-transparent circles indicate the centroid of the geographical group. For abbreviations and information about the geographical origin of each population see the Supporting information. (b) Genetic structure including 110 Scandinavian populations of *Gymnadenia conopsea,* showing admixture proportions for a spatial model using K = 3 in 'conStruct'. Insert shows four Czech populations.

was the best fit, but it was only marginally better than the model using four (Supporting information).

Effective population size was largest in the middle genetic cluster based on 'conStruct' (N_e = 202.0, 95% confidence interval (CI): 198.9–205.1, n = 43), smallest in the northern (N_e = 103.9, CI: 102.8–105.1, n = 22), and intermediate in the southern cluster (N_e = 152.5, CI: 149.7–155.4, n = 17).

Population differentiation

Population genetic differentiation varied from weak to strong (Supporting information), with pairwise F_{ST} values ranging from 0.02 to 0.52, and a mean of 0.19. Two groups of populations had low average F_{ST}, i.e. weak differentiation towards most other populations. One group consisted of fen populations in central Scandinavia along the Swedish–Norwegian border, and the other of southern meadow populations. Populations with high average F_{ST} (> 0.26) consisted of a group of northern Norwegian populations (Rol, Sag, Rub, Gjø), one southern fen population (Herr), and a few geographically scattered populations along the Norwegian coast (Ski, Åkr, Kon) (Supporting information). Several of these populations showed strong differentiation to all other populations (minimum F_{ST} > 0.17; Bug, Herr, Ski, Kon, Åkr). The lowest differentiation was observed between fen population pairs in central Norway (Søl and Tev, F_{ST} = 0.021) and meadow populations on Öland in Sweden (Kvi and Mell, F_{ST} = 0.023). Strong isolation by distance (IBD) was indicated from the Mantel test including all populations, showing a significant correlation between geographic and genetic distance (F_{ST}), ($p < 0.001$, Mantel's r = 0.41; Fig. 3).

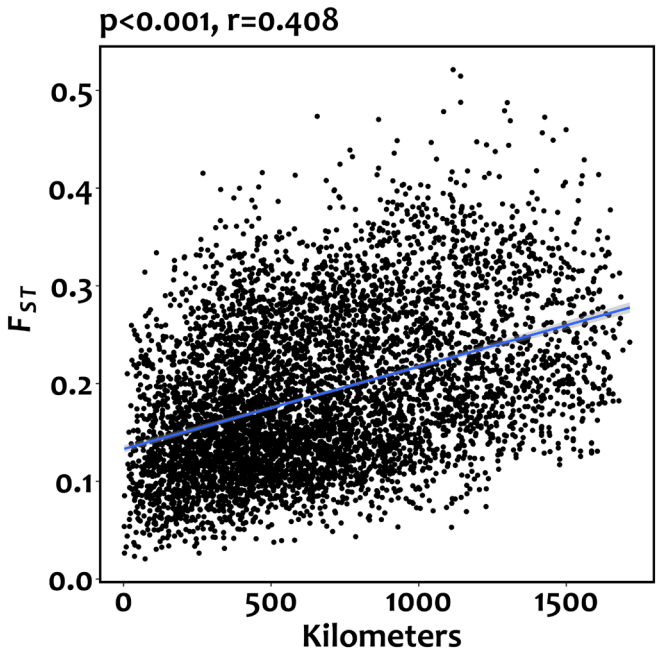


Figure 3. The relationship between geographic distance and genetic differentiation (F_{ST}) among the 110 Scandinavian populations of *Gymnadenia conopsea*. Statistics refer to a Mantel test.

Habitat differentiation

In the southern group, IBD was significant and of similar strength in both habitats (fen: $p < 0.001$, Mantel's r = 0.43, meadow: $p < 0.001$, r = 0.40, Fig. 4a), whereas in the northern group, genetic distance tended to marginally increase with geographic distance in coastal populations (p = 0.08, Mantel's r = 0.30), but not in fen populations (p = 0.37, Mantel's r = 0.03, Fig. 4b).

There was no indication that the difference in flowering phenology caused any isolation by habitat in the southern region. When including all populations, genetic distances were not higher between populations in different habitats compared to populations within the same habitat (p = 0.20, Mantel's r = 0.10; Fig. 4a, Supporting information). Neither was there any effect of habitat type on genetic differentiation between closely located population pairs (one-way ANOVA; $F_{2,21}$ = 0.33, p = 0.72, n = 24). Mean F_{ST} (± SD) between populations in contrasting habitats was 0.11 ± 0.065, compared to 0.10 ± 0.046 and 0.09 ± 0.027 in meadows and fens, respectively.

EEMS analysis

The EEMS analysis revealed multiple potential barriers to gene flow as well as a few corridors (Fig. 5a, b). Significant, large areas of decreased gene flow stretched along most of the central and northern coasts of Norway, separating coastal and inland populations. Other areas of decreased gene flow were detected in northern inland Sweden, running parallel to the Scandinavian mountains. Several small areas of decreased gene flow were scattered across southern Scandinavia, including most of the meadow populations in both Norway and Sweden. Areas of increased gene flow were identified in a few locations along the Scandes and the Swedish coast. Areas with high genetic diversity (q) included the southern part of Sweden, especially meadow populations close to the Baltic coast, and fen populations in central Scandinavia (Supporting information). Areas with low genetic diversity were found along the Norwegian coast and in scattered locations across Scandinavia.

Landscape resistance

Landscape factors explained more of the observed population genetic structure than geographic distance, and connectivity was limited by pronounced terrain ruggedness, seasonality, and weak winds. The bootstrap analysis indicated that the best model, based on the average Akaike information criterion (AIC), included a composite surface of terrain ruggedness (TRI) and precipitation seasonality (BIO15), where the TRI surface contributed most to landscape resistance (68.6%; Supporting information). Resistance increased rapidly from level landscapes to landscapes with a ruggedness corresponding to approximately 100 m variation in altitude, with increasing resistance moving west across Scandinavia (Supporting information). Resistance was low across most

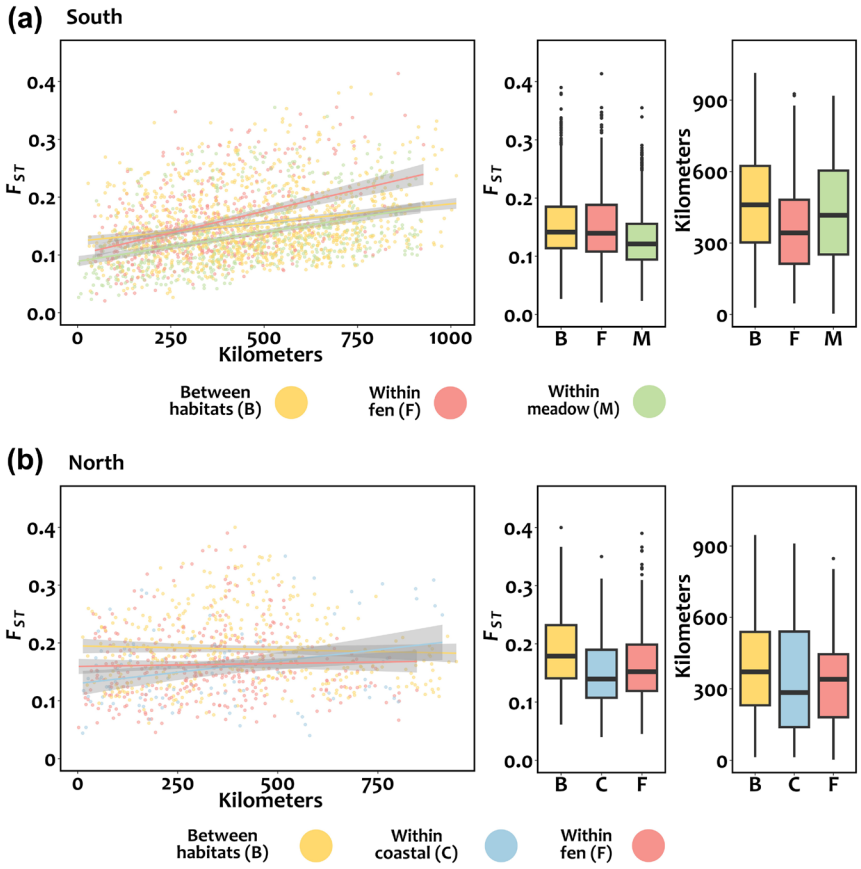


Figure 4. The relationship between geographic distance and genetic differentiation (F_{ST}) among the Scandinavian populations of *Gymnadenia conopsea* in (a) fen (F) and meadow (M) habitats in the southern group (n = 42), and in (b) coastal (C) and fen (F) habitats in the northern group (n = 66). Panels also include between-habitat values for comparison, as well as the distribution of genetic differentiation (F_{ST}), and geographic distances between population pairs in different habitat types (B) and within the same habitat type (F, M, C). For corresponding summary statistics see the Supporting information.

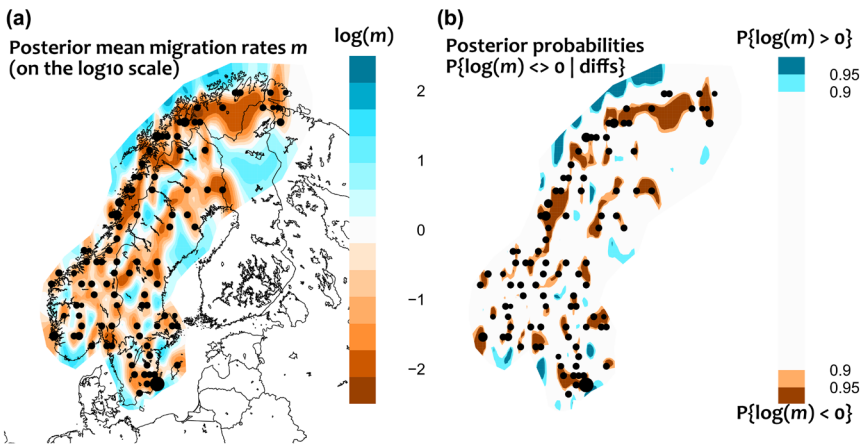


Figure 5. EEMS model results showing (a) effective migration rates, and (b) areas of significantly higher or lower effective migration compared to the average. Orange indicates areas with lower migration and blue areas with higher migration when compared to the average. Results were averaged over 8 runs for deme densities of 800 and 1000, respectively, and combined. Black dots indicate locations of sampling, and dot size indicates the number of samples in each deme.

of the observed interval in seasonality but rapidly accelerated at values corresponding to strongly continental areas (Supporting information). The best single surface model was annual mean wind speed, and this model also explained most of the observed variation in genetic differentiation (63.5%) but was ranked sixth (Supporting information). Resistance rapidly declined with wind speed, and was close to zero above ca 3 m s^{-1}, which corresponds to decreasing resistance moving west across Scandinavia (Supporting information). The bootstrap analysis indicated a minor influence of forest cover as a categorical factor, and geographic distance alone was ranked last (Supporting information). The normalized cumulative current map for the best composite surface model illustrates how gene flow is hampered by rugged terrain, particularly along the Norwegian coast, and also by strong seasonality along the eastern side of the Scandes mountain range in northernmost Scandinavia (Fig. 6a). The corresponding current map for the best single surface model was similar, but shows strong channelization of gene flow by wind (Fig. 6b).

Genetic diversity in relation to census population size and habitat

In the 82 Scandinavian populations with 11 sampled individuals, the proportion of polymorphic loci (P) ranged from 0.29 to 0.81, with an average of 0.65, and the expected heterozygosity (H_E) ranged from 0.11 to 0.24 (mean 0.21; Supporting information). The distribution of the inbreeding coefficient (F_{IS}) was centered on zero, indicating that most populations are under random mating. The highest values for p and H_E were found in central inland and southeastern populations (Supporting information). The proportion of polymorphic loci significantly increased with census population size ($R^2 = 0.039$, $p = 0.041$, $n = 82$), explaining 4% of the observed variation (Fig. 7a). There was no statistically significant association between census population size and H_E (Fig. 7b). When we removed the outlier population, Ski, the relationship between p and census population size was no longer significant, but the overall pattern remained (Supporting information). There was no statistically significant difference between habitat types for any summary statistic (Supporting information). In the southern group (S), all means and ranges were similar in fen and meadow populations (Supporting information). In the northern group (N), fen populations generally showed a higher mean and wider range for all estimates, and the proportion of polymorphic loci tended to be higher in fen populations compared to coastal ones (ANCOVA, $F_{1,29} = 2.954$, $p = 0.096$, $n = 32$; Supporting information).

Discussion

Spatial structure and postglacial colonization

Clear population structure of *G. conopsea* was observed across the Scandinavian peninsula supporting a strong legacy of historical events. Both discrete and continuous models supported three main clusters: a northwestern (NW), middle (M) and southeastern (SE) (Fig. 2b). The populations assigned to the NW cluster, and their admixture proportions, were consistent in all analyses, and for all examined values of K. This indicates a strong differentiation from the M and SE clusters, possibly corresponding to two different colonization routes. A combined northeastern and southern postglacial colonization of Scandinavia has been inferred for many plant and animal species (Nordström and Hedrén 2008, Knopp and Merilä 2009, Thörn et al. 2021, Li et al. 2022, Nota et al.

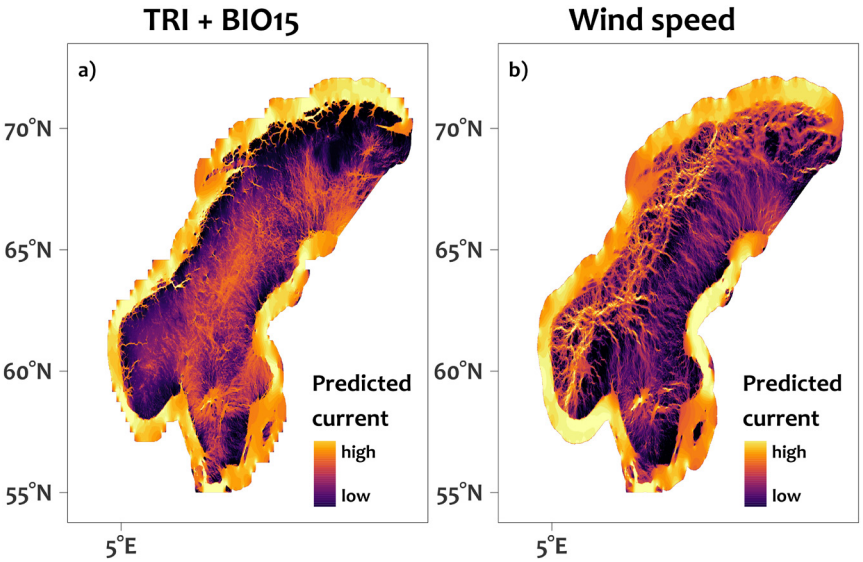


Figure 6. Normalized cumulative current maps (the sum of values at each grid cell across all iterations) of potential gene flow for the best model, including (a) composite resistance surfaces of terrain ruggedness index (TRI) and precipitation seasonality, and (b) the best single resistance surface of annual mean wind speed at 10 m above ground level (wind speed).

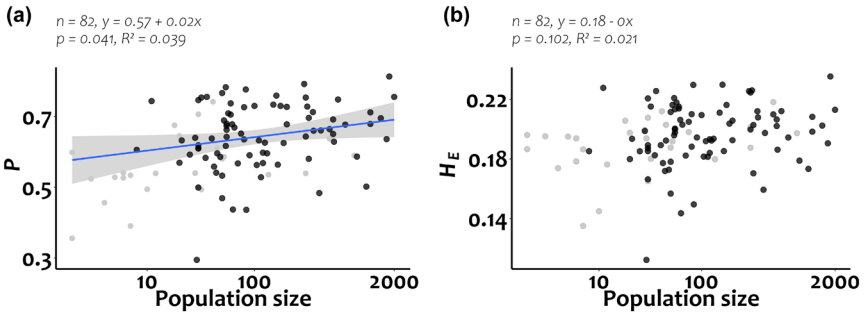


Figure 7. The relationship between population size (ln scale) and (a) proportion polymorphic loci (*P*), and (b) expected heterozygosity (H_E). Black dots represent the 82 populations with 11 samples per population included in the statistical analysis. Gray dots represent 28 populations with fewer than 11 samples per population that were excluded from the analyses.

2022), and the observed pattern in *G. conopsea* fits a similar scenario. Southern meadow populations in Sweden were indeed similar to the Czech populations, supporting a southern colonization route, without a significant loss of genetic variation compared to central Europe (Supporting information). Populations generally had higher pairwise F_{ST} values and lower genetic diversity towards the Atlantic coast, consistent with a stronger influence of genetic drift along the likely range of expansion. The same east–west pattern occurred in northern Scandinavia, but additional samples (Finland/Russia/Baltics) are needed to evaluate a potential eastern colonization route. Similar reductions in genetic diversity along postglacial range expansions have also been observed in other orchids, such as *Himantoglossum hircinum* (Pfeifer et al. 2009) and *Epipactis helleborine* (Tranchida-Lombardo et al. 2011).

It is also possible that the NW cluster represents the remains of a glacial refugial population. Evidence exists for deglaciation of the north Atlantic coast as early as ~ 26 000 years ago, and potential glacial refugia have been documented for other plant species, including *Picea abies* (Parducci et al. 2012), *Betula pubescens* (Kullman 2008), and *Carex scirpoidea* (Westergaard et al. 2019). Today, *G. conopsea* is found at altitudes up to 1500 m a.s.l. in Scandinavia, and the species could possibly have survived in tundra-like habitats. Populations in the vicinity of the potential refugia show limited admixture and have low genetic variation (Sag, Rol, Ørn, Tra; Supporting information), consistent with historically small population sizes and strong isolation. However, an early colonization event along the Atlantic coast could produce a similar pattern, and ice-free corridors are thought to have been present along the Atlantic coast during most of the middle and late Weichselian (Parducci et al. 2012). Regardless of the origin of the ancestral populations, the middle (M) cluster likely represents a secondary contact zone, where populations are highly admixed, resulting in increased genetic variation (Petit et al. 2003, Knopp and Merilä 2009, Thörn et al. 2021, Chiocchio et al. 2022, Li et al. 2022, Nota et al. 2022). Low pairwise F_{ST} values and high genetic diversity were found in central populations stretching along most of the latitudinal gradient, and the EEMS identified significant diversity (*q*) hotspots located in areas where populations are admixed (Supporting information). Presently, the three genetic clusters are well supported, but it is not possible to separate between alternative colonization scenarios without additional data.

Gene flow, population differentiation and landscape resistance

We found strong IBD across the whole dataset, and pronounced geographic structuring was further supported by a close relationship between PCA axis one and latitude. In the southern group of populations, the strength of IBD was similar to that of the entire dataset, suggesting a gradual decline in gene flow with distance. In the northern group, only weak and statistically non-significant IBD was found, in combination with high F_{ST} values, suggesting that populations have remained relatively isolated after colonization, with limited gene flow (Slatkin 1993). This was further supported by the EEMS analysis, which indicated a significant area of reduced gene flow along the western side of the Scandinavian mountains (Fig. 5), corresponding to the rugged fjord landscape along the Norwegian coast (Supporting information), with high resistance to gene flow (Fig. 6a). In contrast, inland populations on both sides of the national border between Norway and Sweden, running along the Scandes mountain range, are more connected. In general, population differentiation was strong compared to many previous studies on orchids (Phillips et al. 2012, Evans et al. 2023), but this could be due to differences in both molecular marker type and geographical scale.

The landscape resistance analyses indicated that a combination of terrain ruggedness and seasonality contributed to the continued isolation between coast and inland. Landscapes with more than around 100 m difference in altitude had high resistance to gene flow, including most parts of western and northern Norway. Also in southern Sweden, the main area with reduced gene flow identified by EEMS corresponded to an area with predicted high landscape resistance due to topographic variation (Fig. 6a). Rugged topography probably strongly limits wind dispersal of seeds, but could also act as a barrier to pollen dispersal. Hawkmoths have been found to prefer a downwind flight direction (Reynolds et al. 2007), and may be expected to follow level parts of the landscape.

Pollinators of *G. conopsea* also include migratory moths such as *Autographa gamma* (Chapurlat et al. 2020, Joffard et al. 2022), which can use tailwinds to move across large distances in the landscape, both within and outside of the migratory period (Chapman et al. 2013). The current map based on wind speed alone illustrates how gene flow is expected to follow valleys and water bodies across the landscape (Fig. 6b), and a more detailed windscape model that includes wind direction (Kling and Ackerly 2021) would be useful to explore in future studies. Because Scandinavia has prevailing westerly winds, this will counter gene flow towards marginal *G. conopsea* populations on the Atlantic coast, and contribute to continued isolation.

Most of the sampled gradient in seasonality had low landscape resistance, except in the most continental parts of southern Norway and northern Sweden. In these areas, pronounced seasonality should limit pollen-mediated gene flow between inland and coast, as highly continental populations will have little flowering overlap with the majority of populations. Although gene flow mediated by seeds is still possible, the association between seasonality and resistance suggests that seed dispersal is too limited to prevent isolation between inland and coast. We also expected that limited overlap in flowering time between habitats within a region would cause population differentiation, but found no support for this. At this smaller spatial scale, seed dispersal may be high enough to prevent differentiation. The strength of IBD in the southern genetic group was similar in both habitat types, and population pairs in contrasting habitats were not more differentiated than population pairs within fens or meadows. Thus, landscape heterogeneity seems to influence gene flow more than variation in local habitat. Neither was there any support for forests acting as barriers to gene flow, despite the fact that forest cover is expected to strongly reduce wind speed , and thereby the efficiency of seed dispersal by wind. This could, to some degree, reflect that forest cover was the only categorical variable in our analysis. It should also be noted that ruggedness, forest cover and wind speed tend to covary across the Scandinavian peninsula (Supporting information), and that differences in the spatial scale most relevant for affecting gene flow may vary among factors. Landscape patterns can be sensitive to scale of measurement and analysis (Wu 2004, Cushman and Landguth 2010), and forest cover could be important if characterized in more detail, or at a smaller spatial scale, where correspondence with terrain ruggedness is likely to be weaker. Finally, our large-scale approach covering the whole of Scandinavia prevented the inclusion of landscape features that vary more locally, such as the amount of meadow habitats, or different land-use types, that could govern genetic patterns at a regional scale.

Genetic diversity in relation to census population size and habitat

The proportion of polymorphic loci increased with population size, in line with theoretical predictions and earlier empirical findings (Frankham 1996, Lammi et al. 1999, Leimu et al. 2006, Peterson et al. 2008, Hague and Routman 2016). However, census population size only explained 4% of the variation in genetic diversity. A weak relationship between genetic diversity and population size can be partly explained by a strong legacy of colonization history at this large spatial scale. Southern meadow populations retain a high number of polymorphic loci even in smaller populations (Supporting information), suggesting that the ongoing decline (Tyler 2007, Johansson et al. 2016, Ståhl and Berglund 2016) has not yet caused substantial genetic erosion. In contrast, even large populations located along the Atlantic coast harbour low genetic diversity, probably owing to founder effects resulting in low genetic diversity upon colonization, combined with subsequent low gene flow. In comparison, population size explained 28% of the variation in genetic diversity among 30 *G. conopsea* populations on the Baltic island of Öland (Söderquist et al. 2024). In the northern and admixed region, some of the small sampled populations may also be the result of recent expansions that have not yet reached equilibrium. Finally, historical fluctuations in population size and variation in flowering fractions and reproductive output among individuals could cause census and effective population size to deviate (Frankham 1995).

None of the estimates of genetic diversity differed significantly between habitats. In the northern group, coastal populations tended to have a lower proportion of polymorphic loci than fen populations. This is likely because coastal populations are fringe populations with limited gene flow, and are also probably colonized later than fen populations. However, the same pattern could emerge if the coastal populations were established from a bottlenecked glacial refugial population or a very early colonization event. The results did not support any discrete groups of populations requiring specific management action, i.e. ESUs (lineages with highly restricted gene flow from other lineages, reviewed by Fraser and Bernatchez 2001).

Implications for management

Gymnadenia conopsea is a typical example of a species that was favoured by the human land-use of previous centuries, and today is in decline. Its presence is considered to indicate a favourable status of species-rich grasslands (https://artfakta.se/taxa/219811/information), a habitat type that is red-listed in Europe, and currently targeted for restoration efforts (Janssen et al. 2016). Here, we document high genetic diversity and low degree of inbreeding in the core distribution area of *G. conopsea* in Scandinavia, indicating that both current viability and future adaptation potential is high, and that restoration and management efforts hold considerable potential. The long lifespan of the species (> 50 years; Tholstrup et al. 2024) likely causes a substantial delay in genetic responses to fragmentation, allowing time to intervene. Efforts to restore original habitat management practices have been successful for boosting population growth of similar orchids

(Jacquemyn et al. 2006, Sletvold et al. 2010), suggesting that documented population losses and rapid declines of genetically variable southern populations (Söderquist et al. 2024, 2025a) can be reversed. Moreover, the lack of genetic separation between populations in southern Scandinavia and Czechia suggests that material for reinforcements and reintroductions in northern Europe can be sourced broadly. Scandinavia includes a major part of the total *G. conopsea* population in northern Europe, and the declining southern populations provide a link to the continental species distribution. Securing that management-dependent meadows are maintained, and that connectivity among them is increased by grassland restoration, will be important for long-term persistence of *G. conopsea* in northern Europe, as well as for many other threatened species linked to the grassland habitat.

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Author contributions

Linus Söderquist: Conceptualization (supporting); Data curation (supporting); Formal analysis (lead); Methodology (equal); Project administration (equal); Visualization (lead); Writing - original draft (lead); Writing - review & editing (lead). **Nina Joffard**: Formal analysis (equal); Writing - review & editing (supporting). **Douglas G. Scofield**: Methodology (supporting); Writing - review & editing (supporting). **Pascal Milesi**: Methodology (supporting); Writing - review & editing (supporting). **Sophie Karrenberg**: Methodology (supporting); Project administration (supporting); Supervision (supporting); Writing - review & editing (supporting). **Nina Sletvold**: Conceptualization (lead); Formal analysis (supporting); Funding acquisition (lead); Methodology (equal); Project administration (lead); Supervision (lead); Writing - original draft (supporting); Writing - review & editing (supporting).

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Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.5x69p8dfp (Söderquist et al. 2025b).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Alexander, D. H., Novembre, J. and Lange, K. 2009. Fast model-based estimation of ancestry in unrelated individuals. – Genome Res. 19: 1655–1664.
Anantharaman, R., Hall, K., Shah, V. B. and Edelman, A. 2020. Circuitscape in Julia: high performance connectivity modelling to support conservation decisions. – Juliacon Proc. 1: 58.
Archer, F. I., Adams, P. E. and Schneiders, B. B. 2017. stratag: an R package for manipulating, summarizing and analysing population genetic data. – Mol. Ecol. Resour. 17: 5–11.
Austerlitz, F., Jung-Muller, B., Godelle, B. and Gouyon, P.-H. 1997. Evolution of coalescence times, genetic diversity and structure during colonization. – Theor. Popul. Biol. 51: 148–164.
Behr, A. A., Liu, K. Z., Liu-Fang, G., Nakka, P. and Ramachandran, S. 2016. pong: fast analysis and visualization of latent clusters in population genetic data. – Bioinformatics 32: 2817–2823.
Bradburd, G. 2019. conStruct: models spatially continuous and discrete population genetic structure. – R package ver. 1.0.4.
Bradburd, G. S., Coop, G. M. and Ralph, P. L. 2018. Inferring continuous and ciscrete population genetic structure across space. – Genetics 210: 33–52.
Chapman, J. W., Lim, K. S. and Reynolds, D. R. 2013. The significance of midsummer movements of *Autographa gamma*: implications for a mechanistic understanding of orientation behavior in a migrant moth. – Curr. Zool. 59: 360–370.
Chapurlat, E., Ågren, J. and Sletvold, N. 2015. Spatial variation in pollinator-mediated selection on phenology, floral display and spur length in the orchid *Gymnadenia conopsea*. – New Phytol. 208: 1264–1275.
Chapurlat, E., Le Roncé, I., Ågren, J. and Sletvold, N. 2020. Divergent selection on flowering phenology but not on floral morphology between two closely related orchids. – Ecol. Evol. 10: 5737–5747.
Chiocchio, A., Zampiglia, M., Biaggini, M., Biello, R., Di Tizio, L., Leonetti, F. L., Olivieri, O., Sperone, E., Trabalza-Marinucci, M., Corti, C. and Canestrelli, D. 2022. Unveiling a hotspot of genetic diversity in southern Italy for the endangered Hermann's tortoise *Testudo hermanni*. – BMC Ecol. Evol. 22: 131.
Cushman, S. A. and Landguth, E. L. 2010. Scale dependent inference in landscape genetics. – Landsc. Ecol. 25: 967–979.
Dahan-Meir, T., Ellis, T. J., Mafessoni, F., Sela, H., Rudich, O., Manisterski, J., Avivi-Ragolsky, N., Raz, A., Feldman, M., Anikster, Y., Nordborg, M. and Levy, A. A. 2024. 36-year study reveals the effects of microhabitats on a wild wheat population. – Mol. Ecol. 33: e17512.
Dray, S. and Dufour, A.-B. 2007. The ade4 package: implementing the duality diagram for ecologists. – J. Stat. Softw. 22: 1–20.

Drechsler, M., Eppink, F. V. and Wätzold, F. 2011. Does proactive biodiversity conservation save costs? – Biodiv. Conserv. 20: 1045–1055.
Evans, A., de Kort, H., Brys, R., Duffy, K. J., Jersáková, J., Kull, T., Selosse, M.-A., Tsiftsis, S., Minasiewicz, J. and Jacquemyn, H. 2023. Historical biogeography and local adaptation explain population genetic structure in a widespread terrestrial orchid. – Ann. Bot. 131: 623–634.
Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. – Genet. Res. 66: 95–107.
Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. – Conserv. Biol. 10: 1500–1508.
Frankham, R., Ballou, J. D., Eldridge, M. D. B., Lacy, R. C., Ralls, K., Dudash, M. R. and Fenster, C. B. 2011. Predicting the probability of outbreeding depression. – Conserv. Biol. 25: 465–475.
Fraser, D. J. and Bernatchez, L. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. – Mol. Ecol. 10: 2741–2752.
Grulich, V. 2012. Red List of vascular plants of the Czech Republic, 3rd edn. Vol. 84. – Preslia, pp. 631–645.
Gustafsson, S. and Lönn, M. 2003. Genetic differentiation and habitat preference of flowering-time variants within *Gymnadenia conopsea*. – Heredity 91: 284–292.
Hague, M. T. J. and Routman, E. J. 2016. Does population size affect genetic diversity? A test with sympatric lizard species. – Heredity 116: 92–98.
Hewitt, G. 2000. The genetic legacy of the quaternary ice ages. – Nature 405: 907–913.
Hoban, S. et al. 2021. Genetic diversity is considered important but interpreted narrowly in country reports to the Convention on Biological Diversity: current actions and indicators are insufficient. – Biol. Conserv. 261: 109233.
Hultén, E. and Fries, M. 1986. Atlas of north European vascular plants: north of the Tropic of Cancer. – Koeltz Scientific Books.
Hyvärinen, E., Juslén, A., Kemppainen, E., Uddström, A. and Liukko, U.-M. 2019. The 2019 Red List of Finnish species. – Ympäristöministeriö & Suomen ympäristökeskus.
Jacquemyn, H., Brys, R., Vandepitte, K., Honnay, O. and Roldán-Ruiz, I. 2006. Fine-scale genetic structure of life history stages in the food-deceptive orchid *Orchis purpurea*. – Mol. Ecol. 15: 2801–2808.
Janssen, J. A. M. et al. 2016. European Red List of habitats. Part 2. Terrestrial and freshwater habitats. – Publications Office of the European Union.
Jeffries, D. L., Copp, G. H., Lawson Handley, L., Olsén, K. H., Sayer, C. D. and Hänfling, B. 2016. Comparing RADseq and microsatellites to infer complex phylogeographic patterns, an empirical perspective in the crucian carp, *Carassius carassius*, L. – Mol. Ecol. 25: 2997–3018.
Jersáková, J. and Malinová, T. 2007. Spatial aspects of seed dispersal and seedling recruitment in orchids. – New Phytol. 176: 237–241.
Joffard, N., Olofsson, C., Friberg, M. and Sletvold, N. 2022. Extensive pollinator sharing does not promote character displacement in two orchid congeners. – Evolution 76: 749–764.
Johansson, B. G., Petersson, J., Ekman, A., Hedgren, S. and Ingmansson, G. 2016. Gotlands flora. – Swe. Bot. Soc..
Kling, M. M. and Ackerly, D. D. 2021. Global wind patterns shape genetic differentiation, asymmetric gene flow, and genetic diversity in trees. – Proc. Natl Acad. Sci. USA 118: e2017317118.
Knopp, T. and Merilä, J. 2009. The postglacial recolonization of northern Europe by *Rana arvalis* as revealed by microsatellite and mitochondrial DNA analyses. – Heredity 102: 174–181.
Kotilínek, M., Těšitelová, T., Košnar, J., Fibich, P., Hemrová, L., Koutecký, P., Münzbergová, Z. and Jersáková, J. 2020. Seed dispersal and realized gene flow of two forest orchids in a fragmented landscape. – Plant Biol. 22: 522–532.
Kullman, L. 2008. Early postglacial appearance of tree species in northern Scandinavia: review and perspective. – Quart. Sci. Rev. 27: 2467–2472.
Laikre, L. et al. 2010. Neglect of genetic diversity in implementation of the convention on biological diversity. – Conserv. Biol. 24: 86–88.
Lammi, A., Siikamäki, P. and Mustajärvi, K. 1999. Genetic diversity, population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*. – Conserv. Biol. 13: 1069–1078.
Landau, V. A., Shah, V. B., Anantharaman, R. and Hall, K. R. 2021. Omniscape.jl: software to compute omnidirectional landscape connectivity. – J. Open Source Softw. 6: 2829.
Leimu, R., Mutikainen, P., Koricheva, J. and Fischer, M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? – J. Ecol. 94: 942–952.
Lewis, E. M., Fant, J. B., Moore, M. J. and Skogen, K. A. 2023. Hawkmoth and bee pollinators impact pollen dispersal at the landscape but not local scales in two species of *Oenothera*. – Am. J. Bot. 110: e16156.
Li, L., Milesi, P., Tiret, M., Chen, J., Sendrowski, J., Baison, J., Chen, Z. Q., Zhou, L., Karlsson, B., Berlin, M., Westin, J., Garcia-Gil, M. R., Wu, H. X. and Lascoux, M. 2022. Teasing apart the joint effect of demography and natural selection in the birth of a contact zone. – New Phytol. 236: 1976–1987.
Love Stowell, S. M., Pinzone, C. A. and Martin, A. P. 2017. Overcoming barriers to active interventions for genetic diversity. – Biodivers. Conserv. 26: 1753–1765.
Luck, G. W., Daily, G. C. and Ehrlich, P. R. 2003. Population diversity and ecosystem services. – Trends Ecol. Evol. 18: 331–336.
Maes, D., Brosens, D., T'jollyn, F., Desmet, P., Piesschaert, F., Van Hoey, S., Adriaens, T., Dekoninck, W., Devos, K., Lock, K., Onkelinx, T., Packet, J., Speybroeck, J., Thomaes, A., Van Den Berge, K., Van Landuyt, W. and Verreycken, H. 2020. Validated Rred Lists of Flanders, Belgium. Ver. 1.7. – Research Institute for Nature and Forest (INBO).
McCormick, M. K. and Jacquemyn, H. 2014. What constrains the distribution of orchid populations? – New Phytol. 202: 392–400.
McRae, B. H. 2006. Isolation by resistance. – Evolution 60: 1551–1561.
Metzing, D., Hofbauer, N., Ludwig, G. and Matzke-Hajek, G. 2018. Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutschlands. Band 7: Pflanzen. – Naturschutz und Biologische Vielfalt 70, Münster (Landwirtschaftsverlag).
Moeslund, J. E. et al. 2023. Den danske rødliste. Aarhus universitet, DCE. – Nationalt Center for Miljø og Energi.
Moritz, C. 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. – Syst. Biol. 51: 238–254.
Nazareno, A. G., Bemmels, J. B., Dick, C. W. and Lohmann, L. G. 2017. Minimum sample sizes for population genomics: an empirical study from an Amazonian plant species. – Mol. Ecol. Resour. 17: 1136–1147.

Nordström, S.and Hedrén, M. 2008. Genetic differentiation and postglacial migration of the *Dactylorhiza majalis* ssp. *traunsteineri/lapponica* complex into Fennoscandia. – Plant Syst. Evol. 276: 73–87.
Nota, K., Klaminder, J., Milesi, P., Bindler, R., Nobile, A., van Steijn, T., Bertilsson, S., Svensson, B., Hirota, S. K., Matsuo, A., Gunnarsson, U., Seppä, H., Väliranta, M. M., Wohlfarth, B., Suyama, Y. and Parducci, L. 2022. Norway spruce postglacial recolonization of Fennoscandia. – Nat. Commun. 13: 1333.
Novembre, J., Williams, R., Pourreza, H., Wang, Y. and Carbonetto, P. 2019. PCAviz: visualizing principal components analysis. – R package ver. 0.3-37, http://github.com/NovembreLab/PCAviz.
Oksanen, J. et al. 2020. vegan: community ecology package (ver. 2.5-7). – https://github.com/vegandevs/vegan.
Parducci, L. et al. 2012. Glacial survival of boreal trees in northern Scandinavia. – Science 335: 1083–1086.
Peterman, W. E. 2018. ResistanceGA: an R package for the optimization of resistance surfaces using genetic algorithms. – Methods Ecol. Evol. 9: 1638–1647.
Peterson, A., Bartish, I. V. and 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.
Petit, R. J., Aguinagalde, I., de Beaulieu, J.-L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck, G., Demesure-Musch, B., Palmé, A., Martín, J. P., Rendell, S. and Vendramin, G. G. 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. – Science 300: 1563–1565.
Petkova, D., Novembre, J. and Stephens, M. 2016. Visualizing spatial population structure with estimated effective migration surfaces. – Nat. Genet. 48: 94–100.
Pfeifer, M., Schatz, B., Picó, F. X., Passalacqua, N. G., Fay, M. F., Carey, P. D. and Jeltsch, F. 2009. Phylogeography and genetic structure of the orchid *Himantoglossum hircinum* (L.) Spreng. across its European central–marginal gradient. – J. Biogeogr. 36: 2353–2365.
Phillips, R. D., Dixon, K. W. and Peakall, R. 2012. Low population genetic differentiation in the Orchidaceae: implications for the diversification of the family. – Mol. Ecol. 21: 5208–5220.
QGIS Development Team. 2022. QGIS geographic information system (ver. 2.16.2). – QGIS Association.
Ralls, K., Ballou, J. D., Dudash, M. R., Eldridge, M. D. B., Fenster, C. B., Lacy, R. C., Sunnucks, P. and Frankham, R. 2018. Call for a paradigm shift in the genetic management of fragmented populations: genetic management. – Conserv. Lett. 11: e12412.
Reynolds, A. M., Reynolds, D. R., Smith, A. D., Svensson, G. P., Löfstedt, C. 2007. Appetitive flight patterns of male *Agrotis segetum* moths over landscape scales. – J. Theor. Biol. 245: 141–149.
Román-Palacios, C. and Wiens, J. J. 2020. Recent responses to climate change reveal the drivers of species extinction and survival. – Proc. Natl Acad. Sci. USA 117: 4211–4217.
Seljestad, G. W., Quintela, M., Faust, E., Halvorsen, K. T., Besnier, F., Jansson, E., Dahle, G., Knutsen, H., André, C., Folkvord, A. and Glover, K. A. 2020. "A cleaner break": genetic divergence between geographic groups and sympatric phenotypes revealed in ballan wrasse (*Labrus bergylta*). – Ecol. Evol. 10: 6120–6135.
Skogen, K. A., Overson, R. P., Hilpman, E. T. and Fant, J. B. 2019. Hawkmoth pollination facilitates long-distance pollen dispersal and reduces isolation across a gradient of land-use change. – Ann. Miss. Bot. Gard. 104: 495–511.
Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. – Evolution 47: 264–279.
Slatkin, M. and Excoffier, L. 2012. Serial founder effects during range expansion: a spatial analog of genetic drift. – Genetics 191: 171–181.
Sletvold, N. and Ågren, J. 2010. Pollinator-mediated selection on floral display and spur length in the orchid *Gymnadenia conopsea*. – Int. J. Plant Sci. 171: 999–1009.
Sletvold, N., Øien, D.-I. and Moen, A. 2010. Long-term influence of mowing on population dynamics in the rare orchid *Dactylorhiza lapponica*: the importance of recruitment and seed production. – Biol. Conserv. 143: 747–755.
Söderquist, L., Karrenberg, S. and Sletvold, N. 2024. Genetic differentiation and diversity do not explain variation in heterosis or inbreeding depression: empirical evidence from a longlived iteroparous plant. – Conserv. Genet. 25: 1303–1312.
Söderquist, L., Dahlgren, J. P. and Sletvold, N. 2025a. Population viability of the orchid *Gymnadenia conopsea* increases with population size but is not related to genetic diversity. – J. Ecol. doi:10.1111/1365-2745.14484.
Söderquist, L., Joffard, N., Scofield, D. G., Milesi, P., Karrenberg, S. and Sletvold, N. 2025b. Data from: Genetic structure and diversity of the declining orchid Gymnadenia conopsea in Scandinavia: implications for conservation and management. – Dryad Digital Repository, https://doi.org/10.5061/dryad.5x69p8dfp.
Söderquist, L., Broberg, A., Rosenberg, V. and Sletvold, N. 2020. Predicting heterosis and inbreeding depression from population size and density to inform management efforts. – J. Appl. Ecol. 57: 1459–1468.
Ståhl, P. and Berglund, B. 2016. Gästriklands flora. – Swe. Bot. Soc..
Taberlet, P., Fumagalli, L., Wust-Saucy, A. G. and Cosson, J. F. 1998. Comparative phylogeography and postglacial colonization routes in Europe. – Mol. Ecol. 7: 453–464.
Tholstrup, D. W., Sletvold, N., Øien, D.-I., Moen, A., Colchero, F. and Dahlgren, J. P. 2024. Actuarial senescence progresses similarly across sites and species in four boreal orchids. – J. Ecol. 112: 443–445.
Thörn, F., Rödin-Mörch, P., Cortazar-Chinarro, M., Richter-Boix, A., Laurila, A. and Höglund, J. 2021. The effects of drift and selection on latitudinal genetic variation in Scandinavian common toads (*Bufo bufo*) following postglacial recolonisation. – Heredity 126: 656–667.
Tranchida-Lombardo, V., Cafasso, D., Cristaudo, A. and Cozzolino, S. 2011. Phylogeographic patterns, genetic affinities and morphological differentiation between *Epipactis helleborine* and related lineages in a Mediterranean glacial refugium. – Ann. Bot. 107: 427–436.
Turvey, S. T. and Crees, J. J. 2019. Extinction in the Anthropocene. – Curr. Biol. 29: R982–R986.
Tyler, T. 2007. Floran i Skåne: arterna och deras utbredning. – Lunds Botaniska Förening.
Vanden Broeck, A., Van Landuyt, W., Cox, K., De Bruyn, L., Gyselings, R., Oostermeijer, G., Valentin, B., Bozic, G., Dolinar, B., Illyés, Z. and Mergeay, J. 2014. High levels of effective long-distance dispersal may blur ecotypic divergence in a rare terrestrial orchid. – BMC Ecol. 14: 20.
Waples, R. K., Larson, W. A. and Waples, R. S. 2016. Estimating contemporary effective population size in non-model species

using linkage disequilibrium across thousands of loci. – Heredity 117: 233–240.
Weir, B. S. and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. – Evolution 38: 1358–1370.
Westergaard, K. B., Zemp, N., Bruederle, L. P., Stenøien, H. K., Widmer, A. and Fior, S. 2019. Population genomic evidence for plant glacial survival in Scandinavia. – Mol. Ecol. 28: 818–832.
Willi, Y., Kristensen, T. N., Sgrò, C. M., Weeks, A. R., Ørsted, M. and Hoffmann, A. A. 2022. Conservation genetics as a management tool: the five best-supported paradigms to assist the management of threatened species. – Proc. Natl Acad. Sci. USA 119: e2105076119.
Wright, S. 1943. Isolation by distance. – Genetics 28: 114–138.
Wu, J. 2004. Effects of changing scale on landscape pattern analysis: scaling relations. – Landsc. Ecol. 19: 125–138.