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Genetic structure of the European white elm (*Ulmus laevis* Pall., Ulmaceae) in Switzerland

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Abstract

Key message Populations of the European white elm (*Ulmus laevis* Pall.) in Switzerland can be considered natural. They show no evidence of genetic differentiation from other European populations. In the past, the *U. laevis* populations were probably more widespread and continuous in Switzerland with a larger gene flow.

Context In Switzerland, at the margin of its distribution range, *U. laevis* is rare and considered endangered. Whether the species is native to Switzerland has been disputed, and it is often surmised to be solely cultivated, without any natural population in the country.

Aims The structure of genetic diversity among Swiss populations of *U. laevis* and comparison to European populations are expected to shed light on the origin of local populations and support their management.

Methods We analyzed 19 populations (194 individuals) in Switzerland and 15 populations (158 individuals) from other European countries, using a set of five microsatellite loci.

Results (1) 90% of the genetic variation in European and Swiss populations occurs within populations. (2) We did not detect isolation by distance at the regional or continental scale. (3) Clustering analysis did not reveal any spatial pattern in the level of admixture of individuals within Swiss or other European populations.

Conclusion Moderate levels of genetic diversity and evidence for recent gene flow between populations indicate that habitat deterioration, loss, and fragmentation are the main threats to the persistence of *U. laevis* populations in Switzerland.

Keywords Elms, Floodplain forests, Microsatellites, Phylogeography, Rhine, SSR

1 Introduction

Riparian forests, or floodplain forests, characterized by dynamic environmental conditions, are valuable habitats due to their high level of biodiversity, which supplies a multitude of environmental, economic, and aesthetic benefits (Gregory et al. 1991; Sakio and Tamura 2008). Floodplain forests are, however, rare and endangered habitats in Europe, and it is estimated that up to 88% of their potential range has disappeared (Hughes and Rood 2003). The major threats to floodplain forests are linked to human disturbance, particularly due to clearing for agriculture and urban development, river channelization, and disruption of river ecosystems through the

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construction of artificial barriers, such as dams (Imbert and Lefèvre 2003; Smulders et al. 2008; Lachat 2010). Currently, there is increasing interest in restoring riparian habitats (Janssen et al. 2021). Understanding the genetic structure of populations of threatened species helps the management and restoration of their habitats (Holderegger and Wagner 2008; Yang et al. 2015) and is particularly crucial for riparian forest trees (Wei et al. 2015).

We focused our study on the European white elm (*Ulmus laevis* Pall.), a large deciduous temperate tree that is distributed principally near rivers, streams, large lakes, and floodplains at low elevations (Collin 2003; Caudullo and De Rigo 2016). It is a distinctive element of riparian forest communities (Mackenthun 2004; Müller-Kroehling 2019). *U. laevis* reproduces through short-distance seed dispersal by wind and long-distance dispersal by water as well as through vegetative propagation (Caudullo and De Rigo 2016). The species is self-incompatible and strictly outcrossing, and no hybrid with the main sympatric species in Europe, such as *U. glabra* Huds. and *U. minor* Mill. has been described (Mittempergher and Porta 1991).

The natural distribution of *U. laevis* ranges from central France to the Ural Mountains and from South Finland to the Caucasus and Albania. The European white elm is the rarest and least studied among the naturally occurring elm species in Europe, such as the wych elm (*U. glabra*) and field elm (*U. minor*) (Caudullo and De Rigo 2016). The morphological similarity of these elm species is clearly one of the reasons for the deficiency in knowledge about their characteristics, especially in their respective distributions (Fragnière et al. 2022). In many European countries, the abundance of elms has decreased significantly in recent centuries. *U. minor* and *U. glabra* were largely affected by the pandemic waves of Dutch elm disease (Collin 2003; Caudullo and De Rigo 2016). *U. laevis* is more resistant to this disease but is largely impacted by habitat destruction. In the marginal countries of the natural distribution of *U. laevis* (e.g., Spain, Finland, and Denmark), the species is considered endangered (Vakkari et al. 2009; Nielsen and Kjær 2010; Venturas et al. 2015).

In Switzerland, *U. laevis* is rare and considered endangered, with only scattered occurrences along rivers and lakes (Schwab 2001; Fragnière et al. 2024). However, there is no agreement whether *U. laevis* populations are natural or were planted in Switzerland. Indeed, *U. laevis* was considered to be a cultivated tree (Welten and Sutter 1982; Lauber et al. 2018). In contrast, in neighboring France and Germany, the species is considered natural (Rameau et al. 1989; Timbal and Collin 1999; Aas 2019; Müller-Kroehling 2019). A plantation in Lobsigen (canton of Bern) is used for the production of forest reproductive material: 78 mature trees from different Swiss origins have been grown there since the 1990s for the production of seeds. Seedlings produced from these seeds are occasionally planted. However, a recent study of the distribution and ecology of *U. laevis* proposed that several populations should be considered natural in Switzerland (Fragnière et al. 2024).

One of the consequences of the considerable habitat reduction, as well as fragmented distribution and small population sizes of *U. laevis,* is its susceptibility to genetic drift and loss of genetic diversity (Collin 2003). Indeed, previous studies consistently showed low genetic variability in the species, especially at the margin of its distribution in Northern and Western Europe (i.e., Denmark, Finland, and Spain) (Whiteley 2004; Vakkari et al. 2009; Nielsen and Kjær 2010; Fuentes-Utrilla et al. 2014; Kavaliauskas et al. 2022), and moderate genetic diversity at the core of its distribution in Central and Eastern Europe (i.e., Poland and Lithuania) (Tamošaitis et al. 2021; Litkowiec et al. 2022). A recent study of nuclear microsatellite loci and chloroplast DNA (cpDNA) in Spain, northwestern Italy, and Serbia revealed that relict populations serving as a reservoir for the genetic diversity of this species may exist (Fuentes-Utrilla et al. 2014; Torre et al. 2022).

The aim of the present study was to evaluate the diversity within and among populations of *U. laevis* in Switzerland and to compare this diversity with that of a few populations across Europe with available microsatellite markers. Together with a companion study (Fragnière et al. 2024) exploring the distribution, population structure, history, and ecology of the species, this study will bring valuable insights for the management and conservation of *U. laevis*. The following questions were specifically addressed: (1) can we identify individuals of the species *U. laevis* among other *Ulmus* species based on multilocus genotypes at microsatellite markers?, (2) what is the level of genetic structure among individuals and populations of *U. laevis* at continental scale?, (3) do the Swiss populations show particular genetic structures when compared to other populations, and therefore suggest the current populations were introduced to Switzerland?, and (4) does the *U. laevis* tree collection in Lobsigen represent the actual genetic diversity of the species in Switzerland?

2 Materials and methods

2.1 Plant material collection

Detailed information about species status is provided in Fragnière et al. (2024). All study stands fulfilled the following criteria: (1) they consisted of a mixture of trees of different diameters and ages, (2) trees were scattered, neither clustered nor growing in rows, (3) several stands or

individuals exist along the same river or lake, (4) extant or historical habitat was a riparian zone and thus suitable for *U. laevis*, and (5) planting appeared unlikely. Based on field assessment, stands were classified into three categories: (A) *most probably natural*: matching 4–5 of these criteria, (B) *potentially natural*: matching 2–4 of the criteria and C) *known or probably planted stands*: which do not match none of the criteria or are known to be planted (for more details on the criteria see Fragnière et al. 2024). No samples were collected from stands recorded as planted. Ten to 13 individuals of *U. laevis* were collected from 19 stands (Table 1), representing a

Table 1 Genetic diversity within sampled populations of *Ulmus laevis* in Switzerland and Europe based on 5 microsatellite loci

Site ID	Location	*n*	sM	MPI	Na	Ne	Ar	Np	Ho	He	F_{IS}
CH_RoYvo	Yvorne	5	–	0,0092	2.4	1.81	2.10	0	**0.560**	0.489	−0.055
CH_LNGra	Grandson-Concise	13	–	0,0004	**3.6**	**2.82**	**3.1**	0	**0.692**	**0.604**	−0.117
CH_LNCon	Concise-Vaumarcus	12	–	0,0005	**3.4**	**2.78**	**3.00**	0	**0.765**	**0.633**	−0.240
CH_LNMar	Marin-Epagnier	13	–	0,0005	**3.6**	**2.70**	**3.00**	0	**0.646**	**0.614**	−0.041
CH_LNCud	Cudrefin	9	–	0,0009	**3.6**	**2.48**	**3.03**	0	0.533	**0.565**	−0.044
CH_LMGre	Grengspitz	12	–	0,0036	3.2	2.00	2.61	0	0.483	0.496	−0.072
CH_LMFao	Faoug	13	–	0,0023	**3.4**	2.10	**2.73**	0	0.554	**0.529**	−0.141
CH_LBSut	Sutz-Nidau	11	–	0,0029	2.8	2.18	2.46	0	0.545	**0.537**	0.037
CH_SaFri	Fribourg-Rossens	12	–	0,0025	**3.4**	2.1	**2.72**	0	0.468	**0.529**	0.07
CH_SaBro	Broc-Grandvillard	13	–	0,0017	**3.4**	**2.23**	**2.71**	0	**0.615**	**0.564**	−0.088
CH_AaMue	Münsingen-Belp	12	2	0,0016	3.2	**2.45**	**2.75**	0	**0.667**	**0.528**	−0.249
CH_AaUtt	Uttigen-Kiesen	13	–	0,0023	3.2	2.20	**2.69**	0	**0.600**	0.511	−0.134
CH_TSInt	Interlaken	3	–	–	NA	NA	NA	NA	NA	NA	NA
CH_AaAar	Aarberg	5	–	0,0006	**3.4**	**2.55**	**3.09**	0	**0.600**	**0.653**	−0.007
CH_AaBru	Brugg-Aarau	10	–	0,0015	**3.4**	**2.36**	**2.75**	0	**0.640**	**0.565**	−0.182
CH_DbDoe	Döltschi Zürich	9	2	0,0354	2.2	1.51	2.04	0	0.311	0.316	−0.038
CH_ThBis	Bischofszell	10	2,2	0,0184	2.8	1.66	2.17	0	0.480	0.392	−0.159
CH_RhRhe	Rheineck	9	2	0,0044	3.0	2.02	2.52	0	0.550	0.507	−0.048
CH_WiLan	Lange-Erlen	10	–	0,0018	**3.4**	2.17	**2.81**	0	**0.600**	**0.544**	−0.143
FR_ThPul	Pulversheim	13	–	0,0010	**3.6**	**2.33**	**2.98**	0	**0.585**	**0.567**	−0.078
FR_RhRhi	Ile de Rhinau	13	–	0,0036	**3.8**	2.02	2.65	0	0.508	0.508	−0.027
DE_RhOet	Ötigheim	12	–	0,0026	**3.6**	2.15	**2.73**	0	**0.567**	0.509	−0.150
DE_RhKir	Kirrlach	13	–	0,0029	**3.4**	2.16	2.61	0	**0.569**	0.506	−0.126
DE_ItEbe	Eberbach	13	–	0,0024	3.2	2.19	2.62	0	**0.585**	0.525	−0.157
DE_MaElm	Elmuss	13	–	0,0039	**3.6**	2.07	2.66	0	0.446	0.457	−0.001
DE_DoErn	Ernsgaden	13	–	0,0008	**3.6**	**2.65**	**2.99**	0	0.508	**0.564**	0.025
FR_SaGra	Gray	6	–	0,0031	2.8	2.16	2.60	0	0.500	0.524	−0.071
FR_RoGri	Grigny	5	–	0,0022	2.8	**2.24**	2.60	0	**0.760**	**0.600**	−0.322
ES_CcJar	Cáceres Jaraíz	9	2	0,0228	2.0	1.65	1.93	0	0.422	0.378	−0.136
ES_SePal	Segovia Palazuelos	10	–	0,0041	2.8	2.14	2.45	0	0.540	0.489	−0.042
PL_WaDeb	Debina	13	–	0,0031	**3.4**	2.18	2.64	0	0.477	0.480	−0.072
PL_WaRog	Rogalin	13	2	0,0032	**4.4**	2.06	**2.89**	2	0.446	0.465	−0.037
PL_WiKra	Krakow	5	–	0,0013	2.8	**2.43**	**2.72**	0	**0.720**	**0.613**	−0.239
HU_DrBar	Barcs	7	2	0,0019	3.2	2.20	**2.82**	0	0.486	**0.554**	**0.166**
CH_Plant	Plantation Lobsigen	19	–	–	**4.4**	**2.70**	**3.14**	0	**0.621**	**0.583**	−0.125
Mean Switzerland		**10.21**			**3.2**	**2.23**	**2.68**	**0**	**0.573**	**0.532**	**−0.092**
Mean Europe		**10.00**			**3.3**	**2.18**	**2.66**	**0.133**	**0.541**	**0.516**	**−0.084**
Total mean		**10.11**		**0.0045**	**3.2**	**2.21**	**2.67**	**0.061**	**0.558**	**0.525**	**−0.088**

n sample number, *sM* same multilocus, *MPI* multilocus probability of identity, *Na* mean no. of alleles, *Ne* mean no. of effective alleles, *Ar* mean allelic richness, *Np* no. of private alleles, *Ho* observed heterozygosity, *He* expected heterozygosity, F_{IS} Inbreeding coefficient. Different colors refer to the country of collection (*CH* Switzerland, *FR* France, *DE* Germany, *ES* Spain, *PL* Poland, *HU* Hungary, and *BG* Bulgaria). The order mainly follows the water basins starting south to north. Values above the total mean are shown in bold. Only within-population identical genotypes are shown. The plantation values are not included in the means. Due to low population sizes, the Swiss population CH_TSInt was not included in the analysis

total of 194 samples across the whole distribution of the species in Switzerland (Fragnière et al. 2024). Leaf samples from individuals at least 20 m apart were collected to avoid clones (e.g., via root suckers) and dried in silica gel. If the population size was low, as many trees as possible were sampled. Additionally, 19 individuals were also sampled from a seed tree plantation in Lobsigen. In addition, 158 individuals from 15 populations from Germany (5 populations), France (4), Poland (3), Spain (2), and Hungary (1) were collected by the authors or local researchers, and 39 samples of outgroup species (10 *U. glabra*, 10 *U. minor*, and 19 *U. americana* from Illinois and Indiana (USA)) were included in our analysis. Twenty-four among the 34 populations were located along the Rhine River in France and Germany (Dermelj et al 2024).

2.2 DNA extraction, PCR, and nuclear microsatellite (nSSR) genotyping

Approximately 10 mg of lyophilized leaves were ground with a TissueLyser II (QIAGEN). Genomic DNA was extracted using the DNeasy Plant Mini Kit and DNeasy 96 Plant Kit (QIAGEN) according to the manufacturer's instructions. Samples were randomized, and negative controls were included in all of the 96-well plates to monitor possible contamination. DNA concentration, estimated with a spectrophotometer (Nanodrop), was in the range 5–20 ng/µl ($n=371$ of *U. laevis* + 39 reference samples).

The samples were genotyped at seven nuclear microsatellite loci (Ulm2, Ulm3, Ulm6, Ulm8, Ulm9, Ulm12, and Ulm19, Whiteley et al. 2003) grouped into two multiplexes for polymerase chain reactions (PCRs). The primers used for MP1 were Ulm12, Ulm19, and Ulm6, and the primers used for MP2 were Ulm2, Ulm3, Ulm8, and Ulm9. The following PCR program was used: (1) denaturation of 5 min at 95 °C, (2) 26 cycles of 30 s at 95 °C, 30 s at annealing temperature (54 °C for MP1 and 63 °C for MP2), and 30 s at 72 °C, and (3) a final 20-min extension period at 72 °C. Amplification by PCR was achieved in a 10 µl reaction volume with 5 µl of Type-it Multiplex PCR Master Mix (QIAGEN), 0.2 µl BSA (only MP 1), and 0.5 µl of each primer (10 µM, F-primer labeled with fluorochrome). Amplified DNA was diluted with 20 µl of deionized water, and 1 µl of each solution was mixed with 10 µl of Hi-Di formamide containing size standard dye (Orange 500 bp, NimaGen) for capillary electrophoresis on a 3130 Genetic Analyser (Applied Biosystems). Alleles were scored for all 7 microsatellite loci with GeneMarker 2.6.3 (Holland and Parson 2011). We excluded locus Ulm12 (monomorphic) and Ulm8 (difficult to score). Genotyping error was estimated based on 30 (7.3%) duplicate samples (Bonin et al. 2004).

We estimated the probability that two unrelated individuals taken at random in the population share the same multi-locus genotype (PI; Waits et al. 2001) using GENALEX 6.5 (Waits et al. 2001; Peakall and Smouse 2012). Given the low power of our set of five microsatellites, we could not exclude that two unrelated individuals could share the same genotype and we therefore kept identical genotypes for genetic diversity analysis.

2.3 Genome size and ploidy level

The ploidy level of selected samples was estimated by flow cytometry (Bourge et al. 2018). For each collected population, 1–2 individuals were randomly selected and sent to Plant Cytometry Services (Didam, The Netherlands, www.plantcytometry.nl) to estimate genome size using propidium iodide staining and external standards (i.e., *Clivia miniata*, 2C = 35.77 pg, and *Allium schoenoprasum*, 2C = 15.03 pg). The results were returned as 2C DNA contents (2C-values given in picograms, pg). The ploidy level of samples was inferred by comparing 2C DNA in picograms to thresholds published for *Ulmus* taxa (Whittemore and Xia 2017; Šmarda et al. 2019; Zonneveld 2019).

2.4 Genetic diversity analysis

We used GENALEX 6.5 (Peakall and Smouse 2012) to estimate the number of alleles (N_A), effective number of alleles (N_E), number of private alleles (N_{priv}), and observed and expected heterozygosity (H_O, H_E), including deviation from HWE and inbreeding coefficient (F_{IS}).

We used the statistical program R (R Core Team 2022) and the package PopGenReport (Adamack and Gruber 2014) to estimate allelic richness (A_R; El Mousadik and Petit 1996) and to estimate the frequency of null alleles based on apparent excess homozygotes relative to Hardy–Weinberg equilibrium (HWE).

2.5 Genetic structure analysis

The program STRUCTURE v2.3.4 (Pritchard et al. 2000) assigns multilocus genotypes to K clusters to optimize HWE following an MCMC procedure. We set the parameter to correlated allele frequency and admixture models, thus assuming some level of gene flow between populations. We inferred the genetic structure within *U. laevis* population based on 100,000 cycles (initial burn-in of 50,000) and ran 20 iterations for each $K=1–10$. The output was analyzed using Structure Harvester (Earl and VonHoldt 2012), which allowed us to find the best fitting K value (through a LnP(D) plot) and highest ΔK value (Evanno et al. 2005) and visualize Q-values using the program R (R Core Team 2022).

We used the program GenAlEx 6.5 (Peakall and Smouse 2012) to decompose the genetic variability

observed in the European and Swiss White elm population of *U. laevis* into within-individuals, inter-individuals with populations and among-populations components using an analysis of molecular variance (AMOVA), test for genetic structuring based on 999 random permutations, and pairwise F_{ST} estimates between populations in Switzerland and other countries.

We tested for correlation between log-transformed pairwise geographic distance (km) and pairwise linearized F_{ST} ($F_{ST}/(1\text{-}F_{ST})$; Rousset 1997) using Mantel tests (Mantel 1967), as implemented in GENALEX 6.5 (Peakall and Smouse 2012). Mantel tests were performed at three spatial levels: at the European scale with all *U. laevis* populations, among populations along the Rhine River, and among Swiss populations. Additionally, we performed individual-based tests between pairwise geographic distances and pairwise linearized genetic distances.

3 Results

3.1 Genome size and ploidy level

Consistent with the expected diploid samples of *U. laevis*, all populations investigated here presented 2C values ranging from 3.14 to 3.88 pg of DNA (see dataset Dermelj et al 2024, Table S3), matching values from previous studies ranging from 2.76 to 4.17 pg (Šmarda et al. 2019; Zonneveld 2019). Our flow cytometry analysis also revealed two ploidy levels in the *U. americana* populations, with two diploid samples showing 2C values of 3.73 and 4.23 pg, respectively, and two tetraploid samples showing 2C values of 6.74 and 7.04 pg, respectively. Matching values ranging from 6.00 to 6.57 pg for tetraploid *U. americana* were reported in Whittemore and Xia (2017).

3.2 Genetic diversity of *U. laevis* populations

The estimated frequencies of null alleles were well below the threshold of 20% (range 0–7.7%), suggesting that we could neglect the risk of significant underestimation of H_E (Chapuis and Estoup 2007). Based on 30 replicated samples, we could only detect genotyping errors at Ulm19 (11.67% allelic dropout or false alleles) and we therefore kept all loci for further analyses.

Allele frequencies across all populations and loci did not show significant deviation from HWE. We did not observe major differences in genetic diversity between Swiss and other European populations. We observed a total of 29 alleles, with the number of alleles per locus ranging from 2 (Ulm6) to 10 alleles (Ulm9); (see dataset Dermelj et al 2024, Tables S1 and S2). The number of alleles per locus ranged from 2.0 to 4.8, an effective number of alleles (N_E) ranged from 1.6 to 2.9, allelic richness (A_R) ranged from 1.9 to 3.6 and expected heterozygosity (H_E) ranged from 0.36 to 0.66. Samples from a Polish population showed one private allele each at locus Ulm3 and Ulm9. Mean expected heterozygosity (He) and mean observed heterozygosity (Ho) across loci and populations were similar across the Swiss and other European samples (Table 1). Values of inbreeding coefficients (F_{IS}) measured across populations suggested an excess of heterozygosity in the Swiss ($t = -4.4815$, df = 17, $p < 0.01$) and other European populations ($t = -2.8562$, df = 14, p value = 0.01).

Based on multilocus genotypes at the five microsatellites, the 194 samples from Switzerland and the 158 samples from across Europe were clearly distinguishable from other elm species *U. glabra*, *U. minor*, and *U. americana* (Fig. 1). Four individuals from Switzerland, identified as *U. laevis* based on morphological characters were assigned to another elm species based on their multilocus genotype, and were excluded from further analyses. Our set of microsatellite markers did not reliably differentiate between *U. glabra*, *U. minor*, and *U. americana*. In particular, Ulm19 and Ulm9 did not amplify in *U. glabra* and *U. minor*, and Ulm3 failed in 50% of *U. minor* samples.

3.3 Genetic structure and cluster analysis of *U. laevis* populations

Variance decomposition using an AMOVA showed that 90% of the genetic variation observed in Swiss populations occurred within populations ($F_{ST} = 0.10$, $p = 0.001$). Similar figures were observed in other European populations, where 89% of the genetic variation observed was

(See figure on next page.)

Fig. 1 Distribution of genetic diversity estimated from microsatellite data within and among populations of *U. laevis* in Switzerland and Europe. **a** Map showing the genetic structure and admixture of all *U. laevis* sampling localities (for $K = 4$). The assigned genetic groups are represented in different colors (red, yellow, orange, and green) according to **b**. The inset map shows the populations analyzed from Spain, Poland, and Hungary. Numbers in brackets indicate the population size. The outgroups are not represented on the map. **b** Summary of structure outputs, based on the whole dataset and $k = 3$–5, illustrating the power of our set of microsatellite markers to differentiate between *Ulmus laevis* and other *Ulmus* species ($k = 3$) and identify peculiar populations at European and regional scale. Populations showing peculiarities ($k = 4$–5). Each of the 371 *U. laevis* individuals (+ 39 outgroup individuals) is represented by a vertical line assigned to the clusters that are represented in different colors (red, yellow, orange, green, and blue). Populations are labeled with abbreviations as in Table 1 and grouped into countries (CH, FR, DE, ES, PL, and HU). Detailed outputs following the hierarchical approach described in the M&M section are presented in the dataset (Dermelj et al 2024, Figure S2)

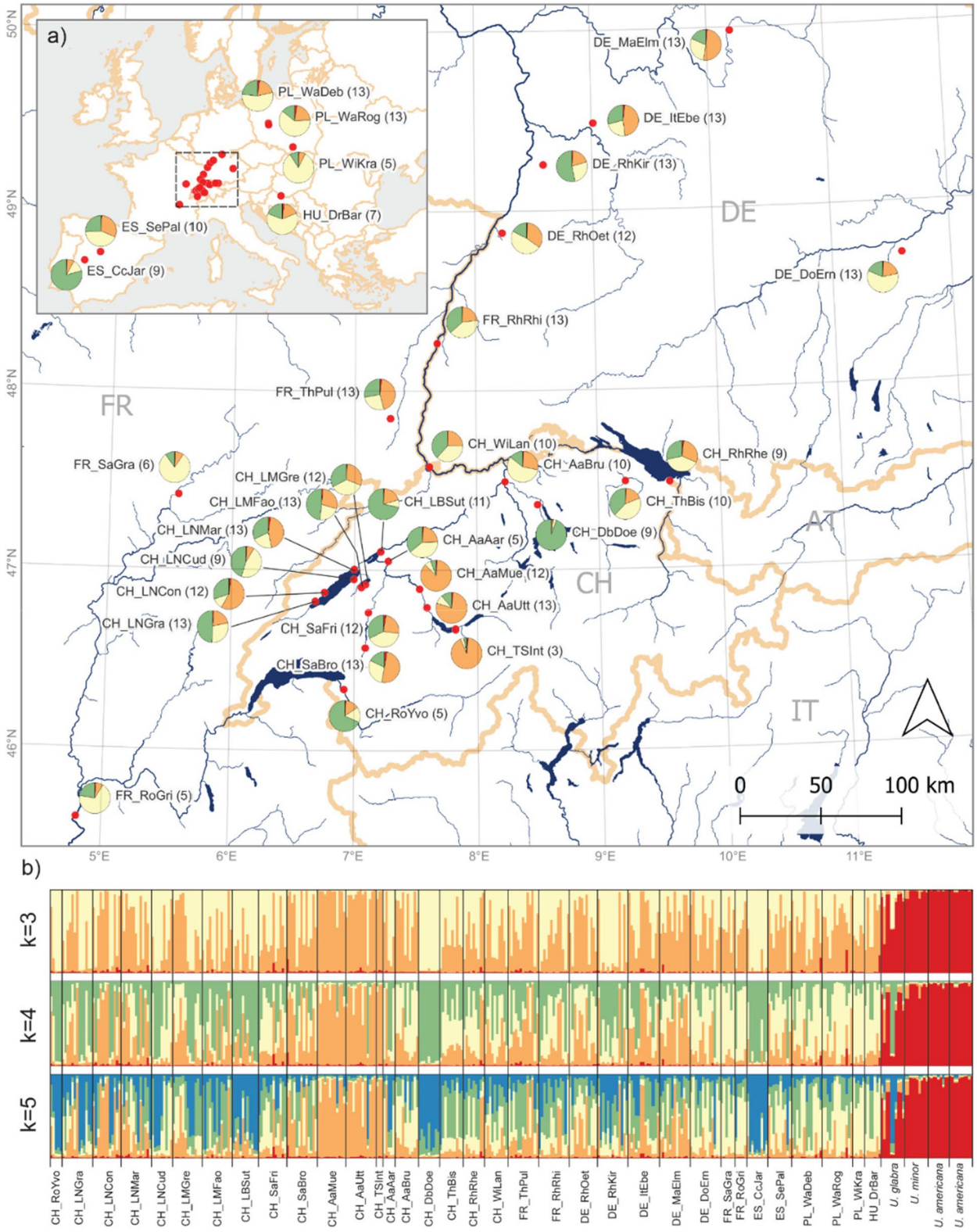

Fig. 1 (See legend on previous page.)

distributed within populations ($F_{ST}=0.11$, $p=0.001$). We detected no pattern of isolation by distance among *U. laevis* populations, but a positive correlation was detected based on analyses at the individual level ($r=0.073$, $p=0.001$). It remains questionable whether such a low level of increase in relatedness, calculated on log-transformed geographic distances, is biologically relevant.

Cluster analysis indicated that individual genotypes of white elms within European and Swiss populations showed various proportions of admixture between two or more genetic clusters (Fig. 1). Analysis using the whole data set unambiguously assigned *U. laevis* individuals to two admixed clusters, whereas other elm species were assigned to a third genetic cluster. We did not observe a spatial pattern in the proportion of the two clusters in white elm. Once restricted to *U. laevis* individuals, the genetic structuring of the species at the continental scale was best explained by the contribution of two genetic clusters, with a second peak in Δk suggesting the contribution of up to five genetic clusters. We did not observe a spatial pattern in the level of admixture at $k=2$ or $k=5$, although the latter analyses better illustrated the genetic peculiarity of the Spanish population ES_CcJar (not further discussed) and Swiss population (CH_DbDoe). Within Switzerland, CH_DbDoe, showed a high level of genetic differentiation from all other Swiss populations, and three populations situated along the Aare River (CH_AaUtt, CH_AaMue, and CH_TSInt; Fig. 1b) showed a strong gradient in allele frequencies at one or more allele at each locus.

The white elm plantation in Lobsigen (CH_Plant) showed a larger number of alleles, a higher level of allelic richness, observed and expected heterozygosity (Table 1) in comparison with other Swiss populations, and showed the lowest mean value of pairwise F_{ST}. The clustering analyses did not show differences in the level of admixture between trees from the plantation in Lobsigen and trees from the other Swiss populations (see dataset Dermelj et al 2024, Figure S1).

4 Discussion

The present work is the first investigation of the distribution of genetic diversity within *U. laevis* across the species distribution range in Switzerland, enabling comparison with other European populations. However, it is important to remember that our study used relatively small sample sizes (5 to 13 trees per population) and was based on only five markers.

Our previous results indicate that *U. laevis* can reliably be identified in the field, based on phenotypic characters and general habitus (Fragnière et al. 2022). With the present study we could additionally confirm that microsatellite genotypes can be used to distinguish *U. laevis* from other European elm species, as well as diploids and tetraploids of *U. americana* (Tamošaitis et al. 2021; Kavaliauskas et al. 2022). Interestingly, markers developed for *U. laevis* appear poorly transferrable among related species, such as *U. glabra* and *U. minor* (Whiteley et al. 2003; Kavaliauskas et al. 2022).

At the regional and continental scale, our results conform with the previous findings that the genetically diverse and closely related populations from Poland and Lithuania are located in the core of the natural range of the species (Tamošaitis et al. 2021; Litkowiec et al. 2022), whereas peripheral populations from Spain, Southern Germany, Switzerland, Denmark, and Finland show low levels of genetic diversity and high levels of genetic differentiation (Vakkari et al. 2009; Fuentes-Utrilla et al. 2014).

We did not observe significant differences in genetic characteristics between Swiss and other European populations, which is consistent with the shared history of European populations. Low levels of genetic differentiation among *U. laevis* populations (this study, Litkowiec et al. 2022; Kavaliauskas et al. 2022) are consistent with the species outcrossing mating system and long-range seed and pollen dispersal through wind and water (Hamrick and Godt 1996). We did not detect evidence for isolation by distance in the species at the regional or continental scale, nor did we observe any spatial trend in the assignment of individuals to genetic clusters within populations, which reflects ongoing or recent gene flow. The observed excess of heterozygotes in the population ($F_{IS}<0$) may be explained by three traits: (1) partial clonality, which maintains higher levels of genetic variation than sexual reproduction, (2) a low number of genetically diverse breeders and (3) by ongoing gene flow. We conclude that the majority of *U. laevis* populations in Switzerland can be considered natural.

Two cases are somewhat uncertain and need some additional explanation. First, the population in Döltschi (next to Zürich, CH_DbDoe) consisted of 31 trees distributed along an urbanized creek in close proximity to settlements. The combination of untypical habitats, peculiar values of genetic diversity, and high levels of genetic differentiation from other Swiss populations, suggests that this stand has been planted or may originate from seeds that escaped from cultivation. This population is probably not of natural origin. Second, the populations situated along the Aare River between Bern and Thun (CH_AaMue, CH_AaUtt, and CH_TSInt) consisted of trees distributed along the levee that colonized the area approximately 100 to 200 years ago, after the Aare was channelized in 1824 (Lachat 2010). Characteristics of the population and moderate levels of genetic differentiation suggest that this population may be of natural

origin (Fragnière et al. 2024). However, as mentioned above, it is important to remember that the present study used relatively small sample sizes and a reduced number of markers. Nevertheless, the observed gradient in allele frequencies between the three populations might suggest a demographic bottleneck followed by a recent expansion. Indices of genetic diversity were marginally lower in the Aare compared to other Swiss populations, which could suggest that the demographic bottleneck was short and/or that the founder population consisted of several unrelated trees.

Finally, the plantation of Lobsigen, founded in the 1990s for seed production, showed high levels of genetic diversity and low levels of genetic differentiation. Trees in the plantation also show a similar pattern of admixture as other Swiss populations (see dataset Dermelj et al 2024, Figure S1). Thus, this plantation adequately represents the genetic diversity of Switzerland, which is encouraging for ex-situ conservation management in Switzerland.

5 Conclusion

The insights gained from our genetic diversity and structure analysis together with the field observations (Fragnière et al. 2024) indicate that most *U. laevis* populations in Switzerland can be considered natural. Swiss populations, being at the southern margin of the distribution range of *U. laevis* with very scattered occurrences, show no evidence of genetic differentiation from other European populations. *U. laevis* was probably more widespread and continuous in Switzerland with a larger gene flow in the past and is now fragmented due to human-induced habitat fragmentation.

U. laevis in Switzerland does not appear to be critically threatened by loss of genetic diversity. Additionally, the genetic diversity of Swiss *U. laevis* is well represented in the nursery plantation of Lobsigen; thus, it can be regarded as a good ex-situ conservation measurement. The threat of habitat loss and population fragmentation in Switzerland is greater than the risk of genetic depression. Therefore, the maintenance and revitalization of riparian forests in Switzerland is of crucial importance.

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Authors' contributions
LD sampled the plant material, carried out the lab analyses, and wrote the first draft of the manuscript. YF developed the general concept of the study, sampled the plant material and field data, and prepared the tables and figures. GJ supervised the lab and data analysis and revised the final version of the manuscript. NK sampled the plant material and field data. JS sampled the plant material and field data. GK developed the general concept of the study, assumed the project administration and funding acquisition, sampled the plant material, and revised the manuscript. CP developed the general concept of the study, supervised the lab work and data analyses, and revised the final version of the manuscript. All authors read and approved the final manuscript.

Code availability
Not applicable.

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Availability of data and materials
The data are available in the Zenodo repository: https://doi.org/10.5281/zenodo.12731265

Declarations

Ethics approval and consent to participate
The authors declare that they follow the rules of good scientific practice. Not applicable.

Consent for publication
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