

RESEARCH ARTICLE

Strong differentiation between source and ex situ populations of rare plant species revealed by next-generation sequencing

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Email: ellen.gradl@ur.de**Societal Impact Statement**

Biodiversity is being lost rapidly worldwide due to factors such as climate change and habitat loss. To help safeguard plant populations, conservation strategies include seed banks and conservation cultures. However, it remains unclear how cultivation affects genetic variation compared with wild populations. Here, we compared genetic diversity and differentiation of wild populations and their corresponding conservation cultures in a conservation nursery using next-generation sequencing. We found considerable differentiation between wild and cultivated populations, highlighting that current conservation culture practices should be improved to ensure genetic biodiversity is maintained for restoration efforts.

Summary

- Ex situ conservation is an important element of modern conservation strategies and contributes significantly to the preservation of species and genetic diversity. However, depending on the specific ex situ approach, genetic variation within and among populations may be affected, mainly due to bottlenecks or drift.
- In this study, the impact of ex situ cultivation on genetic variation was studied. Therefore, source populations of four strongly endangered plant populations (*Ade-nophora liliifolia*, *Dianthus sylvaticus*, *Teucrium scordium* and *Trollius europaeus*) were compared with their respective ex situ populations, which were cultivated in a conservation nursery, using next-generation sequencing (MIG-seq).
- Here, no significant effect of cultivation on genetic diversity and inbreeding was observed, but comparatively high levels of genetic differentiation were found among source and ex situ populations, even though the ex situ populations had only been cultivated for one generation. This might be attributed to either unrepresentative seed collection at the source sites, unconscious selection during plant production or genetic drift in the source populations since the time of seed collection.
- As populations are best adapted to local conditions and show the highest fitness under these conditions, minimising genetic differentiation between source and ex

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situ populations is a key goal for successful reintroduction. Therefore, genetic monitoring and the results it provides are a powerful tool to improve ex situ conservation. Unfortunately, it is rarely applied in nature conservation at present.

KEYWORDS

conservation genetics, ex situ conservation, genetic monitoring, land-use change, MIG-seq, threatened plant species

1 | INTRODUCTION

Biodiversity has been under strong pressure since the beginning of the Industrial Revolution, prompting concerns that a sixth mass extinction may be underway (Barnosky et al., 2011; Ceballos et al., 2015; Cowie et al., 2022). Even under conservative assumptions, vertebrate extinction rates since 1900 are more than 50 times higher than the background extinction rate (Ceballos et al., 2015), whereas plant extinction rates of more than 200 times the background rate have been reported (Le Roux et al., 2019). Plant diversity has also strongly declined at the regional level. According to the Bavarian Red List of Ferns and Flowering Plants, approximately 3% of species are extinct, 61% are endangered and only 36% are not endangered (Klotz et al., 2024). Although the causes of biodiversity loss differ among taxa and have changed over time (Bellard et al., 2022), land-use changes and climate change are widely recognised as major causes of biodiversity decline (Johnson et al., 2017).

Intensive farming has contributed substantially to biodiversity loss (Stein-Bachinger et al., 2022; Tsiafouli et al., 2015). For example, plants adapted to nutrient-poor habitats experience considerable pressure due to excessive fertilisation and the airborne input of nitrogen (Bobbink et al., 2010). In addition, climate change reduces suitable habitats for many species and can negatively affect ecosystem functioning, for example, through changes in phenology and species morphology (Everingham et al., 2024; Parmesan, 2006; Piao et al., 2019). These effects are particularly severe for specialised species that depend on specific species interactions (Saladin et al., 2020).

One strategy to conserve biodiversity is to protect species in situ, that is, within their natural habitats (United Nations, 1992). These, in Central Europe often anthropogenic, habitats, however, require management to maintain their diversity. For example, species-rich calcareous grasslands must be regularly mown or grazed to prevent succession (Kahmen et al., 2002; Poschlod & WallisDeVries, 2002), and on heathlands, turves must be regularly cut or grazed to maintain low nutrient concentrations and prevent vegetation succession (Webb, 1998).

Another strategy is to conserve biodiversity ex situ, that is, outside the species' natural habitats, by collecting and storing seeds or vegetative reproductive units of endangered species from wild populations (United Nations, 1992). Ex situ conservation can be combined with in situ conservation, as the ex situ measures create a kind of 'backup' in case the source population is lost. Besides conserving seeds in seed banks, the cultivation of endangered plant species in botanical gardens or conservation nurseries is a widely applied ex situ

approach. For this purpose, diaspores are first collected from source populations. Then, seeds are germinated and the resulting plants are cultivated under controlled conditions (United Nations, 1992). The genetic variation of ex situ cultivated plants may strongly deviate from the natural genetic patterns, especially if ex situ populations are small, due to seed sampling procedures (Brown & Briggs, 1991; Guerrant et al., 2004), pollination limitation (Ashman et al., 2004), genetic drift (Rauschkolb et al., 2019), crossing processes during cultivation (Hufford & Mazer, 2003; Tallmon et al., 2004) and unintentional 'domestication', that is, adaptation to the conservation conditions (Carroll et al., 2007; Sun et al., 2022). Unfortunately, genetic monitoring of the ex situ populations is rarely conducted (Engels & Ebert, 2021; McLennan et al., 2025).

To close this gap, we analysed the impact of ex situ cultivation on genetic variation of the four strongly endangered plant species: *Adephora liliifolia* L., *Dianthus sylvaticus* Vill., *Teucrium scordium* L. and *Trollius europaeus* L. For each study species, we compared genetic variation within and among source populations with the corresponding ex situ populations and attempted to answer the following questions:

- i. Is there a significant loss of genetic variation within ex situ compared to in situ populations?
- ii. How high is the level of genetic variation among populations?
- iii. What lessons can be learned for future ex situ conservation efforts?

2 | MATERIALS AND METHODS

2.1 | Study species, study sites and sampling procedure

For our study, we selected four endangered species to determine the impact of ex situ cultivation on genetic variation. *A. liliifolia* (L.) A.DC. is threatened by inappropriate forest management and shrubby vegetation, is classified as critically endangered in Bavaria (red list level 1) and is also included in Annex IV of the EU Habitats Directive, which lists species in need of strict protection (Council Directive 92/43/EEC) (Klotz et al., 2024; Prausová et al., 2016). *D. sylvaticus* Hoppe ex Willd. (Seguier's pink, syn.: *Dianthus seguieri* subsp. *sylvaticus* [Hoppe ex Willd.] Arcang., *Dianthus seguieri* subsp. *glaber* Čelak.) is endangered in Bavaria (red list level 2), and the main threats are eutrophication, loss of habitats (e.g., nutrient-poor fringes) and inappropriate forest

management (Klotz et al., 2024; Schneider, 2009). *T. scordium* (water germander) is also endangered (red list level 2, Klotz et al., 2024). It is a mesophyte (Lakušić et al., 2010), and as wetlands have declined drastically worldwide and in Europe (Čížková et al., 2013; Fluet-Chouinard et al., 2023), it is probably affected by habitat loss. *T. europaeus* (globeflower) is classified as vulnerable (red list level 3) as it is threatened by the abandonment of extensive land use (Muncaciuc et al., 2010) and, as a species of moist habitats, is probably also affected by the decline of wetlands. All species occur in Central Europe, but their distributions extend differently into other parts of the continent (Euro+Med, 2006+): Only *T. europaeus* and *T. scordium* also occur in northern Europe. Both species reach Russia in the east and Spain in the west and extend south to Greece. However, the range of *T. scordium* further includes Portugal, Morocco and parts of the Near East. In contrast, the distribution of *D. sylvaticus* is restricted to Central Europe, Spain, Italy and Turkey. *A. liliifolia* spans from France to Russia but does not extend further south than Italy and the Balkans. All species are perennial hemicryptophytes and show limited, mostly local dispersal, except for *T. europaeus*, which is also dispersed by ants (Chytrý et al., 2024).

Ex situ populations of the four species were maintained in a plant conservation nursery in Straubing, Germany. These ex situ populations originated from seeds collected from source populations

between 2014 and 2021 (Table 1). As these populations were not established within the framework of a scientific study, but rather as a practical nature conservation measure, no data on the cultivation process have been collected. The only available information is that the individuals still belong to the original generation derived from the collected seeds. As we aimed to investigate how well these practical approaches, which are often applied by nature conservation stakeholders, conserve biodiversity, we studied both the ex situ populations and their corresponding wild source populations. Three source-ex situ population pairs were included for each species, except for *D. sylvaticus*, for which four pairs were investigated. The source populations were distributed across south-eastern Germany (Figure 1).

For next-generation sequencing (NGS)-based studies with a large number of single-nucleotide polymorphisms (SNPs), fewer than 10 individuals have been shown to accurately reflect the genetic diversity of populations (Nazareno et al., 2017). As the number of SNPs recovered for each species is not known in advance with the MIG-seq method used in this study, leaf samples were collected from 12 individuals per population (Table 1), where possible. These leaf samples were dried and stored on silica gel until DNA extraction. Leaf sampling of source populations took place in 2024, 3–10 years after seeds for the ex situ populations had been collected, whereas leaf material from the ex situ populations was collected in 2022.

TABLE 1 Expected heterozygosity (H_e), inbreeding index (F_{is}), mean kinship coefficient (K) and Holm-adjusted p -value of the bottleneck analysis. The means and standard deviations (SDs) were calculated for each species (in bold). Additionally, the year of seed collection for the ex situ populations is provided, along with the number of DNA samples used per population (N).

Species	Site	Source populations (S)					Ex situ populations (E)					Year
		N	H_e	F_{is}	BN	K	N	H_e	F_{is}	BN	K	
<i>Adenophora liliifolia</i>	01	11	0.205	0.023	<0.0001	−0.01	12	0.151	−0.076	<0.0001	0.23	2015
	02	12	0.204	0.025	0.0537	0.03	12	0.2	−0.002	<0.0001	0.06	2014
	03	12	0.208	0.025	<0.0001	−0.03	12	0.191	−0.042	<0.0001	0.14	2021
	Mean		0.21	0.024		0.00		0.18	−0.04		0.14	
	SD		0	0.001		0.025		0.02	0.03		0.071	
<i>Dianthus sylvaticus</i>	01	12	0.156	−0.098	<0.0001	0.26	12	0.188	−0.082	<0.0001	0.20	2017
	02	12	0.186	−0.031	<0.0001	0.11	12	0.183	−0.029	<0.0001	0.11	2015
	03	12	0.172	−0.094	<0.0001	0.25	12	0.178	−0.038	<0.0001	−0.01	2017
	04	12	0.19	−0.019	<0.0001	0.13	12	0.202	−0.021	<0.0001	0.10	2017
	Mean		0.18	−0.06		0.19		0.19	−0.04		0.10	
	SD		0.01	0.036		0.065		0.01	0.024		0.072	
<i>Teucrium scordium</i>	01	12	0.214	−0.257	<0.0001	0.39	12	0.228	−0.126	<0.0001	0.25	2019
	02	12	0.239	−0.163	<0.0001	0.32	11	0.248	−0.102	<0.0001	0.23	2018
	03	12	0.258	−0.114	<0.0001	0.22	12	0.252	−0.083	<0.0001	0.18	2018
	Mean		0.24	−0.18		0.31		0.24	−0.1		0.22	
	SD		0.02	0.059		0.073		0.01	0.018		0.031	
<i>Trollius europaeus</i>	01	3	0.124	0.013	<0.0001	−0.07	12	0.159	0.026	<0.0001	−0.04	2018
	02	5	0.143	0.037	0.0344	−0.16	12	0.157	0.009	<0.0001	0.03	2018
	03	12	0.184	0.088	0.3723	−0.19	12	0.179	0.084	0.3723	−0.17	2020
	Mean		0.15	0.046		−0.14		0.17	0.04		−0.06	
	SD		0.03	0.031		0.051		0.01	0.032		0.081	

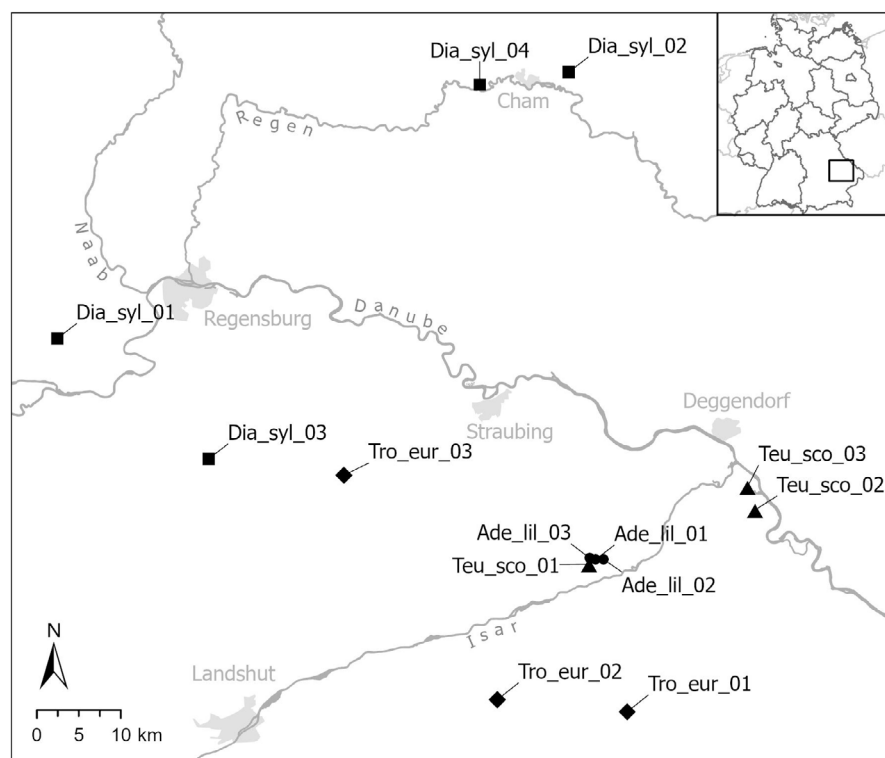


FIGURE 1 Locations of the source populations of *Adenophora liliifolia*, *Dianthus sylvaticus*, *Teucrium scordium* and *Trollius europaeus* in south-east Germany.

2.2 | MIG-seq analysis and SNP detection

The DNA extraction was carried out using the cetyltrimethylammonium bromide (CTAB) method by Rogers and Bendich (1989), with modifications as detailed by Reisch (2007). DNA concentrations were determined using a NanoDrop One spectrophotometer from Thermo Fisher Scientific (Germany). The samples were diluted with deionised water to a final concentration of 15 ng/μL and stored at −20°C until further analysis.

Genome-wide SNPs were analysed using multiplexed ISSR (inter-simple sequence repeats) genotyping-by-sequencing (MIG-seq) following the protocol by Suyama and Matsuki (2015) to analyse the genetic variation of all samples. This method enables the rapid and cost-effective acquisition of SNPs for ecological research and has already been successfully applied to various species groups (Grädl et al., 2026; Hirano et al., 2019; Kusuma et al., 2024; Sakaba et al., 2023; Takata et al., 2019).

The MIG-seq library was prepared following a two-step polymerase chain reaction (PCR) protocol, and DNA fragments ranging from 300 to 800 bp were selected for sequencing by applying a size selection with magnetic beads (AMPure Xp, Beckman Coulter). Sequencing was performed on an Illumina MiSeq platform (Illumina, USA) using the MiSeq Reagent Kit v3 (150 cycles), following the method described by Suyama and Matsuki (2015). Following the MiSeq run, FastQC (version 0.11.5, Andrews, 2010) was utilised to check sequencing quality.

Trimmomatic (version 0.36, ILLUMINACLIP:\${ADAPTER_FASTA}:2:30:10, SLIDINGWINDOW:4:15, CROP75, HEADCROP:1 MINLEN:74, Bolger et al., 2014) was employed to remove adapter

sequences from the reads. Following this process, the data quality was reassessed, and individuals with fewer than 10,000 reads were excluded from the statistical analysis.

Subsequently, Stacks (version 2.66, Catchen et al., 2013) was used to build loci from the short-read sequences. Stacks included the functions *ustacks*, *cstacks*, *sstacks*, *tsv2bam* and *gstacks*. The settings that deviated from the default included *ustacks* (m 3) and *cstacks* (n 2), which corresponded to the parameters described by Tsuzuki et al. (2022). Afterwards, the *populations*-function was applied, using the following settings: *R* (the minimum percentage of individuals across populations required to process a locus, with values ranging from 0 to 1), *min-maf* (the minimum minor allele frequency required to process a nucleotide site at a locus, with values ranging from 0 to 0.5), *write-single-snp* (which restricts data analysis to only the first SNP per locus) and *p* (the minimum number of populations in which a locus must be present to be processed, with *p* = 1). Various values for *R* and *min-maf* were tested, and the results were compared. Ultimately, a value of *R* = 0.5 and *min-maf* = 0.02 were selected. Similar combinations of *R* and *min-maf* values were used by Kusuma et al. (2019), Park et al. (2020) and Takata et al. (2019). This function generated multiple files that were later utilised for Bayesian structure analyses and analyses of molecular variance (AMOVAs). Additionally, it calculated the expected heterozygosity (H_e) and the inbreeding index (F_{is}).

2.3 | Statistical analysis

For all species, a paired Wilcoxon signed-rank test was performed in RStudio (version: 2025.5.0.496, Posit team, 2025; R version: 4.5.0, R

Core Team, 2024) to compare the source and ex situ populations' H_e and F_{is} .

To detect heterozygosity excess, which indicates a recent bottleneck effect, the software Bottleneck 1.2.02 (Cornuet & Luikart, 1996) was used, which has been used to study bottleneck effects in multiple studies using SNP markers (Robiansyah et al., 2026; Setzke et al., 2022; Stojanova et al., 2020). The infinite allele model (IAM) was used, and significance was evaluated using the sign test.

Pairwise kinship coefficients were calculated using the KING-robust estimator implemented in PLINK 2.0 (--make-king-table) (Chang et al., 2015; Manichaikul et al., 2010). Mean pairwise kinship coefficients were subsequently calculated within each population to compare levels of relatedness between ex situ and wild populations.

The molecular variance was analysed in an AMOVA using GenAlEx 6.52 (Peakall & Smouse, 2006). The AMOVA allowed for determining the percentage of variance within and among populations. For distance calculation, codom-allelic was selected, and within-individual analysis was suppressed. Output labelled pairwise F_{st} matrix was selected, and 999 was defined for the permutations. A linear regression was used to determine a relationship between the F_{st} values between source populations and their corresponding ex situ population and source population size. A Mantel test was also performed, based on F_{st} values and geographic distances between source populations.

Additionally, a Bayesian structure analysis was performed for all source and ex situ populations combined using the program Structure 2.3.4 (Pritchard et al., 2000). The program identifies the number of genetically homogeneous groups (K) without requiring prior knowledge of the actual number of populations. The model determining the true K is an evaluation of the posterior likelihood of the data for a given K . The analysis employed the settings 'admixture model' and 'correlated allele frequencies'. For each analysis, K_{min} was set to 1, and the value for K_{max} was $N + 2$. The length of the burn-in period was set to 10,000, and the number of Markov chain Monte Carlo (MCMC) iterations after burn-in was set to 100,000. The analysis was repeated 20 times for each K . Output from the structure program was analysed via StructureSelector (Li & Liu, 2018). StructureSelector and the Evanno method (Evanno et al., 2005) were used to determine the most likely value for K . To quantify differences in cluster assignment between source populations and their corresponding ex situ populations, mean cluster assignment was calculated for each population. Ancestry overlap was then calculated as the sum of the minimum ancestry proportions across clusters.

A principal component analysis (PCA) was performed based on the allele frequencies to accompany the cluster analysis with an analysis that is not restricted to a fixed number of clusters.

3 | RESULTS

3.1 | Genetic diversity

MIG-seq analysis resulted in 1035 loci for *A. liliifolia* (Ade_lil), 989 for *D. sylvaticus* (Dia_syl), 498 for *T. scordium* (Teu_sco) and 1752 for

T. europaeus (Tro_eur). Concerning H_e , no statistically significant differences between source and ex situ populations were found ($p_{Ade_lil} = .25$, $p_{Dia_syl} = .25$, $p_{Teu_sco} = .5$, $p_{Tro_eur} = .5$, Table 1). The same was the case for F_{is} among source and ex situ populations ($p_{Ade_lil} = .25$, $p_{Dia_syl} = .25$, $p_{Teu_sco} = .25$, $p_{Tro_eur} = .75$, Table 1).

Heterozygosity excess was present in almost all populations, indicating a recent bottleneck (Table 1). No significant heterozygosity excess was detected for source population O2 of *A. liliifolia* and for the source and ex situ populations O3 of *T. europaeus*.

Kinship coefficients were higher in the ex situ population than in the source population in *A. liliifolia* and *T. europaeus* but lower in *D. sylvaticus* and *T. scordium*.

3.2 | Genetic differentiation

The two-way AMOVA showed that the mean genetic differentiation between *A. liliifolia* source and ex situ populations was 10.4% (Table 2). When comparing source and ex situ populations separately in a two-way AMOVA, it was revealed that the differentiation among source populations was lower at 6% (p -value = .001) than the 17% differentiation among ex situ populations (p -value = .001, Table 2).

For *D. sylvaticus*, the mean genetic differentiation between source and ex situ populations was 9.5% (Table 2). Furthermore, the differentiation among source populations was higher at 19% (p -value = .001) than the 14% differentiation among ex situ populations (p -value = .001, Table 2).

The mean genetic differentiation between *T. scordium* source and ex situ populations was 9.4% (Table 2). When comparing source and ex situ populations separately in two-way AMOVAs, it was found that the differentiation among source populations was higher at 21% (p -value = .001) than the 14% differentiation among ex situ populations (p -value = .001, Table 2).

Finally, the AMOVAs for *T. europaeus* revealed a mean genetic differentiation of 11% among source and ex situ populations (Table 2). The differentiation among ex situ populations was only slightly higher at 12% (p -value = .001) than the 11% differentiation among source populations (p -value = .001, Table 2).

Due to the low number of source-ex situ population pairs per species, no significant correlation between F_{st} values and source population size was detected ($p > .05$). However, the estimate was negative for all species, except *D. sylvaticus* (Figure S1). The Mantel test was not significant for any species ($p > .05$).

The most likely number of clusters found using the Bayesian cluster analysis was $K = 2$ for *A. liliifolia*, *T. scordium* and *T. europaeus*, whereas the genetic structure of *D. sylvaticus* was best explained by $K = 3$ (Figure 2). When comparing the source populations with the ex situ populations using a Bayesian cluster analysis, only the spatial genetic structure of *D. sylvaticus*'s and *T. scordium*'s source populations was still fully reflected in the genetic structure of the ex situ populations, whereas for *A. liliifolia* and *T. europaeus*, the cluster assignment of some, but not all, populations changed. This visual impression was supported by the ancestry overlap, which exceeded

TABLE 2 AMOVA results. Each source population was compared with the respective ex situ population. The differentiation among (AP) and within (WP) the two populations was determined. Mean F_{st} values and the standard deviation (SD) were calculated, as well, shown in bold. Furthermore, the source populations were compared with each other and the ex situ populations with each other.

Species	Test	AP	WP	p-value	F_{st}
<i>Adenophora liliifolia</i>	S_01 vs. E_01	16	84	.001	0.157
	S_02 vs. E_02	8	92	.001	0.082
	S_03 vs. E_03	7	93	.001	0.074
	Mean				0.104
	SD				0.037
	Source	6	94	.001	0.064
	Ex situ	17	83	.001	0.168
<i>Dianthus sylvaticus</i>	S_01 vs. E_01	18	82	.001	0.177
	S_02 vs. E_02	10	90	.001	0.096
	S_03 vs. E_03	3	97	.003	0.032
	S_04 vs. E_04	7	93	.001	0.073
	Mean				0.095
	SD				0.053
	Source	19	81	.001	0.185
<i>Teucrium scordium</i>	S_01 vs. E_01	8	92	.002	0.081
	S_02 vs. E_02	14	86	.001	0.144
	S_03 vs. E_03	6	94	.002	0.057
	Mean				0.094
	SD				0.037
	Source	21	79	.001	0.206
	Ex situ	14	86	.001	0.139
<i>Trollius europaeus</i>	S_01 vs. E_01	12	88	.001	0.117
	S_02 vs. E_02	14	86	.001	0.141
	S_03 vs. E_03	7	93	.001	0.066
	Mean				0.108
	SD				0.031
	Source	11	89	.001	0.108
	Ex situ	12	88	.001	0.121

90% in all populations of *D. sylvaticus* and *T. scordium*. In contrast, ancestry overlap ranged from 0% to 100% (mean: 58.6%) in *A. liliifolia* and from 2% to 100% (mean: 67.6%) in *T. europaeus*. The PCA also showed that in *D. sylvaticus* and *T. scordium*, source and ex situ individuals clustered, whereas in *A. liliifolia* and *T. europaeus*, some ex situ individuals associated with other clusters (Figure 3).

4 | DISCUSSION

4.1 | Genetic diversity

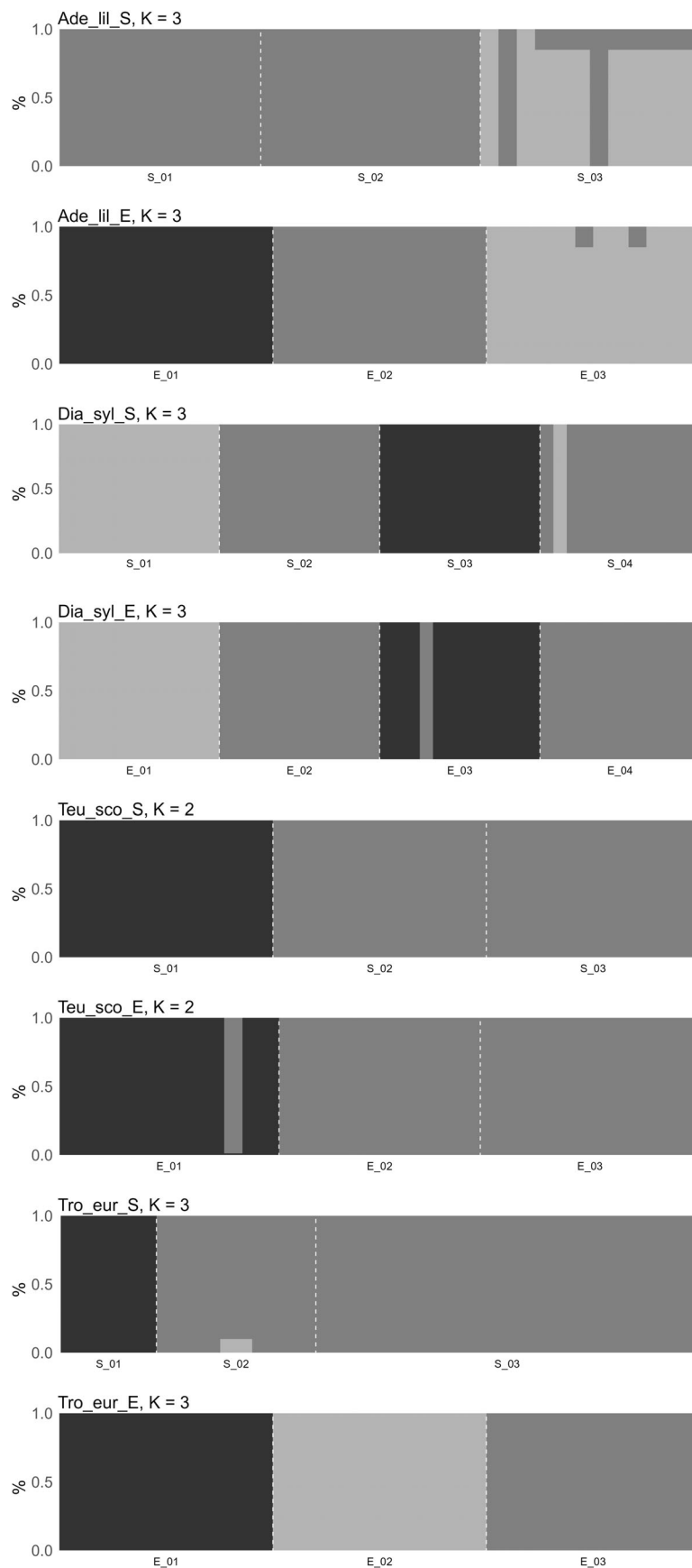
Regarding genetic diversity, we observed no significant differences in the level of genetic diversity between source and ex situ populations. The same pattern was observed for the inbreeding index as well. As demonstrated in previous studies, ex situ conservation can lead to a

loss of genetic diversity (Lauterbach et al., 2012; Rucińska & Puchalski, 2011). This phenomenon can be attributed to ex situ cultures of insufficient size, maintained over several generations, which can then experience inbreeding (Wei & Jiang, 2020).

However, there are also studies that have documented an increase in genetic diversity in ex situ cultures. On the one hand, this can be traced back to the cultivation procedure, for example, the repeated addition of new plants to the ex situ populations (Forgiarini et al., 2023). On the other hand, hybridisation with closely related species during the cultivation (Zhang et al., 2010) or the unintentional mixing of ex situ populations from different sources (Vergeer et al., 2005) could also increase genetic diversity.

Because the ex situ populations in this study were cultivated over several years, but never went beyond the first generation, factors like inbreeding or mixing of ex situ populations most likely did not affect the current genetic diversity. Generally, non-representative seed

FIGURE 2 Bayesian structure analysis of the source (S) and ex situ populations (E) of *Adenophora liliifolia*, *Dianthus sylvaticus*, *Teucrium scordium* and *Trollius europaeus*. K is the most likely number of groups the individuals were assigned to.



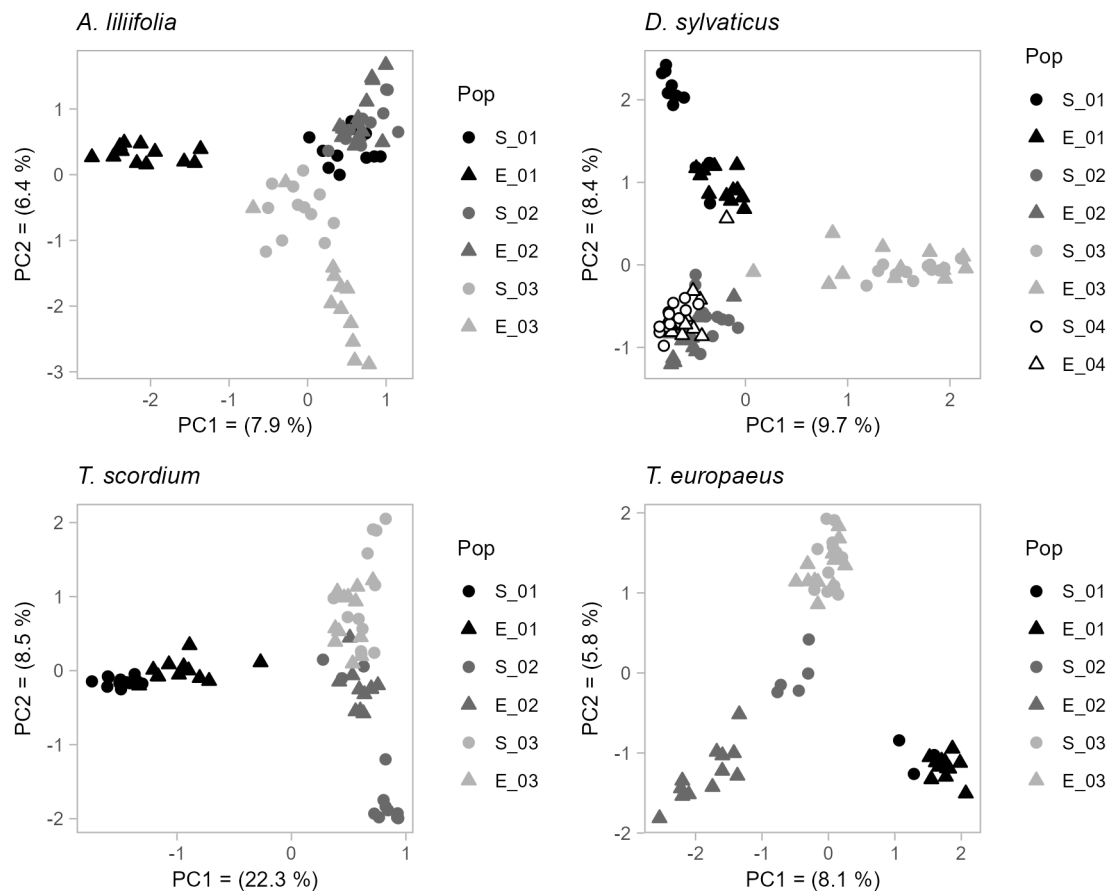


FIGURE 3 Principal component analysis based on allele frequencies of *Adenophora liliifolia*, *Dianthus sylvaticus*, *Teucrium scordium* and *Trollius europaeus*. Colours indicate the source–ex situ population pairs, whereas shapes indicate source versus ex situ populations.

sampling may lead to a bottleneck, reducing genetic diversity. It is also possible that certain genotypes may be lost because they encounter difficulties germinating and growing under conditions that deviate from those of the source site (Vander Mijnsbrugge et al., 2010). Similar levels of genetic diversity between source and ex situ populations may therefore indicate that genetic diversity was successfully captured during establishment. However, when populations are assessed long after the ex situ population was founded, as in our study, similar diversity levels could also result from both populations experiencing genetic diversity loss independently over time, for example, through bottlenecks. Indeed, a recent bottleneck was detected for most of the source and ex situ populations. Additionally, a higher relatedness between individuals in ex situ populations in two species may indicate they originated only from a subset of the population. Our results therefore indicate that although genetic diversity was similarly high in ex situ and source populations, some parts of genetic diversity may still have been lost when ex situ populations were founded.

4.2 | Genetic differentiation

The study presented here revealed a comparably strong level of genetic differentiation between source and ex situ populations.

Moreover, Bayesian structure analyses revealed that only the spatial genetic structure of *D. sylvaticus*'s and *T. scordium*'s source populations was still reflected in the genetic structure of the ex situ populations. Similar results have been found in other studies. For example, Brütting et al. (2013) reported genetic differentiation between source and ex situ populations ranging from 8% to 61%. Furthermore, Forgiarini et al. (2023) observed an average genetic differentiation of 13%. Lauterbach et al. (2012) showed an even greater average genetic differentiation of 28% between source and ex situ populations. However, genetic differentiation between source and ex situ populations should remain minimal, as exemplified by Pierson et al. (2007), who detected a genetic differentiation of only 3%.

As mentioned above, ex situ populations never went beyond the first generation. Consequently, inbreeding due to small ex situ population size and adaptation processes (Carroll et al., 2007; Sun et al., 2022), hybridisation with other species (Zhang et al., 2010) and mixing of ex situ populations (Vergeer et al., 2005) can most likely be excluded as reasons for the observed genetic patterns. However, seed sampling, cultivation conditions and genetic changes in the source population may have affected genetic differentiation (Ensslin et al., 2015; Iglesias-Andreu et al., 2017).

If seed sampling has not been conducted representatively across the whole population, optimally at different points in time, certain

genotypes might not be included in the ex situ populations (Brown & Briggs, 1991; Guerrant et al., 2004), which may have caused the genetic differentiation. This effect was shown for previous ex situ cultivations of wild populations (Iglesias-Andreu et al., 2017; Yokogawa et al., 2013). This explanation may be particularly relevant for *A. liliifolia* and *T. europaeus*. As mentioned above, these species show higher relatedness among individuals in the ex situ populations than among individuals in the source populations. They are also the species for which the genetic structure of the source populations is only partially reflected in the ex situ populations.

Furthermore, cultivation conditions may also have affected genetic differentiation. Different conditions in the ex situ environment compared to the wild environment may lead to different selection pressures, and plants may also be subjected to artificial selection by gardeners (Ensslin et al., 2015). If individuals with specific traits or certain genotypes have been selected unintentionally during cultivation, this may have increased genetic differentiation among source and ex situ populations while decreasing genetic differentiation between ex situ populations. This could explain why the ex situ populations of *D. sylvaticus* and *T. scordium* are more similar than the source populations. However, the pattern could also have been caused by accidental interbreeding within or among the ex situ populations, resulting in unintended second-generation individuals. Though the conservation cultures were intended to maintain only the original generation derived from wild-collected seeds and inflorescences were cut to prevent seed set, such offspring may have gone unrecognised and been inadvertently included in the analysis. However, *A. liliifolia* and *T. europaeus* show the opposite pattern, and cultivation conditions may not lead to unintended selection in some species. Although genome-scan approaches can identify candidate loci potentially under selection (de Villemereuil & Gaggiotti, 2015; Foll & Gaggiotti, 2008), the limited number of independent wild-ex situ comparisons in our dataset restricts the power of such analyses, and they were therefore beyond the scope of this study.

Finally, high levels of genetic differentiation between source and ex situ populations may have resulted from genetic drift in the source populations. This drift could be explained by disturbance events during which a large part of the source population, and therefore parts of genetic diversity, can be lost (Banks et al., 2013; García-Montes et al., 2020; Godhe & Ryneerson, 2017; Lazcano-Cruz et al., 2025; Markert et al., 2010). This may have occurred in Tro_eur_01 and Tro_eur_02. Compared to Tro_eur_03, they show much higher genetic differentiation between the source and ex situ populations, possibly because both source populations were reduced to three and five individuals, respectively. This interpretation is supported by the observed trend that, in three of the four species, smaller source populations are more strongly differentiated from their ex situ populations than larger source populations and by the fact that many source populations show signs of a recent bottleneck.

Although closely capturing the genetic variation and structure of wild populations remains a primary goal of ex situ conservation, there may be cases in which genetic differentiation between wild and ex situ populations is not necessarily detrimental. Differentiation

resulting from the decline of wild populations, and the associated genetic drift, may preserve genetic variation in ex situ populations that has already been lost in the wild. Furthermore, genetic differentiation may prove beneficial when reintroduction under the original environmental conditions is not possible, as the presence of different alleles could facilitate the colonisation of alternative habitats. However, because the advantages or disadvantages of specific alleles under altered environmental conditions cannot be predicted in advance, these considerations should not alter the primary objective of maintaining low genetic differentiation between source and ex situ populations.

5 | CONCLUSIONS WITH RESPECT TO CONSERVATION

As populations are adapted to local conditions and often show the highest level of fitness under these conditions (Vander Mijnsbrugge et al., 2010), keeping genetic differentiation between wild and ex situ populations low is a main goal of ex situ conservation. However, this was not the case in our study. A combination of unrepresentative sampling, cultivation conditions and genetic drift in the source populations is likely responsible for this differentiation. These results emphasise that ex situ populations should be cultivated for as short a time as possible (Carroll et al., 2007). This could minimise the impact of genetic drift in source populations and the effects of unintentional selection. The strong genetic differentiation could further be reduced by regularly introducing seeds from the source population (Forgiarini et al., 2023), which would also increase genetic diversity in the ex situ population. Finally, this study underlines that genetic monitoring is essential to survey the success of the ex situ cultivation.

AUTHOR CONTRIBUTIONS

Ellen Gradl and Christoph Reisch developed the study design; Maximilian Alexander Steurer collected the data; Maximilian Alexander Steurer, Ellen Gradl and Lena Winkler analysed the data; and Maximilian Alexander Steurer led the writing of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study can be found at the UR data hub under <https://www.doi.org/10.5283/epub.80471>.

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SUPPORTING INFORMATION

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