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RESEARCH PAPER

Assessment of genetic diversity among seed transfer zones for multiple grassland plant species across Germany

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ABSTRACT

Species diversity and intraspecific genetic diversity play a critical role in conservation and restoration of grassland ecosystems. To maintain regional adaptations of native wild plants, seeds for restoration projects are produced regionally. The delineation of regions is organised by seed transfer zones (STZs). Generalised STZs that apply uniformly to many species have been established in several European countries. Ideally, generalised STZs should be based on comprehensive data of intraspecific genetic and phenotypic diversity for a larger number of species. However, such underlying data is missing. The project RegioDiv aims to fill this gap and generate empirical data on genetic variation of multiple grassland plant species across Germany. Here we describe the driving principles and main methods of the project. A total of 33 species were collected at an average density of ~1 sample/1000 km^2 across the 22 existing STZs, and a total of 11,976 samples were genotyped with SNP markers. The analysis of genetic population structure included cluster analysis and analyses of isolation-by-distance and isolation-by-environment. An exemplary within-species analysis for *Agrostis capillaris,* a widespread grass, revealed five intraspecific genetic clusters, distributed in spatially coherent ranges that did not fully match the STZs. Most of the STZs differed genetically following a pattern of isolation-by-distance and isolation-by-environment. In an across-species analysis, genetic differentiation was affected by mating system and ploidy. Outcrossed and polyploid species were less differentiated than self-compatible and diploid species. However, genetic differentiation did not significantly differ between grasses and herbs, highlighting the variability among species within these groups. The dataset of the RegioDiv project will advance both basic and applied research on genetic variation of grassland plant species. The results will allow the assessment of the current German STZ system and guide potential improvements.

Introduction

Intraspecific genetic diversity is an essential component of biodiversity as recognised by the Convention on Biological Diversity (CBD) and plays a crucial role in conservation and restoration of ecosystems. However, genetic variation remains largely understudied and is not sufficiently implemented in conservation and restoration (Hogg, 2024). Genetic diversity drives phenotypic variation observed within and among populations, acting both as the raw material for, and the outcome of, selective processes. Therefore, local adaptation plays a critical role in restoration as locally or regionally adapted material proved to have increased reclamation success (Knapp & Rice, 1994; Mayeur et al., 2024). Comparative common-garden experiments have highlighted species-specific local adaptations to varying climate and soil conditions (Joshi et al., 2001, Bucharova et al., 2017). In addition to selection, structuring of intraspecific diversity can be influenced by intraspecific ploidy variation (Kolář et al., 2017) and phylogeographic history. Particularly in Central Europe, differentiated lineages may have immigrated post-glacially, potentially forming distinct boundaries (Taberlet et al., 1998). Conservation and restoration measures should aim at increasing resilience and adaptability of populations. Thus, a comprehensive understanding of the drivers of genetic diversity within species is necessary and must be based on a joint assessment of geographically structured genetic variation and patterns of adaptation.

In Central Europe, grasslands are among the most species-rich ecosystems and are integral to the cultural landscape, having evolved over time through natural post-glacial colonisation and human influence (Leuschner and Ellenberg, 2017). Characterised by the prevalence of grasses and a high diversity of dicotyledonous herbs, these ecosystems provide invaluable ecosystem services, including carbon sequestration (Spohn et al., 2023) and pollination service (Klaus et al., 2021). However, species-rich grasslands face threats from habitat destruction, degradation, fragmentation, eutrophication, land-use and climatic changes, leading to a significant decline in the diversity of grassland types, plant species (Wesche et al., 2012), and genetic variation (Honnay & Jacquemyn, 2007).

In degraded landscapes where only a few fragmented high-value habitats are left, restoration relies on seed input (Orrock et al., 2023). To ensure the use of locally adapted seeds in restoration projects and to protect existing genetic diversity, seed transfer zones (STZs) have emerged as a key strategy (Erickson and Halford, 2020). These zones delineate areas where seeds are collected, propagated and utilised, aiming to preserve local adaptation and enhance restoration success (e.g. Bucharova et al., 2019, McKay et al., 2005). STZs have been defined for a number of European countries (De Vitis et al., 2017). They are typically delineated on the basis of (bio)geographical ecoregions (e.g. Miller et al., 2011, Prasse et al., 2010). In contrast to species-specific STZs (e.g. Massatti, 2020), generalised STZs apply uniformly to many species. In Germany, the obligation to use native provenances for restoration in the open landscape results from §40 of the Federal Nature Conservation Act (BNatSchG, 2009; Ortner, 2005). Thus, a generalised system of 22 STZs for common grassland species defined by Prasse et al. (2010) has been implemented by law (ErMiV, 2011). The German

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regional seed system combines two basic concerns, i.e. “mix and match” (Bucharova et al., 2019), where "mix" indicates that commercially offered seeds within a STZ originate from several source populations within the STZ and thus comprise a large genetic diversity, fostering the adaptability of populations under potentially changing environmental conditions, and “match“ indicates that regionally adapted seeds are most compatible with the prevailing environmental conditions of the STZs.

Generalised STZs derived from ecoregions could be questioned as long as no comprehensive empirical knowledge on genetic diversity and population structure was used for their definition. Moreover, most species for which seeds are produced are common and widespread. Therefore, both population sizes and gene flow among populations are expected to be large, leading to genetic homogenisation and less differentiation. Additionally, grasslands in Europe are heavily affected by human management (Poschlod, 2017) extending seed dispersal by, e.g. transhumance or agricultural machinery, leading to even more homogenisation (Mix et al., 2006). Consequently, larger and fewer STZs than currently defined might be sufficient, which may also allow a more profitable seed production (Mainz & Wieden, 2019). However, patterns of genetic variation differ greatly among species (e.g. Durka et al., 2017) depending on species` life-history traits (Hamrick & Godt, 1996) and are expected to be affected by ploidy level (Soltis & Soltis, 2000) and ecological niche width (Van Valen, 1965). For example, population differentiation is much lower in outcrossing compared to selfing species (Reisch and Bernhardt-Römermann, 2014). Additionally, theory predicts that in polyploid plants genetic drift will have less impact on genetic structure compared to diploids as effective population sizes are higher and migration is more effective (Meirmans et al., 2018). Also, species with constrained ecological niches have been shown to maintain lower levels of (adaptive) genetic diversity than more widespread species that exploit a greater variety of habitats (Hamrick & Godt, 1996). Hence, applying a generalised STZ system uniformly to all species might be inadequate to represent patterns of genetic variation that differ between particular groups of species. Thus, if using generalised STZs, their delineation should be based on scientific evidence (e.g. genetic or phenotypic diversity for a larger number of species), but such evidence is mostly lacking so far. The RegioDiv project has been established to serve as a framework for gathering population genomic and ploidy data for multiple grassland plant species. Utilising a comprehensive and spatially representative nation-wide sampling, the project aims at comparing observed genetic diversity patterns across species with the existing STZ system, thus providing valuable insights for future restoration efforts.

In the present paper, we pursue three main objectives: (1) to provide an overview of the methods employed by the RegioDiv project; (2) to present exemplary data illustrating the genetic population structure for one representative species, *Agrostis capillaris* L. (Poaceae), thereby showing the feasibility of the approach; (3) to assess genetic diversity and differentiation of all species and evaluate how they are influenced by species’ life-history traits.

Materials and methods

The main steps of the project consisted of (1) designing a spatial sampling scheme, (2) selecting a list of target species, (3) formulating a sampling protocol, recruiting collectors and the actual sampling, (4) genotyping and ploidy analysis, and (5) data analysis (Fig. 1). In the following we give a short overview of the methodology. For a comprehensive description see Appendix A.

Spatial sampling scheme

The guiding principles for a spatial sampling design were, (a) comprehensiveness aiming at representative sampling across Germany, (b) hierarchical structure allowing to test for homogeneity of individual STZs, and (c) practicality by using the existing STZs as upper-most hierarchical level. The STZs (Prasse et al., 2010) were defined on the spatial reference basis of established ecoregions (Meynen & Schmithüsen, 1953-1962). To obtain manageable sampling regions, we subdivided the 22 zones into 3–4 subregions each using lower order ecoregions, resulting in a total of 72 subregions (Fig. S1).

Species selection

Guiding principles for species selection were (a) representativeness with respect to species used in restoration and (b) stakeholder involvement in the selection process. First, we compiled a list of species of all grassland seed mixtures in the 2020 catalogues of the two major producers of regional seeds in Germany, in total 204 species (Table S3). All species are widespread across large parts of Germany and abundant in grasslands. Second, species were selected encompassing (a) grasses and dicotyledonous herbs, (b) common and less common species, (c) species of mesic, moist or poor/sandy grassland, and (d) both taxonomically non-critical and critical species, such as species aggregates and mixed-ploidy species. The selection process resulted in a set of 33 plant species, including eight grasses, 23 non-legume herbs, and two legumes (Table 1).

Sampling

The guiding principles of site selection were (a) geographic representativeness of the site and (b) autochthony of the sampled population. We aimed at sampling all taxa from at least three sites in each of the 72 subregions. Sampling sites were preferentially chosen in conservation areas, old natural and semi-natural meadows, pastures, fallows, traditional orchard meadows, margins along hedges, woods or banks, whereas recently sown grassland was avoided (Fig. S2). In total, 2,706 sites were sampled by 163 volunteers. Per site and species, leaves from up to three individuals were collected at random, totaling about 19,000 samples (Table S4, Fig. S4).

Sampling site conditions were assessed at two different spatial scales. First, regional conditions were assessed using global databases for climate (www.worldclim.org) and soil (www.isric.org/explore/soilgrids). We extracted the first three axes of a principal component analysis (PCA) for downstream analyses. For climate, the three axes represented (1) mean annual precipitation and temperature, (2) temperature seasonality and (3) diurnal temperature fluctuations. For soil, the axes represented (1) soil density, (2) soil texture and (3) nutrients, cation exchange capacity, and pH. Second, site conditions at the local scale were assessed as mean Ellenberg indicator values for moisture and nutrient availability.

Ploidy analysis and SNP genotyping

Given the high number of sites sampled, in most species one sample per site was randomly chosen as a trade-off between the number of samples per site and the number of sites. Thus, the number of analysed samples ranged between 89 and 961 per species (Table 1). In species with known ploidy variation, we assessed ploidy of individual samples using flow cytometry on dried leaves (Dolezel et al., 2007).

We applied the ddRAD protocol of Peterson et al. (2012) for library preparation and the dDocent 2.9.4 bioinformatic pipeline (Puritz et al., 2014) to generate single nucleotide polymorphism (SNP) genotype data, yielding between 1465 and 11,341 SNPs (mean 5521) (Table S8). We then identified SNP-loci putatively originating from the chloroplast genome representing on average 1.8% of all SNPs. For further details, see Appendix A.

Population genomic data analysis

If not otherwise mentioned, we used R 4.3.2 (R Core Team, 2018) for data analyses. First, we cleaned each species dataset from sequencing batch effects and outgroup samples (see Appendix A). Then, we

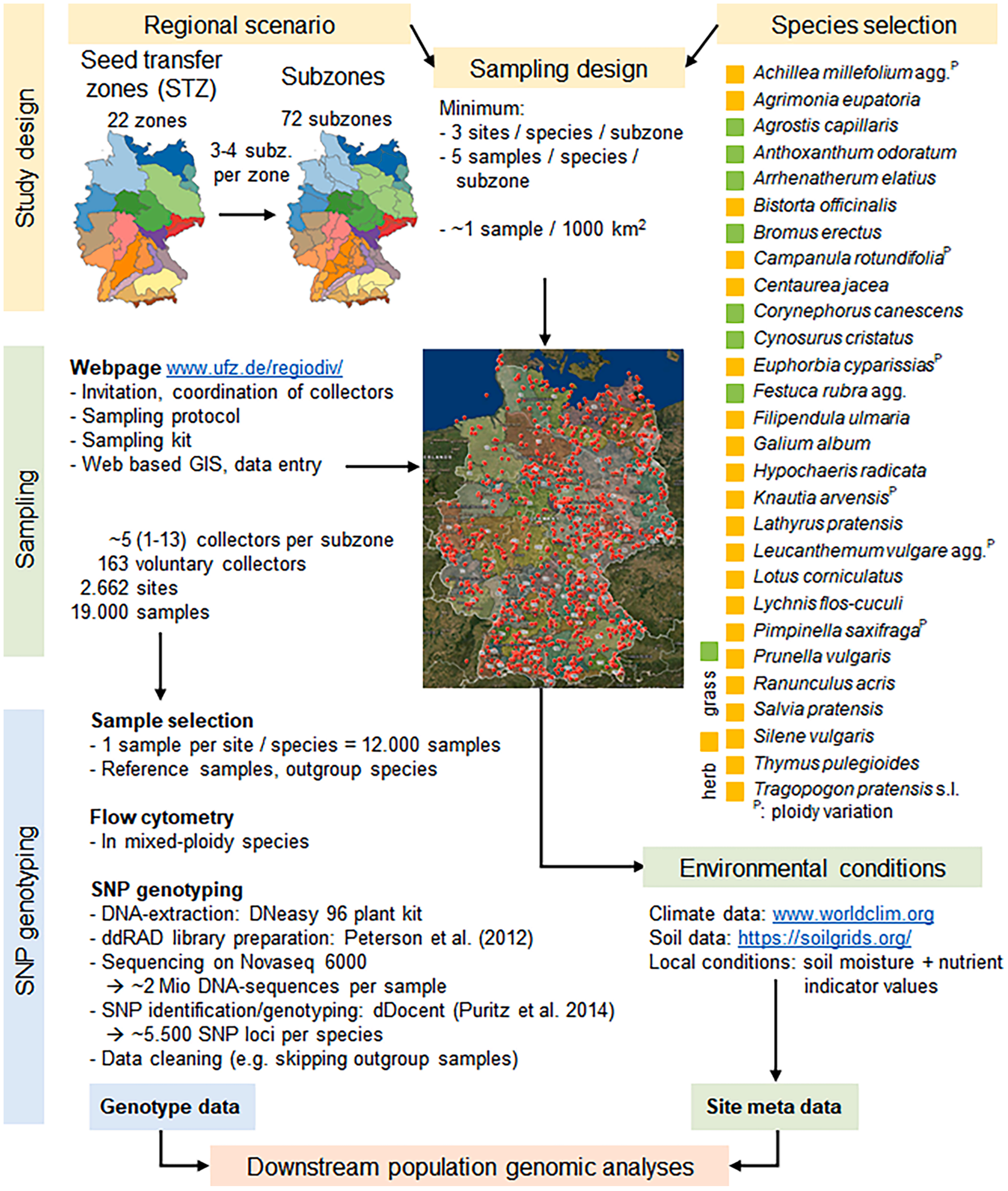

Fig. 1. Design and set up of the RegioDiv project.

visualized genetic relationships among samples with principal component analyses (PCA). We assessed population structure and identified intraspecific genetic groups with model-based Bayesian cluster analysis as implemented in ADMIXTURE (Alexander et al., 2009). To display the distribution of gene pools identified by ADMIXTURE, we spatially interpolated the individual ancestry coefficients on a map.

Considering all samples originating from a single STZ as a population, we quantified overall genetic differentiation among STZ by analysis of molecular variance (AMOVA) as Φ_{ST}. We calculated genetic differentiation (F_{ST}) between pairs of seed zones. We assessed patterns of isolation-by-distance (IBD) by correlating pairwise F_{ST}-values with geographic distance, and used Mantel tests for significance testing. Similarly, we assessed isolation-by-environment (IBE) patterns correlating F_{ST}-values with environmental distances, using the three principal components each of the climate and the soil variables and partial Mantel tests.

We quantified genetic variation (expected heterozygosity, H_e; observed heterozygosity, H_o; inbreeding coefficient F_{IS}) at the individual and seed zone level. Species level estimates were obtained by averaging across STZs.

Patterns of genomic diversity across species

The across-species dataset encompassed 33 taxa, including *Campanula rotundifolia* L. with two separate ploidyspecific datasets, and excluding *Tragopogon minor* due to small sample size (Table S10). We tested whether species-level descriptors of genetic variation vary with respect to plant type, mating system, ploidy, and ecological niche width. Species were classified (1) into grasses and dicotyledonous herbs, (2) into mating system groups exhibiting either self-incompatibility and

Table 1

Species list for RegioDiv, taxonomy following Buttler et al. (2018), chromosome numbers, number of sampling sites (N.sites) and number of samples (N.samples) in the final datasets.

Taxon	Chromosome number	N. sites	N. samples
Achillea millefolium agg.	$2n=2x\|4x\|6x\|8x=18\|36\|54\|72$	577	639
Achillea millefolium L.	$2n=6x=54$	375	378
Achillea collina/pratensis	$2n=4x=36$	167	173
Agrimonia eupatoria L.	$2n=4x=28$	343	355
Agrostis capillaris L.	$2n=4x=28$	365	368
Anthoxanthum odoratum L. s. str.	$2n=4x=20$	382	598
Arrhenatherum elatius (L.) J. Presl & C. Presl	$2n=4x=28$	497	517
Bistorta officinalis Delarbre	$2n=4x=(44,46)48$	209	222
Bromus erectus Huds.	$2n=8x=56$	201	224
Campanula rotundifolia L. s. str.	$2n=2x\|4x\|6x=34\|68\|102$	337	348
Campanula rotundifolia L. (2*x*)	$2n=2x=34$	149	153
Campanula rotundifolia L. (4*x*)	$2n=4x=68$	188	195
Centaurea jacea L. s. str.	$2n=4x=44$	492	522
Corynephorus canescens (L.) P. Beauv.	$2n=2x=14$	136	188
Cynosurus cristatus L.	$2n=2x=14$	260	270
Euphorbia cyparissias L.	$2n=2x\|4x=20\|40$	367	389
Festuca rubra agg.	$2n=4x\|6x\|8x=28\|42\|56$	327	332
Festuca nigrescens Lam.	$2n=6x=42$	152	155
Festuca rubra L.	$2n=6x\|8x=42\|56$	154	156
Filipendula ulmaria (L.) Maxim.	$2n=2x=14$	420	431
Galium album Mill.	$2n=4x=44$	518	538
Hypochaeris radicata L.	$2n=2x=8$	315	345
Knautia arvensis (L.) Coult. s. str.	$2n=2x\|4x=20\|40$	533	961
Knautia arvensis (2*x*)	$2n=2x=20$	28	56
Knautia arvensis (4*x*)	$2n=4x=40$	515	891
Lathyrus pratensis L.	$2n=2x=14$	423	428
Leucanthemum vulgare agg.	$2n=2x\|4x=18\|36$	387	507
Leucanthemum vulgare Lam.	$2n=2x=18$	95	113
Leucanthemum ircutianum DC.	$2n=4x=36$	305	394
Lotus corniculatus L.	$2n=4x=24$	460	477
Lychnis flos-cuculi L.	$2n=2x=24$	293	454
Pimpinella saxifraga L.	$2n=2x\|4x=18,20\|40$	317	347
Prunella vulgaris L.	$2n=2x=28$	288	295
Ranunculus acris L.	$2n=2x=14$	448	458
Salvia pratensis L.	$2n=2x=18$	216	220
Silene vulgaris (Moench) Garcke	$2n=2x=24$	275	297
Thymus pulegioides L.	$2n=4x=28$	306	318
Tragopogon pratensis L. s. l.	$2n=2x=12$	354	380
Tragopogon minor Mill.	$2n=2x=12$	7	16
Tragopogon orientalis L.	$2n=2x=12$	83	89
Tragopogon pratensis L. s. str.	$2n=2x=12$	229	245

obligate outcrossing (SI/outcr) or self-compatibility (SC), and (3) into diploids (2*x*) or polyploids (>2*x*).

Ecological niche width was calculated as the area of the smallest convex hull that enclosed all sampled sites for a species in a two-dimensional environmental space spanned by the scaled indicator values for moisture and nutrient availability assessed at the local scale. To describe genetic variation, we used mean pairwise F_{ST} values, overall Φ_{ST} values, and species-level estimates of H_{o}, H_{e} and F_{IS}. We are aware that in across-species comparisons, traits may not be independent of phylogenetic relationships among considered species, possibly introducing a bias if species are sampled unevenly across clades. However, practical management decisions are likely to be based on realised trait expression on species-level. Therefore, we initially performed all comparisons without considering phylogeny, using linear regression. We then repeated the analyses accounting for phylogenetically caused covariance among samples using the 'phylolm' package (Ho & Ané 2014) and a phylogeny extracted from the DaPhnE supertree (Durka & Michalski, 2012).

Results and discussion

Samples and data

With 2,706 sites, the samples covered most of the geographical and environmental space of Germany and all 22 STZs (Fig. S4, Fig. S6). Across all species, on average 358 sites were sampled, which corresponds to ~1 site/1,000 km^2. For most species, the number of sites and samples was well above 200, the minimum number suggested for analyses of landscape genomics for species with limited dispersal or even larger than 400, as suggested for random-mating populations (Selmoni et al., 2020).

Using the reduced representation sequencing approach of ddRAD-Seq, we obtained on average 5,521 biallelic SNP markers. Considering the ultimately generated number of SNP markers, the species-specific genome size (Smarda et al., 2019), and an assumed mean fragment length of 400 bp, the proportion of the genome represented in the final datasets ranged between 0.01% (*Lathyrus pratensis*) and 0.74% (*Filipendula ulmaria*) with a mean value of 0.18%. Thus, although the datasets had about 10–50 times more loci than typical AFLP marker studies (e.g. Alvarez et al., 2009), the overall genomic coverage is still relatively low. However, these numbers are typical for the ddRAD approach and allow detailed population-genomic analyses and to search for candidate adaptive loci (e.g., Diaz-Martin et al., 2023, Höfner et al., 2024).

Case study *Agrostis capillaris*

In total, 506 *Agrostis* samples were genotyped based on 2.50E+09 sequences, i.e. on average 4,944,708 sequences per sample. After exclusion of outgroups identified via reference samples of *A. stolonifera, A. vinealis,* and *A. canina*, the final dataset of *A. capillaris* comprised 368 samples from 365 sites, on average 16.7 samples per STZ, genotyped at 11,341 SNPs, including 60 chloroplast markers. SNPs were genotyped as diploid, but highly similar results were obtained for tetraploid genotyping (Fig. S10).

Population structure

In the PCA depicting genetic relationships (Fig. 2A), the distribution of the samples reflects their spatial origin, with individuals from northern Germany being separated from samples of eastern, central, and southern origin. For the maternally inherited chloroplast markers, we distinguished three groups (Fig. 2B). The smallest cpDNA group 3 (orange) was largely absent in northern Germany, whereas the other two groups occurred everywhere (Fig. 2C). This may indicate that the three cpDNA lineages were involved in colonisation of Germany, one of which did not reach northern Germany.

The ADMIXTURE analysis showed a rather steep decline of the cross entropy value until $k = 3$ (Fig. S10) indicating that the uppermost hierarchical level consists of three genetic groups distributed in the south, northwest and northeast (Fig. 3, Fig. 4). However, minimum cross entropy was reached at $k = 4$ indicating additional genetic differentiation. Furthermore, at $k = 5$ an additional gene pool emerged which was represented by many individuals with large ancestry proportions ($Q >$ 0.6–0.9) occurring in a coherent geographic area in western Germany (Figs. 3 and 4). Thus, we considered five groups to represent biologically relevant gene pools. The initial western group at $k = 2$ is hierarchically structured as from $k = 3$ to $k = 5$, the regions in the northwest, very north (Schleswig-Holstein), and west separated, respectively. Each of these five gene pools covered one to several STZs, but without obvious congruence.

Genetic differentiation between pairs of STZs was significant in 96% of all comparisons with a mean F_{ST} value of 0.012. Similarly, AMOVA quantified 1.17% of molecular variance to reside among STZs. In a hierarchical AMOVA, 0.93% of variation resided among STZs and 0.72% among subzones within STZs, indicating that STZs are not homogenous

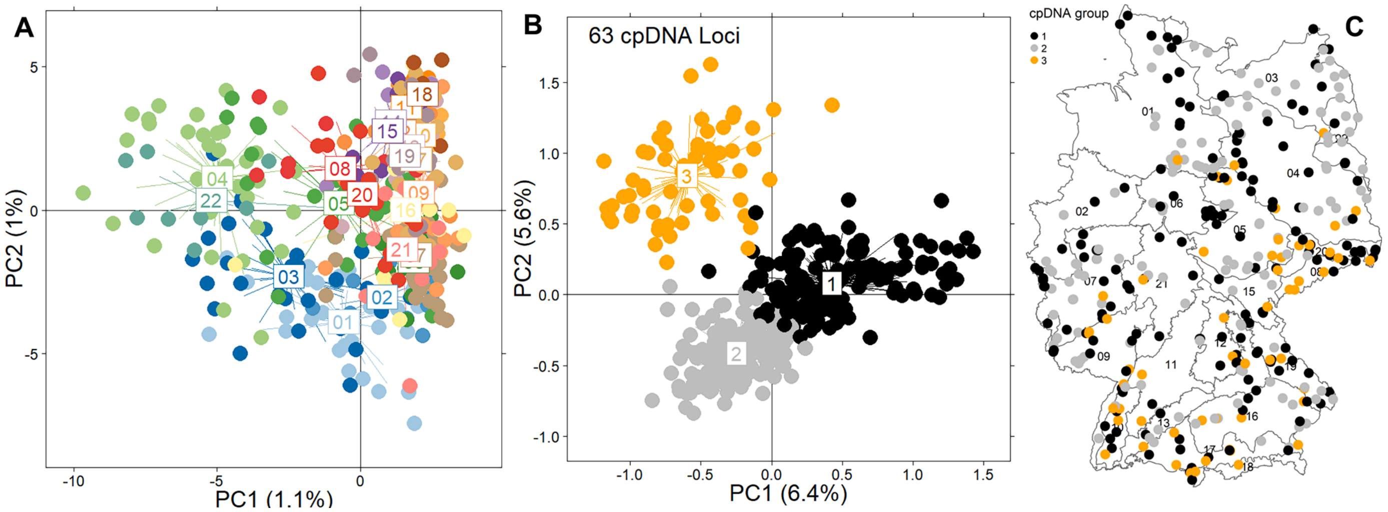

Fig. 2. Genetic structure of 368 samples of *Agrostis capillaris* originating from 22 STZs across Germany. (**A**) Principal component analysis based on all 11,341 SNPs, with numbers and colours indicating STZs (see Fig. 1, Fig. S1). (**B**) PCA based on only 63 cpDNA SNP loci grouped into $k = 3$ groups by k-means clustering, with numbers and colours represent cpDNA groups. (**C**) Map of samples and their cpDNA group.

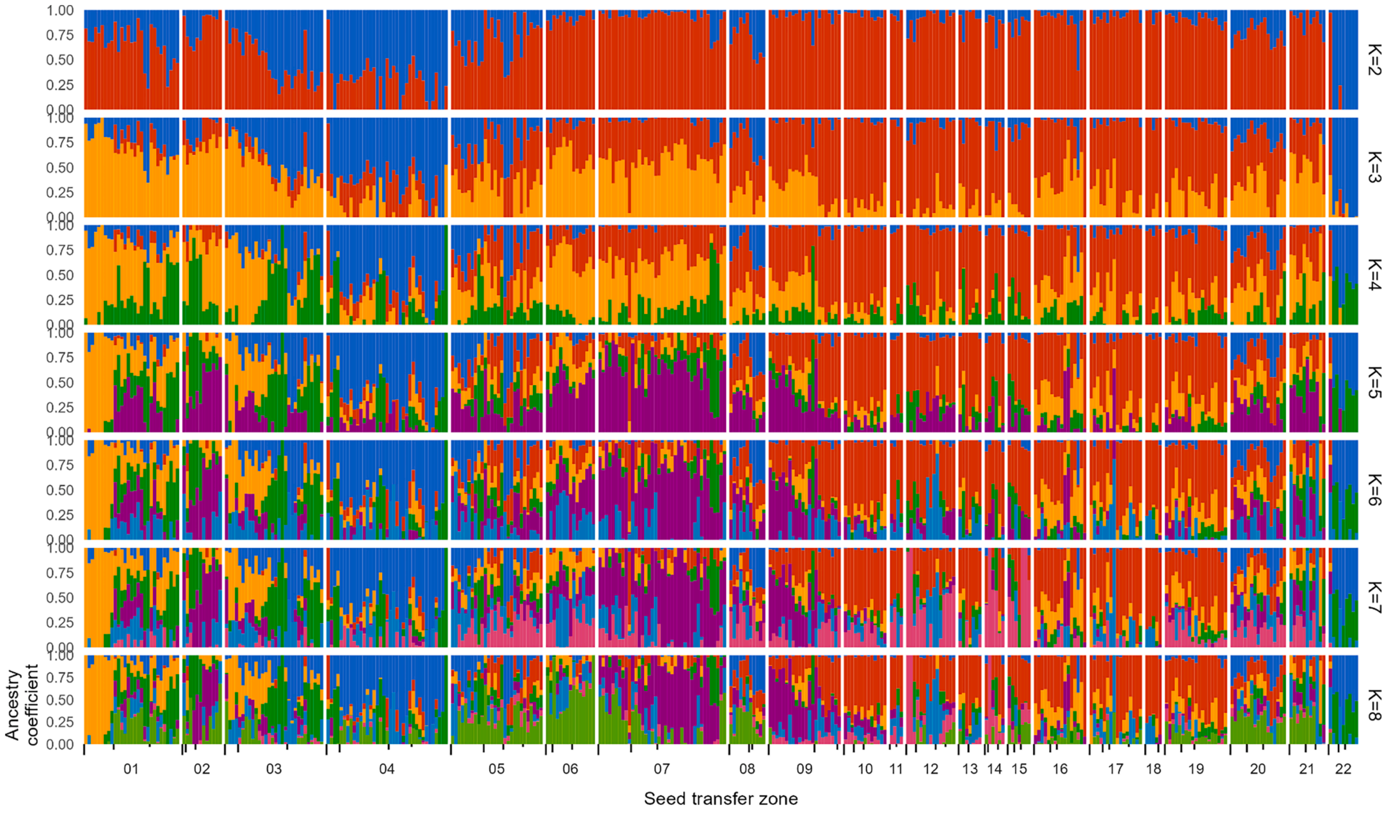

Fig. 3. Bar charts of the ADMIXTURE analysis of 368 samples of *Agrostis capillaris* with the number of gene pools ranging from $k = 2$–8. An individual plant is represented by a narrow bar with stacked colours indicating the proportional ancestry coefficients. Samples are ordered by seed transfer zones and within zones by subzones.

but that subzones again reflect substantial genetic structure. *Agrostis capillaris* showed a significant pattern of isolation-by-distance (IBD; Fig. 5), both among the STZs and within geographic regions dominated by the different ADMIXTURE groups (Fig. S11). Thus, these groups were not genetically homogenous, but again spatially structured. Isolation-by-environment (IBE) was found for the climatic factors of temperature and precipitation, and for the three soil factors representing density, sand content, and nitrogen/pH (Table S9), suggesting adaptation to environmental conditions.

Relevance for the German seed transfer zone system

So far, in comparison to the German STZ system, genetic structure has only been analysed for a single grass species *Arrhenatherum elatius*, which showed the lowest level of genetic differentiation among the species compared (Durka et al., 2017). This contrasts with our results for *A. capillaris,* a similarly common, widespread, wind-pollinated,

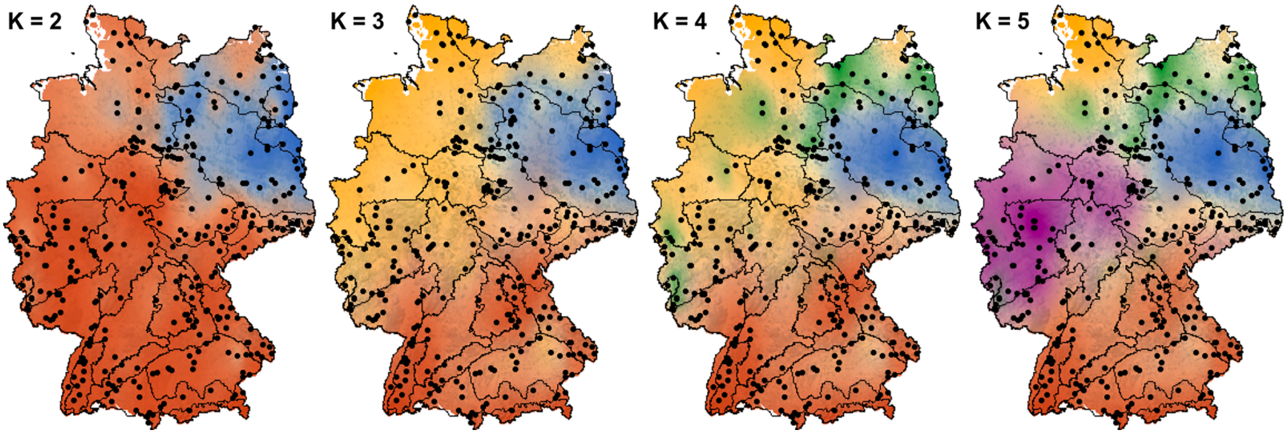

Fig. 4. Distribution of intraspecific genetic groups of *Agrostis capillaris* according to spatial interpolation of ADMIXTURE ancestry coefficients for $k = 2$–5 (colour coding follows Fig. 3).

outcrossing grass species. Here, genetic differentiation is significantly spatially and hierarchically structured and affected by environmental gradients and its strength is well within the range of other grassland species (Table S10). This finding, based on thoroughly sampling at a representative spatial scale, principally justifies the use of STZs. We show that five spatially coherent and genetically differentiated groups can be distinguished (ADMIXTURE $k = 5$). If we were to design a single-species management system for *A. capillaris*, these groups could be used to delimit a minimal number of STZs for the species. Yet, patterns of IBD within these groups suggest genetic differentiation on a smaller spatial scale. Therefore, using just five STZs – or even three STZs, considering the uppermost hierarchical level – for whole Germany would homogenise this differentiation and would disregard potential adaptation to the regional environments. Large regions like the southern German ADMIXTURE cluster should therefore be further subdivided, unless alternative, e.g., distance-based seed sourcing rules for specific target sites are implemented.

Moreover, the borders of the STZs did not match the spatial outline of ADMIXTURE clusters. On the one hand, genetic clusters extended across different STZs, on the other hand, existing zones encompassed multiple genetic clusters (Fig. 4). This suggests that the ecoregions used to define STZs do not match with intraspecific genetic patterns, at least not for *A. capillaris*. However, the German STZs are means of a generalised system valid for all common and widespread grassland species (Prasse et al., 2010). Thus, spatial genetic patterns of a single species deviating from the STZs do not disqualify the system. A multi-species analysis of intraspecific genetic groups and a discussion of the relevant spatial and genetic differentiation scales are necessary to comprehensively address the STZ system.

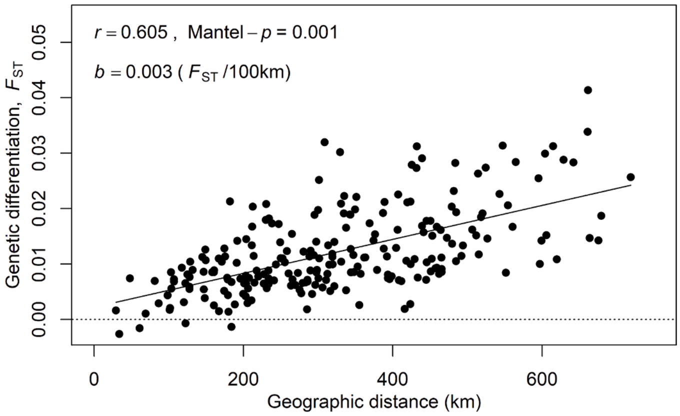

Fig. 5. Isolation-by-distance plot of genetic differentiation among pairs of STZs as a function of geographic distance for *Agrostis capillaris*. The slope is expressed as change of F_{ST} per 100 km.

Patterns of genomic diversity across species

Across species, the species-level estimates of genetic diversity ranged from $H_e = 0.22$ (*Salvia pratensis, Leucanthemum vulgare*) to 0.29 (*Cynosurus cristatus*). Species level estimates of genetic differentiation among STZs ranged between $F_{ST} = 0.003$ and 0.07 (Fig. 6), with the lowest levels detected in three grass species (*Arrhenatherum elatius, Festuca rubra, Bromus erectus*) and the highest levels in the herbs *Tragopogon pratensis, T. orientalis, Prunella vulgaris, Lychnis flos-cuculi,* and *Agrimonia eupatoria*. The F_{ST} values were in the typical range for SNP datasets of grassland plant populations (e.g. Reinula et al., 2021, Conrady et al., 2022), with grasses rather at the lower end of the range (e.g., Michalski et al., 2017) compared to herbs. Note, however, that for identical species, the absolute F_{ST} values of the current SNP data set are systematically lower than those of our previous study (Durka et al., 2017) and cannot be directly compared, because of differences in marker type, respective data analysis and spatial sampling design.

The linear regression analysis testing for an effect of plant type (grass vs. herb), mating system, ploidy and ecological niche width on genomic diversity (Table 2) showed that species-level values of genetic differentiation among STZ (Φ_{ST}) were significantly lower in self-incompatible or outcrossing species than in self-compatible species. In contrast, grasses, although showing lower mean Φ_{ST} values, did not differ significantly from herbs due to large variations in both plant types. Genetic differentiation was negatively correlated with environmental niche width, but only when phylogeny was considered, indicating that species with large environmental niches tend to be less genetically differentiated than more specialised species. Expected heterozygosity (H_e) did not differ among groups. However, when considering phylogeny, H_e was slightly lower in diploids than in polyploids and positively correlated with environmental niche width. Observed heterozygosity (H_o) was affected by mating system, plant type and ploidy. Expectedly, the inbreeding coefficients (F_{IS}) were lower in obligately outcrossing species and polyploids but surprisingly, were not significantly different between grasses and herbs. Only when considering phylogeny, F_{IS} was negatively correlated to environmental niche width suggesting that the smaller the niche, the higher the tendency for selfing.

In summary, genetic diversity and structure of our study species was most affected by mating system and ploidy. The higher differentiation in self-compatible species corroborates previous studies (e.g. Hamrick & Godt, 1996; Vekemans & Hardy, 2004). The effect of ploidy on genetic structure, although rarely studied (but see Rosche et al., 2016), confirms the expectation of higher diversity within and lower differentiation

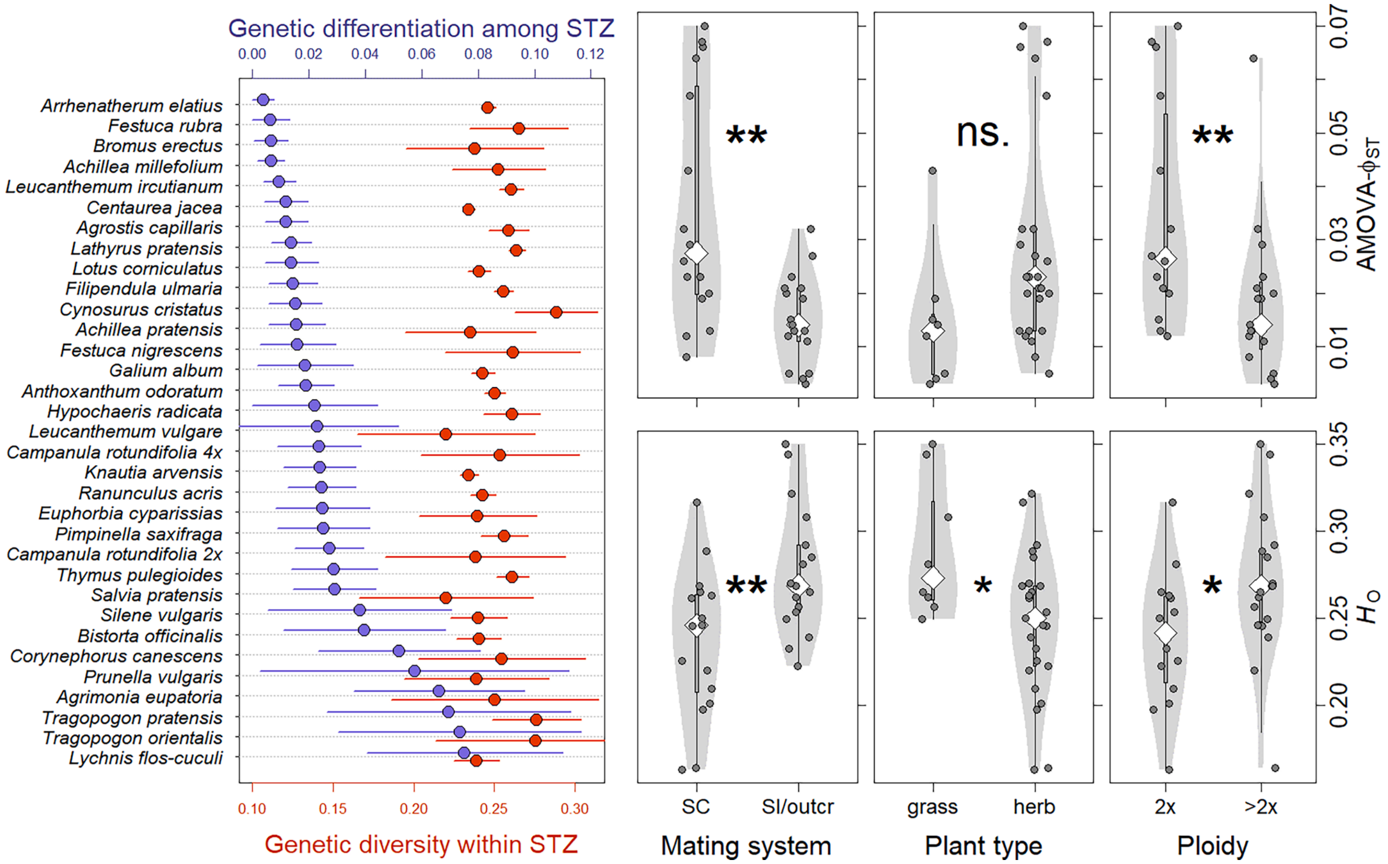

Fig. 6. **Left panel:** Species-level estimates of genetic differentiation among seed transfer zones (mean pairwise $F_{ST} \pm$ SD) and genetic diversity (mean $H_e \pm$ SD across STZs) for 33 grassland taxa; **Right panel:** Violin plots for genetic differentiation (AMOVA -Φ_{ST}) and genetic variation (observed heterozygosity (H_o)) within species, compared between mating system groups, plant type and ploidy levels. Asterisks mark significant differences between group means (see Table 2, significance levels: $^{**}p < 0.01$, $^{*}p < 0.05$).

Table 2
Relationship between life history traits and environmental niche width and genetic diversity descriptors on STZ level for 33 grassland plant species. Significant differences in means between groups and slopes (b) are indicated in bold ($p < 0.05$). Means and slopes from a phylogenetic linear regression are given in parentheses.

Life history trait		n	AMOVA-Φ_{ST} mean	H_e mean	H_o mean	F_{IS} mean
Mating system	SI/ outcr	17	**0.02** **(0.01)**	0.25 (0.26)	**0.24** (0.28)	**-0.01** (-0.03)
	SC	16	**0.04** **(0.03)**	0.25 (0.25)	**0.28** (0.29)	**0.12** (0.05)
Plant type	Grass	8	0.01 (0.01)	0.26 (0.25)	**0.29** (0.29)	-0.01 (-0.02)
	Herb	25	0.03 (0.03)	0.25 (0.24)	**0.25** (0.24)	0.07 (0.06)
Ploidy	2x	14	**0.04** **(0.02)**	0.25 **(0.24)**	**0.24** **(0.26)**	**0.13** **(0.09)**
	>2x	18	**0.01** **(0.01)**	0.25 **(0.26)**	**0.27** **(0.30)**	**-0.01** **(-0.06)**
		n	b	b	b	b
Environmental niche width		33	0.00 (**-0.003**)	0.00 **(0.004)**	0.00 (0.003)	0.00 **(-0.01)**

among populations in polyploids. This supports findings at the community level that polyploid species have lower extinction risk in fragmented landscapes (Plue et al., 2018), which, by maintaining gene flow among populations, contributes to lower genetic differentiation.

These results have some practical implications. Larger STZs would simplify seed production (Mainz & Wieden, 2019) and larger zones for particular species groups, e.g. wind pollinated grasses would be one option. However, grasses and herbs encompass both weakly and more strongly differentiated species and do not significantly differ. Thus, we did not find support for separate systems for grasses and herbs.

Perspectives

Grassland biodiversity is threatened worldwide, hence grassland restoration is a global task (United Nations, 2019) and has recently been implemented in the European nature restoration law. EU member states will need to fulfill legally binding restoration targets and put into place restoration measures on about 20% of the area by 2030 (European Parliament, 2024). Concomitantly to increasing restoration efforts, conservation of intraspecific genetic diversity is not well implemented (Hogg, 2024). The demand for seed material for restoration and other biodiversity boosting measures such as flowering strips will strongly increase. For both restoration and conservation, STZs can play an important role and empirical support is warranted, as they provide a framework for the production of genetically diverse and regionally adapted seed. The dataset collected within RegioDiv is the largest and most comprehensive assessment of intraspecific genetic diversity for a set of herbaceous grassland species across a medium-sized country so far. Our data indicates the presence of spatial genetic differentiation in almost all species, which supports the spatially segregated management of intraspecific genetic entities. The dataset represents a baseline for future basic and applied research and will inform decision making in national restoration management. Our data covers all STZs enabling us to compare genetic diversity and ploidy variation patterns across species to the current seed zone system in Germany. This will allow us to identify strengths and weaknesses of single STZ in representing - and thus being able to conserve and utilise - the genetic variation. Management of whole plant communities as done with grassland seed mixtures is a complex task facing the disparity of species-specific genetic patterns and generalised STZs. Thus seed zone design needs to integrate across multiple species, finding a reasonable compromise between over-splitting and homogenisation. Ultimately, the dataset will allow us to suggest alternative, generalised designs of STZs in Germany and recommendations for species-specific exchange rules between STZs based on genetic distance, which should further facilitate the practical implementation of legal requirements (e.g. Skowronek et al., 2023).

In times of global climate change, static STZs may be regarded as inappropriate and additional management options such as assisted gene flow and assisted migration need to be considered (Aitken & Bemmels, 2016). These concepts depend on an assessment of the risks of non-adaptedness to changing environmental conditions. The genomic resources available here can be exploited to describe current gene-environment associations (Catchen et al., 2017). These can be combined with climate change scenarios for subsequent risk assessment and the identification of suitable donor and recipient regions for seed transfer (e.g. Lachmuth et al., 2024). A functioning seed production system of wild plants, as established in Germany, will facilitate any such future management adaptations. Our dataset of more than 30 plant species sampled at dense spatial resolution, provides a unique opportunity for the assessment and potential improvement of the German STZ system.

Data availability

For *Agrostis capillaris*, demultiplexed individual raw sequence data are available under sample accession numbers ERS17591121-ERS17591682 at https://www.ebi.ac.uk/ena and SNP data in genlight format including sample metadata are available at https://doi.org/10.5281/zenodo.10977130.

Code availability

Shell scripts for running dDocent and SNP filtering are available at https://doi.org/10.5281/zenodo.10977130.

https://zenodo.org/uploads/10977130?token=eyJhbGciOiJIUzUxMiIsImlhdCI6MTcxODk2NTY5OCwiZXhwIjoxNzM1NjAzMTk5fQ

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CRediT authorship contribution statement

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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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.baae.2024.11.004.

References

Aitken, S. N., & Bemmels, J. B. (2016). Time to get moving: assisted gene flow of forest trees. *Evol. Appl., 9*, 271–290. https://doi.org/10.1111/eva.12293

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Res, 19*, 1655–1664. https://doi.org/10.1101/gr.094052.109

BNatschG. (2009). Gesetz über Naturschutz und Landschaftspflege (Bundesnaturschutzgesetz - BNatSchG). Zuletzt geändert durch Art. 421 V v. 31.8.2015 I 1474. *Bundesgesetzblatt, Teil I Nr, 51*, 2542–2579.

Bucharova, A., Bossdorf, O., Hölzel, N., Kollmann, J., Prasse, R., & Durka, W. (2019). Mix and match! Regional admixture provenancing strikes a balance among different seed-sourcing strategies for ecological restoration. *Conservation Genetics, 20*, 7–17. https://doi.org/10.1007/s10592-018-1067-6

Bucharova, A., Michalski, S. G., Hermann, J. M., Heveling, K., Durka, W., Hölzel, N., Kollmann, J., & Bossdorf, O. (2017). Genetic differentiation and regional adaptation among seed origins used for grassland restoration: Lessons from a multi-species transplant experiment. *Journal of Applied Ecology, 54*, 127–136. https://doi.org/10.1111/1365-2664.12645

Buttler, K. P., May, R., & Metzing, D. (2018). Liste der Gefäßpflanzen Deutschlands - Florensynopse und Synonyme. *BfN Skripten, 519*. https://doi.org/10.19217/skr519

Catchen, J. M., Hohenlohe, P. A., Bernatchez, L., Funk, W. C., Andrews, K. R., & Allendorf, F. W. (2017). Unbroken: RADseq remains a powerful tool for understanding the genetics of adaptation in natural populations. *Molecular Ecology Resources, 17*, 362–365. https://doi.org/10.1111/1755-0998.12669

Conrady, M., Lampei, C., Bossdorf, O., Durka, W., & Bucharova, A. (2022). Evolution during seed production for ecological restoration? A molecular analysis of 19 species finds only minor genomic changes. *Journal of Applied Ecology, 59*, 1383–1393. https://doi.org/10.1111/1365-2664.14155

De Vitis, M., Abbandonato, H., Dixon, W. K., Laverack, G., Bonomi, C., & Pedrini, S. (2017). The European native seed industry: Characterization and perspectives in grassland restoration. *Sustainability, 9*, 14. https://doi.org/10.3390/su9101682

Diaz-Martin, Z., De Vitis, M., Havens, K., Kramer, A. T., MacKechnie, L. M., & Fant, J. (2023). Species-specific effects of production practices on genetic diversity in plant reintroduction programs. *Evolutionary Applications, 16*, 1956–1968. https://doi.org/10.1111/eva.13614

Doležel, J., Greilhuber, J., & Suda, J. (2007). Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols, 2*, 2233–2244. https://doi.org/10.1038/nprot.2007.310

Durka, W., & Michalski, S. G. (2012). DaPhnE: A dated phylogeny of a large European flora for phylogenetically informed ecological analyses. *Ecology, 93*. https://doi.org/10.1890/12-0743.1, 2297-2297.

Durka, W., Michalski, S. G., Berendzen, K. W., Bossdorf, O., Bucharova, A., Hermann, J. M., Hölzel, N., & Kollmann, J. (2017). Genetic differentiation within multiple common grassland plants supports seed transfer zones for ecological restoration. *Journal of Applied Ecology, 54*, 116–126. https://doi.org/10.1111/1365-2664.12636

Erickson, V. J., & Halford, A. (2020). Seed planning, sourcing, and procurement. *Restoration Ecology, 28*, S219–S227. https://doi.org/10.1111/rec.13199

ErMiV. (2011). Verordnung über das Inverkehrbringen von Saatgut von Erhaltungsmischungen (Erhaltungsmischungsverordnung), zuletzt geändert durch Artikel 6 der Verordnung vom 28. September 2021 (BGBl. I S. 4595). *Bundesgesetzblatt, Teil I, Nr, 65*, 2641.

European Parliament. (2024). European Parliament legislative resolution of 27 February 2024 on the proposal for a regulation of the European Parliament and of the Council on nature restoration, P9_TA(2024)0089.

Hamrick, J. L., & Godt, M. J. (1996). Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society London B, 351*, 1291–1298.

Ho, L. S. T., & Ané, C. (2014). A linear-time algorithm for Gaussian and non-Gaussian trait evolution models. *Systematic Biology, 63*, 397–408. https://doi.org/10.1093/sysbio/syu005

Höfner, J., Bucharova, A., Durka, W. & Michalski, S.G. (2024). Spatial patterns of genomic variation and temporal genomic offset in a common grassland plant and their relation to seed transfer zones. *Authorea.* August 13, 2024. DOI: 10.22541/au.172114963.35436738/v2.

Hogg, C. J. (2024). Translating genomic advances into biodiversity conservation. *Nature Reviews Genetics, 25*, 362–373. https://doi.org/10.1038/s41576-023-00671-0

Honnay, O., & Jacquemyn, H. (2007). Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology, 21*, 823–831.

Joshi, J., Schmid, B., Caldeira, M. C., Dimitrakopoulos, P. G., Good, J., Harris, R., Hector, A., Huss-Danell, K., Jumpponen, A., Minns, A., Mulder, C. P. H., Pereira, J. S., Prinz, A., Scherer-Lorenzen, M., Siamantziouras, A. S. D., Terry, A. C., Troumbis, A. Y., & Lawton, J. H. (2001). Local adaptation enhances performance of common plant species. *Ecology Letters, 4*, 536–544. https://doi.org/10.1046/j.1461-0248.2001.00262.x

Klaus, F., Tscharntke, T., Uhler, J., & Grass, I. (2021). Calcareous grassland fragments as sources of bee pollinators for the surrounding agricultural landscape. *Global Ecology and Conservation, 26*, e01474. https://doi.org/10.1016/j.gecco.2021.e01474

Knapp, E. E., & Rice, K. J. (1994). Starting from seed: Genetic issues in using native grasses for restoration. *Restoration & Management Notes, 12*, 40–45.

Kolář, F., Čertner, M., Suda, J., Schönswetter, P., & Husband, B. C. (2017). Mixed-ploidy species: Progress and opportunities in polyploid research. *Trends in Plant Science, 22*, 1041–1055. https://doi.org/10.1016/j.tplants.2017.09.011

Lachmuth, S., Capblancq, T., Prakash, A., Keller, S. R., & Fitzpatrick, M. C. (2024). Novel genomic offset metrics integrate local adaptation into habitat suitability forecasts and inform assisted migration. *Ecological Monographs, 94*, e1593. https://doi.org/10.1002/ecm.1593

Leuschner, C., & Ellenberg, H. (2017). Ecology of Central European non-forest vegetation: Coastal to alpine. *Natural to man-made habitats: Vegetation ecology of Central Europe*. Volume II.

Mainz, A. K., & Wieden, M. (2019). Ten years of native seed certification in Germany – A summary. *Plant Biology, 21*, 383–388. https://doi.org/10.1111/plb.12866

Massatti, R. (2020). *Genetically-informed seed transfer zones for Cleome lutea and Machaeranthera canescens across the Colorado Plateau and adjacent regions*. Cooperator Report for the Bureau of Land Management's Colorado Plateau Native Plant Program.

Mayeur, A., Carpentier, F., Soki-Makilutila, A., & Colas, B. (2024). Assessing seed mixtures for roadside revegetation – richness and origin as predictors for erosion control. *Journal of Ecological Engineering, 25*, 8–24. https://doi.org/10.12911/22998993/187147

McKay, J. K., Christian, C. E., Harrison, S., & Rice, K. J. (2005). How local is local?" - A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology, 13*, 432–440. https://doi.org/10.1111/j.1526-100X.2005.00058.x

Meirmans, P. G., Liu, S., & van Tienderen, P. H. (2018). The analysis of polyploid genetic data. *Journal of Heredity, 109*, 283–296. https://doi.org/10.1093/jhered/esy006

Meynen, E., & Schmithüsen, J. (1953–1962). *Handbuch der naturräumlichen Gliederung Deutschlands*. Selbstverlag der Bundesanstalt für Landeskunde.

Michalski, S. G., Malyshev, A. V., & Kreyling, J. (2017). Trait variation in response to varying winter temperatures, diversity patterns and signatures of selection along the latitudinal distribution of the widespread grassland plant *Arrhenatherum elatius*. *Ecology and Evolution, 7*, 3268–3280. https://doi.org/10.1002/ece3.2936

Miller, S. A., Bartow, A., Gisler, M., Ward, K., Young, A. S., & Kaye, T. N. (2011). Can an ecoregion serve as a seed transfer zone? Evidence from a common garden study with five native species. *Restoration Ecology, 19*, 268–276. https://doi.org/10.1111/j.1526-100X.2010.00702.x

Mix, C., Arens, P. F. P., Rengelink, R., Smulders, M. J. M., van Groenendael, J. M., & Ouborg, N. J. (2006). Regional gene flow and population structure of the wind-dispersed plant species *Hypochaeris radicata* (Asteraceae) in an agricultural landscape. *Molecular Ecology, 15*, 1749–1758. https://doi.org/10.1111/j.1365-294X.2006.02887.x

Orrock, J. L., Brudvig, L. A., Damschen, E. I., Mattingly, W. B., Cruz, J., Veldman, J. W., Hahn, P. G., & Larsen-Gray, A. L. (2023). Long-term, large-scale experiment reveals the effects of seed limitation, climate, and anthropogenic disturbance on restoration of plant communities in a biodiversity hotspot. *Proceedings of the National Academy of Sciences, 120*, Article e2201943119. https://doi.org/10.1073/pnas.2201943119

Ortner, D. (2005). Zur naturschutzrechtlichen Verpflichtung der Verwendung autochthonen Saat- und Pflanzguts bei der Straßenbegleitbegrünung. *Natur und Recht, 27*, 91–99. https://doi.org/10.1007/s10357-004-0534-7

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for *De Novo* SNP discovery and genotyping in model and non-model species. *Plos ONE, 7*, e37135. https://doi.org/10.1371/journal.pone.0037135

Plue, J., Kimberley, A., & Slotte, T. (2018). Interspecific variation in ploidy as a key plant trait outlining local extinction risks and community patterns in fragmented landscapes. *Functional Ecology, 32*, 2095–2106. https://doi.org/10.1111/1365-2435.13127

Poschlod, P. (2017). *Geschichte der Kulturlandschaft*. Ulmer.

Prasse, R., Kunzmann, D., & Schröder, R. (2010). *Entwicklung und praktische Umsetzung naturschutzfachlicher Mindestanforderungen an einen Herkunftsnachweis für gebietseigenes Wildpflanzensaatgut krautiger Pflanzen. Abschlussbericht zum Forschungsprojekt (DBU FKZ: 23931*Hannover.

Puritz, J. B., Hollenbeck, C. M., & Gold, J. R. (2014). dDocent: A RADseq, variant-calling pipeline designed for population genomics of non-model organisms. *PeerJ, 2*, e431. https://doi.org/10.7717/peerj.431

R Core Team. (2018). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing. https://www.R-project.org/.

Reinula, I., Träger, S., Hernández-Agramonte, I. M., Helm, A., & Aavik, T. (2021). Landscape genetic analysis suggests stronger effects of past than current landscape structure on genetic patterns of *Primula veris*. *Diversity and Distributions, 27*, 1648–1662. https://doi.org/10.1111/ddi.13357

Reisch, C., & Bernhardt-Römermann, M. (2014). The impact of study design and life history traits on genetic variation of plants determined with AFLPs. *Plant Ecology, 215*, 1493–1511. https://doi.org/10.1007/s11258-014-0409-9

Rosche, C., Durka, W., Hensen, I., Mráz, P., Hartmann, M., Müller-Schärer, H., & Lachmuth, S. (2016). The population genetics of the fundamental cytotype-shift in invasive *Centaurea stoebe* s.l.: Genetic diversity, genetic differentiation and small-scale genetic structure differ between cytotypes but not between ranges. *Biological Invasions, 18*, 1895–1910. https://doi.org/10.1007/s10530-016-1133-2

Selmoni, O., Vajana, E., Guillaume, A., Rochat, E., & Joost, S. (2020). Sampling strategy optimization to increase statistical power in landscape genomics: A simulation-based approach. *Molecular Ecology Resources, 20*, 154–169. https://doi.org/10.1111/1755-0998.13095

Skowronek, S., Eberts, C., Blanke, P., & Metzing, D. (2023). Leitfaden zur Verwendung von gebietseigenem Saat- und Pflanzgut krautiger Arten in der freien Natur Deutschlands. Hinweise zur Umsetzung des § 40 Abs. 1 BNatSchG. *BfN Skripten, 647*, 1–98. https://doi.org/10.19217/skr647

Smarda, P., Knapek, O., Brezinova, A., Horova, L., Grulich, V., Danihelka, J., … Bures, P. (2019). Genome sizes and genomic guanine plus cytosine (GC) contents of the Czech vascular flora with new estimates for 1700 species. *Preslia, 91*, 117–142. https://doi.org/10.23855/preslia.2019.117

Soltis, P. S., & Soltis, D. E. (2000). The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 7051–7057.

Spohn, M., Bagchi, S., Biederman, L. A., Borer, E. T., Bråthen, K. A., Bugalho, M. N., Caldeira, M. C., Catford, J. A., Collins, S. L., Eisenhauer, N., Hagenah, N., Haider, S., Hautier, Y., Knops, J. M. H., Koerner, S. E., Laanisto, L., Lekberg, Y., Martina, J. P., Martinson, H., McCulley, R. L., Peri, P. L., Macek, P., Power, S. A., Risch, A. C., Roscher, C., Seabloom, E. W., Stevens, C., Veen, G. F., Virtanen, R., & Yahdjian, L. (2023). The positive effect of plant diversity on soil carbon depends on climate. *Nature Communications, 14*, 6624. https://doi.org/10.1038/s41467-023-42340-0

Taberlet, P., Fumagalli, L., Wust-Saucy, A. G., & Cosson, J. F. (1998). Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology, 7*, 453–464. https://doi.org/10.1046/j.1365-294x.1998.00289.x

United Nations. (2019). *Resolution adopted by the General Assembly on 1 March 2019 - 73/284*. United Nations Decade on Ecosystem Restoration (2021–2030).

Van Valen, L. (1965). Morphological variation and width of ecological niche. *The American Naturalist, 99*, 377–390.

Vekemans, X., & Hardy, O. J. (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology, 13*, 921–935. https://doi.org/10.1046/j.1365-294X.2004.02076.x

Wesche, K., Krause, B., Culmsee, H., & Leuschner, C. (2012). Fifty years of change in Central European grassland vegetation: Large losses in species richness and animal-pollinated plants. *Biological Conservation, 150*, 76–85.