







RESEARCH ARTICLE

UN Decade on Ecosystem Restoration

Populations restored using regional seed are genetically diverse and similar to natural populations in the region

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Abstract

1. Ecological restoration and plant re-introductions aim to create plant populations that are genetically similar to natural populations to preserve the regional gene pool, yet genetically diverse to allow adaptation to a changing environment. For this purpose, seeds for restoration are increasingly sourced from multiple populations in the target region. However, it has only rarely been tested whether using regional seed indeed leads to genetically diverse restored populations which are genetically similar to natural populations.
2. We used single nucleotide polymorphism (SNP) markers to investigate genetic diversity within and differentiation among populations of *Centaurea jacea* and *Betonica officinalis* on restored and natural meadows in the White Carpathians, Czech Republic. The restoration took place 20 years ago using regional seeds propagated from a mix of multiple regional source populations. We included original regional seeds in our analysis to compare the restored populations with their origin (only in *C. jacea*). Additionally, we analysed conventional seeds without certified origin because these would have constituted a common alternative for restoration seeding in the absence of regional seeds.
3. The differentiation between restored and natural populations (mean pairwise $F_{ST} = 0.018$ in *Centaurea* and 0.021 in *Betonica*) was similar to the differentiation among natural populations ($F_{ST} = 0.023$ and 0.021), and the restored populations were slightly more genetically diverse than the natural populations. In addition, restored populations were relatively similar to their origin, the regional seeds ($F_{ST} = 0.015$). In contrast, conventional seeds were strongly differentiated from all regional populations ($F_{ST} = 0.100$ and 0.059, in *Centaurea* and *Betonica* respectively) and harboured substantially lower genetic diversity. We also found signs of gene flow via pollen or seed dispersal from natural to restored populations but not *vice versa*.

Anna Bucharova and Walter Durka shared last authorship.

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4. *Policy implications.* Regionally sourced seeds can produce genetically diverse populations at natural levels of genetic differentiation.

KEYWORDS

ecosystem restoration, gene flow, genetic population differentiation, grassland, local adaptation, plant reintroduction, seed sourcing, seed transfer zone

1 | INTRODUCTION

Habitat destruction is among the most important factors threatening biodiversity, which in turn compromises ecosystem health and ecosystem services (Díaz et al., 2019). With about 30% of global land degraded (Nkonya et al., 2016), ecological restoration is increasingly important to ensure the persistence of ecosystem services necessary for human well-being (Tolvanen & Aronson, 2016). In terrestrial ecosystems, restoration often requires the active introduction of target plant species (Hölzel et al., 2012) via seeds or other vectors such as fresh hay or seedlings.

The availability of suitable seeds for restoration is a common problem. In many cases, the only available seeds of native species are from conventional providers. Such seeds have no certified origin, lack production standards, and are sometimes produced in remote countries or even in another continent to reduce costs (Mainz et al., 2018). While this type of seed may be convenient in some cases, the lack of documentation makes it impossible to assess the adaptation of the particular genotypes to the specific restoration conditions. Best practice in ecological restoration thus requires using seeds of known geographic origin (Pedrini & Dixon, 2020).

Seeds are often sourced directly from local populations, aiming to maintain the integrity of the local gene pool and potential local adaptation (but see e. g. Lesica & Allendorf, 1999). In contrast, using seeds from non-local populations may introduce genotypes that are not part of the natural genetic composition of populations in that area (Gemeinholzer et al., 2020) and might even be maladapted (Lesica & Allendorf, 1999). However, local populations are often not available in degraded landscapes, and if they are, they may be small and genetically impoverished due to inbreeding and genetic drift (Breed et al., 2018). Additionally, direct harvest in the wild may not be sustainable, especially when large amounts of seeds are needed (Meissen et al., 2015; Merritt & Dixon, 2011). In these cases, it is common to source seeds of native species from large populations in the wider region, often followed by agricultural propagation in seed orchards (Bucharova et al., 2019; Ladouceur et al., 2018).

Both sourcing seed from a wider region and agricultural propagation may introduce genetic differences between restored and neighbouring natural populations. Natural plant populations are commonly genetically differentiated at the landscape or regional scale (Giles & Goudet, 1997). Consequently, sourcing seeds from large populations in the region does not guarantee close genetic similarity between restored populations and natural populations at a particular locality (Kaulfuß & Reisch, 2019). Agricultural propagation implies new challenges: Agricultural practice may reduce genetic diversity (Espeland

et al., 2017), which would negatively affect survival and adaptability of restored populations (Schäfer et al., 2020; Sgrò et al., 2011). Moreover, environmental conditions in agricultural propagation differ from conditions in the wild, which may lead to the selection of particular genotypes (Espeland et al., 2017). It therefore remains unclear whether regionally sourced, agriculturally propagated seeds sufficiently represent the gene pool of local natural populations.

The degree of differences between natural and restored populations varies, depending on the seed source used for restoration. Transfer of material over short distances (e.g. hay transfer) usually leads to higher similarity between source and restored sites (Dittberner et al., 2019). On the other hand, both regional seed sources with a known provenance in the region and conventional seed mixtures of unknown provenance have resulted in restored populations that genetically differed from surrounding natural stands (Gemeinholzer et al., 2020; Kaulfuß & Reisch, 2019). Yet, genetic differentiation among populations is ubiquitous in plants (Gamba & Muchhala, 2020), and a certain level of genetic differentiation is expected among the majority of plant populations. It is the magnitude of differentiation that matters. Thus, any genetic differentiation should be evaluated relative to the genetic distance between adjacent and distant populations or, in the case of restoration, relative to other seed sources potentially available for a given restoration project.

In this study, we focused on a restoration project in the White Carpathian Mts., Czech Republic (Jongepierová, 2008), where we addressed genetic differentiation between restored plant populations and older, natural populations on extensively managed meadows. Although meadows and pastures are human-mediated habitats dependent on mowing and grazing, they are among the most species-rich ecosystems in Central Europe and carry an inherent, historically grown conservation value (Halada et al., 2011; Johnson, 2009; Veen et al., 2014). Therefore, we refer to these older populations as 'natural'. The restored meadows under study here were established between 2000 and 2003 using seeds produced within a non-governmental project on regional seed production (Jongepierová, 2008). Restored and natural meadows are spatially close, which possibly allows gene flow between the two types of habitats. We included plant samples grown from the original seeds produced specifically for this restoration ('regional seeds') to compare contemporary restored populations to their origin. To compare regional seeds to their practical alternative, that is, conventional seeds, we also included seeds bought from a conventional seed producer who provides seeds without certified provenance for restoration in the Czech Republic.

We asked (a) How much do restored populations differ genetically from natural populations? (b) How genetically differentiated are the regional seeds from natural and restored populations? (c) How much do conventional seeds with unknown provenance differ from all regional populations? (d) What is the within-population level of genetic variation in restored populations, natural populations, regional seeds and conventional commercial seeds?

2 | MATERIALS AND METHODS

2.1 | Study design

The study was carried out in the White Carpathian Mts., Czech Republic. The area is a biodiversity hotspot known for species-rich hay meadows. In the second half of the 20th century, numerous meadows were converted to agricultural fields. However, since the 1990s, many fields have been restored to meadows because of low crop productivity. In this study, we focused on sites that had been restored between 2000 and 2003 using a regional seed mixture (Jongepierová, 2008). This mixture was produced from several natural populations in the region, mainly around Suchov (sites SR and SN in Figure 1), complemented by other natural meadows up to 20 km northeast from Suchov. The exact sampling locations are unknown. These regional seeds were agriculturally propagated specifically for this restoration project (Jongepierová, 2008) and harvested in two consecutive years between 1999 and 2002 from the same propagation field. As the species are perennial, we consider all seeds as offspring of the same maternal generation.

We focused on two species that are common at both natural and restored meadows, *Centaurea jacea* L. and *Betonica officinalis* L. (syn. *Stachys officinalis* (L.) Trevis.), hereafter referred to by their genus name. In May 2019, we collected samples at 10 meadows located at five localities (Kněždub, Miladka, Pechové, Suchov and Vojšické louky), each locality including one natural (N) and one restored meadow (R) (Figure 1; Table 1). Within each locality, the meadows were selected as proximal as possible, which resulted in the direct adjacency of natural and restored meadows for the localities Kněždub, Miladka and Vojšické louky, a distance of around 230 m at

Suchov and of 970 m at Pechové. Hedges separated meadows within one locality. We sampled 13 plants per species along a transect at each meadow, leaving 5 m between samples to avoid collecting close relatives. Each of the species was absent in one meadow (Table 1). We carried out the field work with oral consent of the local nature protection authority (I. Jongepierová, Nature Conservation Agency of the Czech Republic). No official permits were needed.

In addition, we obtained a sample of seeds originally used to restore the restored meadows (hereafter referred to as 'regional seeds'), which the restoration project manager had stored. These seeds were two decades old, and only the seeds of *Centaurea* germinated. Furthermore, we bought conventional seeds from a supplier based in the North of the Czech Republic. This seed material is commonly used for native species sowing in the Czech Republic and represents a conventional alternative to the regionally grown seed mixtures. The exact provenance of these conventional seeds is unknown. We germinated both types of seeds in soil-filled germination trays in the greenhouse and randomly harvested 13 young plants per group. In the following, we refer to the samples originating from natural and restored sites as well as those from regional and conventional seeds as 'populations'.

2.2 | Population genomic analysis

To assess genome-wide molecular genetic variation within populations and genetic differentiation among populations, we used a reduced representation sequencing method to sequence parts of the genome of the plant samples and to extract single nucleotide polymorphism (SNP) markers. In short, we used the double digest restriction-site associated DNA sequencing (ddRAD) protocol by Peterson et al. (2012) with minor modifications to generate libraries representing parts of the genomes, which were sequenced and from which co-dominant, biallelic, SNP markers were derived (see Supporting Information for details). SNP markers are considered superior to other marker types because they are distributed across the whole genome and are co-dominant (Morin et al., 2004). The final datasets consisted of 119 individuals genotyped at 6,855 SNPs with 3.22% missing data in *Centaurea* and 117 individuals genotyped at 6,632 SNPs with 2.63% missing data in *Betonica*.

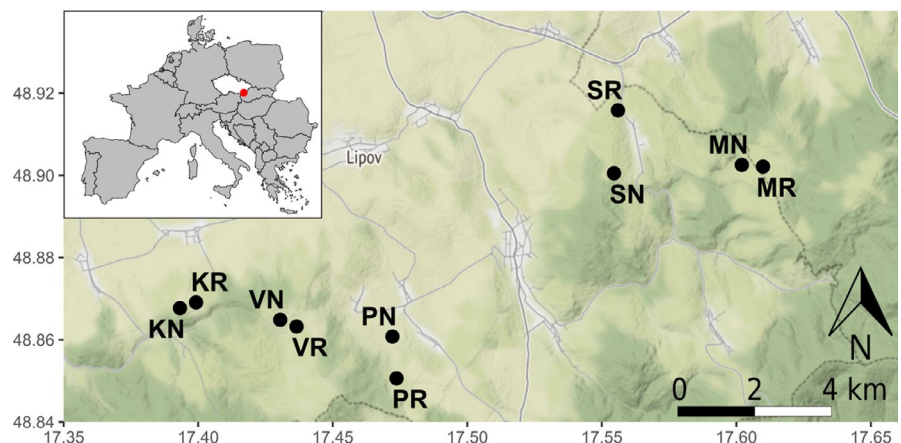


FIGURE 1 Location of the study sites in the White Carpathians. The first letter of the labels indicates locality (K, Kněždub; M, Miladka; P, Pechové; S, Suchov, V, Vojšické louky), the second the populations type (N, natural; R, restored)

TABLE 1 Population level statistics of genetic variation. #ind, number of individuals in the filtered SNP dataset; AR, allelic richness (with sample size); F_{IS} , inbreeding coefficient; H_e , observed heterozygosity, H_o , observed heterozygosity. Averages per population type and coefficients of variation (CV) are for each statistic

Pop.	Locality	Population type	<i>Centaurea jacea</i>					<i>Betonica officinalis</i>				
			#ind	AR ₄	H_e	H_o	F_{IS}	#ind	AR ₁₀	H_e	H_o	F_{IS}
KN	Kněždub	Natural	12	1.472	0.263	0.325	-0.157					
KR		Restored	12	1.516	0.287	0.357	-0.165	13	1.788	0.423	0.324	-0.219
MN	Miladka	Natural	5	1.486	0.277	0.340	-0.186	11	1.765	0.394	0.315	-0.179
MR		Restored	12	1.515	0.287	0.354	-0.162	10	1.787	0.407	0.324	-0.181
PN	Pechové	Natural	9	1.486	0.273	0.332	-0.156	12	1.734	0.416	0.308	-0.263
PR		Restored	11	1.507	0.283	0.343	-0.146	12	1.788	0.429	0.325	-0.230
SN	Suchov	Natural	12	1.503	0.280	0.348	-0.168	12	1.790	0.410	0.323	-0.190
SR		Restored						12	1.779	0.401	0.319	-0.182
VN	Vojšické louky	Natural	11	1.497	0.278	0.338	-0.153	12	1.780	0.409	0.320	-0.197
VR		Restored	11	1.510	0.284	0.349	-0.156	10	1.784	0.423	0.324	-0.225
RS		Regional seeds	12	1.511	0.285	0.361	-0.189					
CS		Conventional seeds	12	1.420	0.236	0.314	-0.257	13	1.712	0.401	0.302	-0.243
	Average natural			1.49	0.27	0.34	-0.16		1.77	0.41	0.32	-0.21
	Average restored			1.51	0.29	0.35	-0.16		1.79	0.42	0.32	-0.21
	CV (%) natural			0.80	2.47	2.57	8.26		1.38	2.30	2.07	18.29
	CV (%) restored			0.28	0.72	1.75	5.33		0.21	2.87	0.74	11.55

If not otherwise stated, all statistical analyses and visualisations were performed in R 4.0.4 (R Core Team, 2021). Based on the two SNP datasets, we visualised genetic relationships of individuals by principal component analysis (function 'gIPCA' from the package 'ADEGENET'; Jombart, 2008), retaining four axes and using the sum of their eigenvalues to calculate the variance explained by the first two principal components. We assessed population structure using the model-based clustering algorithm of STRUCTURE 2.3.4 (Pritchard et al., 2000), which identifies genetic clusters and proportionally assigns individual genotypes to the genetic clusters (details in Supporting Information). We used the criterion developed by Evanno et al. (2005) to identify the most meaningful number of clusters K , which is indicated by the maximum of the ΔK statistic. To assess migration and gene flow between natural and restored sites, we classified individuals based on the cluster membership coefficients (q) as residents if they had $q > 0.8$ for their home gene pool, as migrants if they had $q > 0.8$ for the foreign gene pool, or as hybrids, if $q > 0.2$ for both home and foreign gene pools.

We quantified pairwise genetic differentiation between populations as F_{ST} values based on allele frequencies applying the 'stampFst' function from the 'STAMPP' package (Pembleton et al., 2013) bootstrapping 1,000 times. We tested for significant differences in pairwise F_{ST} among groups of populations (e.g. natural or restored) using analysis of variance (function 'aov') and Tukey's HSD posthoc test.

Overall genetic differentiation among different groups of populations was estimated as Φ_{ST} values derived from an analysis of molecular variance (AMOVA) using the implementation of the package 'POPPR' (Kamvar et al., 2014), which is based on the implementation in 'ADE4' package (Dray & Dufour, 2007). In AMOVA, Φ -statistics are F -statistic analogues calculated in an ANOVA-like procedure based on pairwise genotype distances (Excoffier et al., 1992) and thus comparable to F_{ST} values. We ran three AMOVAs, for all populations, for natural and restored meadow populations separately, and hierarchically for populations nested within one of the two types of meadows.

We assessed within-population genetic diversity as allelic richness AR (El Mousadik & Petit, 1996, thus correcting for differences in sample size), expected and observed heterozygosity (H_e and H_o), and inbreeding coefficient (F_{IS}) with the packages 'POPGENREPORT' (Adamack & Gruber, 2014) and 'HIERFSTAT' (Goudet, 2005) and calculated averages and coefficients of variation (CV).

3 | RESULTS

3.1 | Population structure

3.1.1 | Principle component analysis

In *Centaurea jacea*, the principle component analysis (PCA) revealed a strong separation of the conventional seeds from all other populations (Figure 2A). When the conventional seeds were excluded from

the analysis, it became more evident that all natural populations (blue colours), except for KN, clustered closely together (Figure 2C). The regional seeds (RS) were clearly apart from the main cluster of natural populations. The restored populations (red colours) clustered between the main cluster of natural populations and the regional seeds, substantially overlapping with the regional seeds and partly with some natural populations.

For *Betonica officinalis*, similar patterns were found. Note, however, that no data for the regional seeds were available for this species. Otherwise, the conventional seeds (CS) were clearly separated (Figure 2B) and the natural and the restored populations formed two partially overlapping clusters (Figure 2D). One natural population (PN) stood out of the natural cluster, and among the cluster of restored populations two populations (MR and SR) overlapped with the natural populations.

3.1.2 | Pairwise population differentiation

Analysis of pairwise population differentiation showed for both species that differentiation was smallest between restored populations (RR) and intermediate between natural (NN) populations (Figure 3). The conventional seeds showed the strongest differentiation from both the natural and the restored regional populations (NC and RC in Figure 3). The regional seeds in *Centaurea* were as strongly differentiated from natural populations as natural populations from one another (NS and NN in Figure 3). Similarly, the restored populations were as differentiated from natural populations (NR) as natural populations were from one another, regardless of whether within one locality (NRw) or across localities (NR in Figure 3).

These results were fully corroborated by AMOVA analyses (Table S1). In *Centaurea*, 3.4% of the total genetic variation resided among populations considering all populations ($\Phi_{ST} = 0.034$), 2.6% considering natural populations only ($\Phi_{ST} = 0.023$) and 0.4% considering restored populations only ($\Phi_{ST} = 0.003$). In a hierarchical AMOVA of populations nested within population types (natural and restored), 0.6% of genetic variation resided between population types ($\Phi_{CT} = 0.005$). In *Betonica*, the values were very similar, with the exception that restored populations accounted for even less variation (0.1%, $\Phi_{ST} = 0.008$).

3.1.3 | Clustering and gene flow

The results of the model-based cluster analysis in STRUCTURE reflected those of the PCA analysis. In both species, the ΔK statistic had a maximum at $K = 2$ but similarly high values at $K = 4$ indicating hierarchical structure (Figure S2). At $K = 2$, and also at $K = 4$, the conventional seeds formed an exclusive cluster (brown, Figure S1, Figure 2E,F).

Considering $K = 4$ clusters in *Centaurea*, two clusters (light and dark blue) included all natural populations (Figure 2E). One cluster represented the KN population exclusively (dark blue), while the

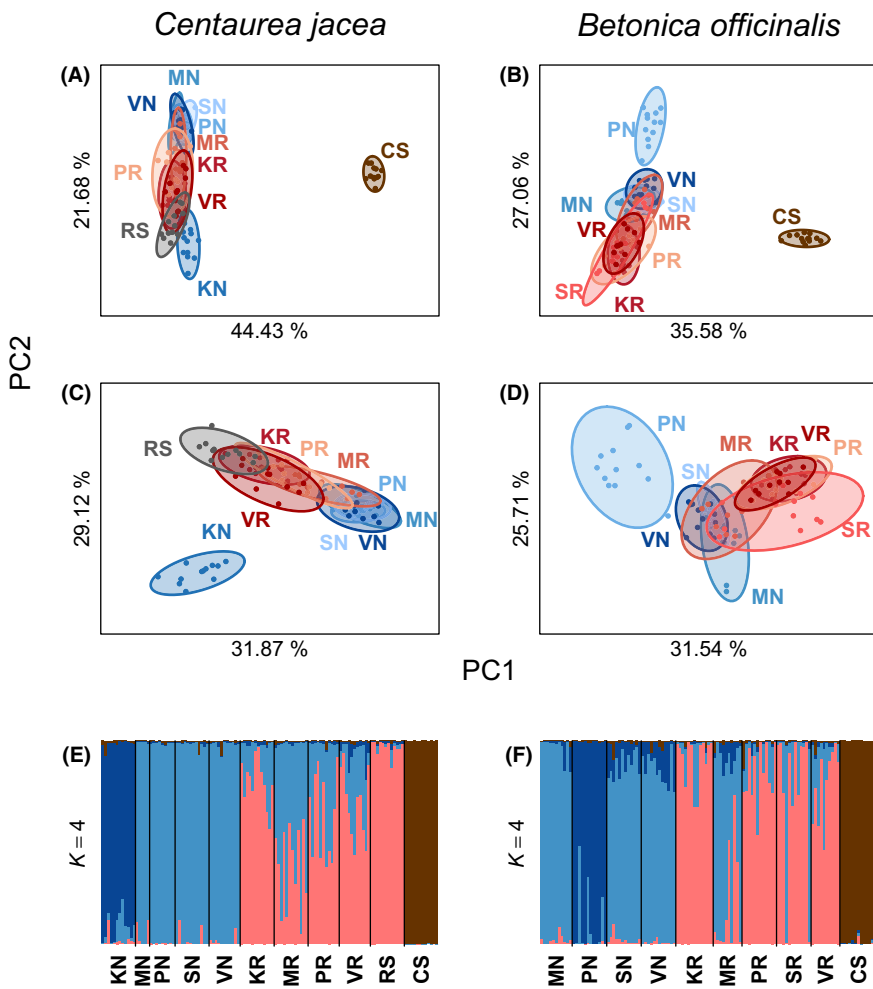


FIGURE 2 Principle component analysis (PCA) and *STRUCTURE* plots. PCA performed on the whole dataset (A and B) and excluding the most divergent group, the conventional seeds (CS) (C and D). First two principle components are presented with their proportion of explained variance. Population type is colour coded: blue for natural, red for restored, grey for regional seeds (only in *C. jacea*) and brown for conventional seeds. Ellipse sizes correspond to 90% confidence intervals. *STRUCTURE* plots (E and D) show ancestry coefficients per individual (q -value) of clusters for $K = 4$ (see Figure S1 for other K). See Table 1 for population labels

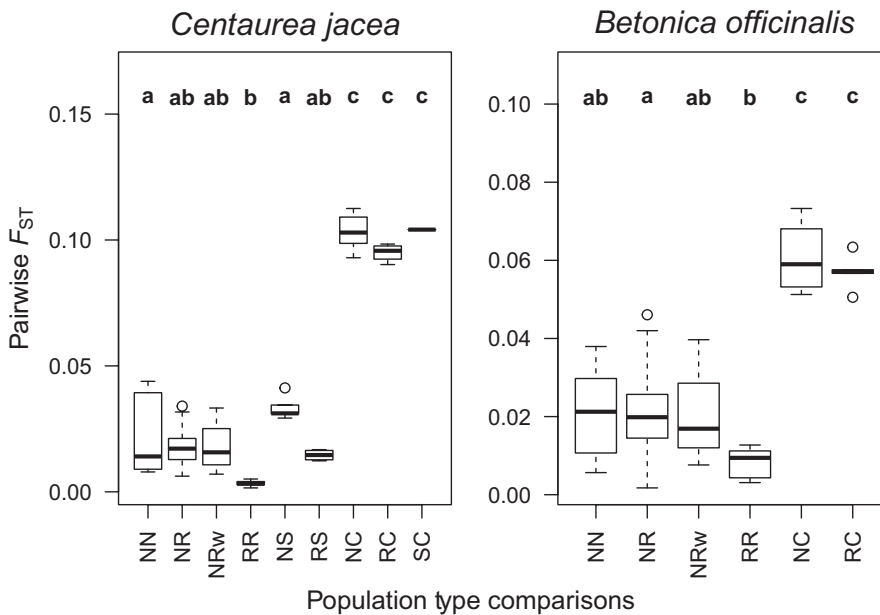


FIGURE 3 Boxplots of pairwise F_{ST} values for different population type combinations. NC, natural/conventional seeds; NN, all possible pairs of natural meadows; NR, natural/restored excluding the within-locality comparisons (KN and KR for example); NRw, natural/restored within locality; NS, natural/regional seeds; RC, restored/conventional seeds; RR, restored/restored; RS, restored/regional seeds; SC, regionals seeds/conventional seeds. Lowercase letters indicate significantly different groups ($p < 0.05$). See Tables S2 and S3 for exact pairwise F_{ST} values

other cluster included all remaining natural populations (light blue) and did also occur in restored populations. The fourth cluster (red) included all plants grown from regional seeds (RS) and dominated

most restored populations (Figure 2E). When we categorised individuals in restored populations into residents (>80% of markers assigned to the restored cluster), hybrids (>20% assigned to both

natural clusters AND the restored cluster) and migrants (>80% assigned to natural clusters), we found per population in *Centaurea* zero to three migrants (6% of all individuals in restored populations) and four to seven hybrids (59% overall), the rest being residents. In contrast, all individuals were considered residents when applying the same categorisation scheme to natural populations (see blue colours in natural populations in Figure 2E).

Considering $K = 4$ clusters in *Betonica*, the conventional seeds (CS) formed a homogenous cluster as did the natural populations (light blue), except for population PN, which formed a separate cluster (dark blue; Figure 2F). The last cluster (red) dominated in the restored populations except for MR, where the main natural cluster (light blue) prevailed. When categorising individuals in restored populations into residents, hybrids and migrants, there were zero to six migrants (15% overall) and two to four hybrids (25% overall), the rest being residents. Like in *Centaurea*, all individuals in natural populations were residents (see blue colours in natural populations in Figure 2F). Across both species, this points towards both seed and pollen dispersal from the natural to the restored populations but not *vice versa*.

3.2 | Population-level diversity

Population-level genomic diversity, assessed as allelic richness, observed or expected heterozygosity, showed pronounced differences between the different groups (Table 1; Figure 4). In both species, the conventional seeds had the lowest level of diversity, that is, lower than both restored and natural populations. The regional seeds, which were only available in *Centaurea*, were slightly more diverse than the natural populations and similarly diverse as the restored populations. The restored populations were, on average, more diverse than the natural populations in *Centaurea*, but despite a similar trend, the difference was not significant in *Betonica* ($\alpha = 0.05$). In addition, levels of genetic diversity varied more among the natural populations (*Centaurea* $CV_{Ar} = 0.80\%$; *Betonica* $CV_{Ar} = 1.38\%$) than among the restored populations, which had very similar levels of genetic diversity (0.28%; 0.21% respectively).

4 | DISCUSSION

We used genome-wide SNP markers to investigate population differentiation and genetic diversity in plant populations of *Centaurea jacea* and *Betonica officinalis* growing in natural and restored meadows. Additionally, we analysed seeds that had been used for restoration (available only for *Centaurea*), as well as conventional commercial seeds without certified origin. We found that 20 years after restoration, restored populations were still differentiated from the surrounding natural populations. However, this differentiation was about as high as the differentiation among natural populations and relatively small compared to the differentiation between regional populations (both restored and natural) and conventional

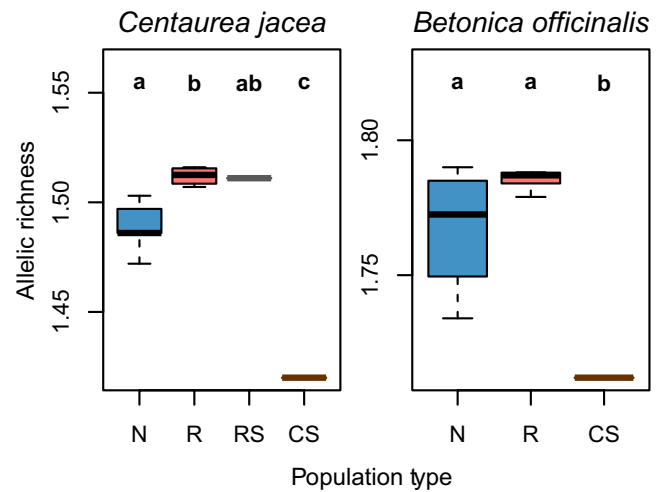


FIGURE 4 Allelic richness per population type. N, natural populations ($n = 5$ for *Centaurea* and $n = 4$ for *Betonica*); R, restored populations ($n = 4$ and 5); RS, regional seeds ($n = 1$); CS, conventional seeds ($n = 1$ for both species). Lowercase letters represent significantly different groups found via Tukey's HSD test (confidence $\geq 95\%$). Rarefaction sample sizes are 4 and 10 alleles in *Centaurea* and *Betonica* respectively. See Table 1 for exact values

seeds. Restored populations were genetically intermediate between the original regional seeds and the natural populations (in *Centaurea*), which—together with our clustering results—suggests ongoing gene flow from natural to restored sites, making this an overall positive example of restoration.

4.1 | Genetic diversity

The genetic diversity within restored populations was comparable (in *Betonica officinalis*) or even higher (in *Centaurea jacea*) than the genetic diversity of natural populations. Increased genetic diversity was already apparent in the regional seeds of *Centaurea*, and thus, the restored populations must have been diverse since establishment. This is a positive observation because genetic diversity is indispensable for adaptation to novel environments (Sgrò et al., 2011). Globally, around half of the restored populations studied by Jordan et al. (2019) are more genetically diverse than natural stands and have thus an enhanced ability to adapt to environmental changes, a feature that is especially important under ongoing climate change.

The higher genetic diversity of restored populations was most likely caused by mixing seeds from multiple regional sources (Jordan et al., 2019). Even though the regional seeds had only been cultivated for one generation to produce seeds for this restoration project, cultivation could have reduced genetic diversity, for example, by the harvesting method or by the time point of harvesting. The latter limits the harvest to those genotypes with ripe seeds at the time of harvest (Espeland et al., 2017). Yet, the resulting regional seeds in our study were more diverse than natural populations. Consequently, if there was any reduction of diversity

during agricultural propagation, it did not lead to a genome-wide reduction of genetic diversity. These results align with previous studies showing that reducing genetic diversity through cultivation is relatively rare, especially if the cultivation takes place only for few generations (Nagel et al., 2019; St. Clair et al., 2020). Notably, the high levels of genetic diversity provided by the regional seeds in our study were successfully translated into diverse restored populations.

4.2 | Differentiation of natural and restored populations

Seeds used for restoration, as well as the restored populations, were differentiated from natural populations. However, the genetic distances corresponded in magnitude to the differentiation among natural populations and therefore represented natural levels of population differentiation in the study region. This is in line with previous works investigating restoration using seeds sourced from the seed transfer zone of the restoration sites, which found genetic differentiation between natural and restored populations of similar magnitude as differentiation among natural sites (Aavik et al., 2012; Kaulfuß & Reisch, 2019). However, even populations restored using regional seeds were strongly differentiated from natural populations in *Knautia arvensis* (Kaulfuß & Reisch, 2019). In that study, *K. arvensis* individuals had two genetically differentiated ploidy levels, that is, diploids and tetraploids (Kolář et al., 2009), which occurred within the same seed transfer zone. Individuals differing in ploidy do not readily interbreed (Kramer et al., 2018), and thus, diploids and tetraploids behave as distinct taxa. It is thus not surprising that they are genetically differentiated. Yet, such ploidy differences were not present for the two species in our study region. On the other hand, substantial genetic differences between natural and restored populations are common when restored populations are established using cheap conventional seeds of unknown origin or even cultivars (Gemeinholzer et al., 2020; Slaymaker et al., 2015).

The restored populations were genetically very similar to each other, with very low values of genetic differentiation. This is expected given their common origin from the same gene pool (Aavik et al., 2012). At the same time, the restored gene pools were genetically highly diverse by mixing and propagating seeds from multiple source populations in one common homogenised gene pool, which has been suggested as a strategy to maximise diversity during seed production (Bucharova et al., 2019; but see St. Clair et al., 2020). It is expected that subsequent site-specific selection and gene flow from surrounding populations will lead to adequate genetic differentiation. Moreover, the homogenisation is spatially restricted, either to the project scale, or to a seed transfer zone, given a respective system. Pooling multiple populations of origin necessarily homogenises the differentiation that existed between them, which may be of concern if populations are pooled from too large regions, for example, whole countries (Tong et al., 2020). Regional seed sourcing, as used in this restoration project, thus represents a compromise between

spatial genetic homogenisation of restored populations on the regional scale, and the feasibility of seed production.

4.3 | Gene flow

We detected signs of recent gene flow from natural to restored populations. On average, restored populations had a share of one third of the natural gene pool (27% for *B. officinalis* and 35% for *C. jacea*). We found both first-generation immigrants and hybrid offspring in restored populations, indicating both seed and pollen dispersal from the surrounding populations into restored sites. In our system, the natural and restored populations were spatially close to each other, which likely allowed such strong gene flow (McRae et al., 2012). While this may seem to be a logical consequence of spatial proximity, other studies have shown that strong gene flow is not always taking place. Even with short distances between populations and more than 10 years since restoration, high differentiation between restored and natural sites can persist (Broadhurst, 2013; Ritchie et al., 2019).

While we detected strong gene flow from natural to restored populations, we did not find any signs of gene flow in the opposite direction. This is surprising because gene flow from restored to natural populations was detected in other restoration projects (Aavik et al., 2013; Cordeiro et al., 2019; Gemeinholzer et al., 2020). A possible reason could be the smaller size of the restored compared to the natural populations resulting in lower propagule pressure from restored gene pools. Additionally, lower vegetation cover on restored sites may have facilitated seedling establishment. In general, the lack of gene flow from restored to natural populations is good news because this direction may be of concern, especially if the genetic material used for restoration strongly differs from natural populations, and gene flow may cause contamination of the natural gene pool (McKay et al., 2005).

4.4 | Conventionally produced seeds of unknown origin

Conventional seeds without certified origin were genetically strongly differentiated from both natural and restored populations. The origin of these seeds is unknown, but they might be from Northern Bohemia where the producer is located. Northern Bohemia and the study area in the White Carpathians belong to different seed transfer zones (Ševčíková et al., 2014) and even different biogeographic zones. Thus, populations of many species belong to different phylogeographic clades (Mráz & Ronikier, 2016). The strong genetic difference between conventional seeds and regional populations is therefore not surprising. In general, conventional seeds represent their populations of origin but are differentiated from populations in any distant target region (Gustafson et al., 2018; Massatti et al., 2018; Tong et al., 2020 but see e.g. Braasch et al., 2021; Shaw & Mummey, 2017). Therefore, using such seed sources could

contaminate the regional gene pool and possibly reduce plant performance due to lacking regional adaptation (Bucharova et al., 2017).

The conventional seeds without certified origin had low genetic variability. As there is no information available on their production, and the seed producer does not declare to have followed any specific standards, we can only speculate about the reasons. The seeds were possibly sourced from only a few wild plants and propagated for many generations, including repeated bottlenecks (Espeland et al., 2017). Establishing and following international standards could reduce uncertainties around seed sourcing and production procedures (Pedrini & Dixon, 2020). In general, conventionally produced seeds may, or may not have reduced genetic variability, depending on the production procedures (Jordan et al., 2019; Massatti et al., 2018; Shaw & Mummey, 2017), which highlights that conventional and regional seeds are two extremes of a continuum spanning from no standards to high standards. While some producers are well aware of the importance of high genetic diversity (Aavik et al., 2012; Bucharova et al., 2019; Kiehl et al., 2014), restoration using single homogeneous cultivars is still common (Gemeinholzer et al., 2020; Massatti et al., 2018; Slaymaker et al., 2015). As genetic variability is a prerequisite of the ability of a population to adapt to environmental changes, using genetically impoverished seeds is risky because the resulting restored populations may lack adaptive potential.

While using conventional seed is suboptimal, there are cases when there is no other option. Restoration is often an urgent endeavour, and regional seeds are not always available. In some species, the detrimental effect of non-regional seeds might be negligible, for example, in species with long-distance pollen and seed dispersal syndromes, in which populations typically show only small genetic differentiation (Durka et al., 2017). In other species, using conventional seeds without certified origin may be suboptimal but necessary in the case of seed scarcity. In any case, as much prior knowledge as possible about population differentiation and genetic diversity should be acquired to minimise latent risks such as genetic swamping of locally adapted genotypes or poor adaptability to environmental change. Detailed discussions on how to optimally source and produce seeds are abundant in the literature (e.g. Espeland et al., 2017; Pedrini & Dixon, 2020; Pedrini et al., 2020).

5 | CONCLUSIONS

Our results show that populations restored using seeds sourced from the region differ genetically from adjacent natural populations to an extent that is typically found among natural populations within the region. Using regional seeds thus successfully maintains the regional gene pool. If regional seeds were not available, restoration would have to rely on wild-sourced or conventional seeds without certified origin. Wild collected seeds are scarce and cannot cover the demand for the growing restoration efforts (Meissen et al., 2015; Merritt & Dixon, 2011). Using conventional seeds without certified origin can result in genetically different, maladapted and impoverished populations. Therefore, their application should be limited to cases where

knowledge about the population structure of the species in question justifies it or where there is no other option. Our results suggest that regional provenancing can strike the balance between providing genetically diverse restored populations and maintaining large-scale patterns of genetic differentiation. Optimally, regional seed sourcing would be based on a tested system of seed transfer zones accounting for genetic structure, environmental factors and adaptability. Our study included only two species of perennial, insect-pollinated herbs from only one region. To provide more general results, future research should focus on collecting data for a broader spectrum of species on a larger geographic scale.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

A.B., C.L. and W.D. conceived the ideas and designed methodology; A.B., J.H., T.K.-R. and O.M. collected the samples; J.H. and W.D. analysed the data; J.H. led the writing of the manuscript under supervision of A.B. and W.D. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Raw data have been deposited in the European Nucleotide Archive (ENA) at EMBL under accession number PRJEB45358 (Höfner et al., 2021b). Genotype-containing vcf files are available via the Dryad Digital Repository <https://doi.org/10.5061/dryad.qbzkx18j0> (Höfner et al., 2021a).

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