Population history, genetic variation, and conservation status of European white elm (Ulmus laevis Pall.) in Poland

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Abstract

Key message: The core populations of the European white elm (Ulmus laevis Pall.) located in Poland maintained slightly higher level of genetic diversity compared to the peripheral populations of this species.

Context: The most severe threat to elms is the loss of natural habitat under the pressures of agriculture and forestry as well as urbanization. The reductions in European white elm populations as well as populations of other elm species have also been caused by Dutch elm disease (DED). Previous studies have indicated a low level of genetic variation in Ulmus leavis Pall. However, in Poland, the genetic resources and demographic history of U. laevis populations remain poorly documented.

Aims: The genetic resources of U. laevis in Poland were identified and characterized. Additionally, tests were performed to identify potential bottleneck signatures and effective population sizes of the examined populations.

Methods: Polymorphism was analyzed using a set of six nuclear microsatellite markers (nSSRs) for 1672 individuals from 41 populations throughout the species range in Poland.

Results: (1) A moderate level of genetic variation was found. (2) A low genetic differentiation and lack of population structuring were identified. (3) Evidence of reduction in population size was found as a consequence of severe, past bottlenecks.

Conclusion: The loss of genetic diversity of U. laevis probably occurred in their refugia or shortly after the postglacial recolonization. This loss may have been affected by past DED pandemics similar to those seen at present.

Keywords: Central-marginal populations, Bottleneck, Microsatellites, DED pandemic, Population structure

1 Introduction

The European white elm (Ulmus laevis Pall.) is a broad-leaved tree whose occupied natural distribution in Europe extends from central France to the Urals. In the northern part of its range, white elm covers only the southern part of Finland; it reaches the southern end of its range in Albania and Bulgaria and grows in several isolated stands in Turkey (Jalas and Suominen 1999; Collin 2003). Across its natural range in Europe, white elm grows mainly in lowlands and sporadically enters mountainous areas along river valleys. Generally, it is more common in eastern Europe than in western Europe (Boratyńska et al. 2015). As the species preferentially occupies lowland areas, its optimal occurrence range covers fertile and moist habitats in river valleys; it is often found in floodplains, is tolerant to humid soils...
and periodic flooding, and typically occurs in damp, low-lying areas and as a component of riparian forests. In Poland, white elm is one of three native species of elm: the European white elm (U. laevis Pall.), wych elm (U. glabra Huds.), and field elm (U. minor Mill.). The area occupied by elms covers 17,654 ha, i.e., 0.24% of the total forested area. There are only about 1000 ha dominated by elms (Napiersa-Filipiak et al. 2014). The vast majority of elm resources are currently formed by white elm. In at least 1/3 of the sites, white elm are of artificial origin. Most often, the species is represented by isolated individuals or by small groups (Napiersa-Filipiak et al. 2016). It seldom occurs in the mountains and does not exceed foothill elevations. U. laevis can sow and grow under the canopies of old trees, and the species often emerges from dense grass covers. Therefore, in areas covered by white elm, several generations of elm forest may coexist (Filipiak and Napiersa-Filipiak 2015).

Today, the most severe threat to elms is the loss of their natural habitat under the pressures of agriculture, forestry, and urbanization. For the last hundred years, European white elm populations and other elm species have also undergone population reductions caused by Dutch elm disease (Brasier 2000), caused by the non-native fungi, Ophiostoma ulmi (Buisman) Nannf. and O. novo-ulmi Brasier, which are spread by bark beetles of Scolytus Geoffroy (Coleoptera, Scolitidae; Brasier 2001). U. laevis is the most resistant to infection (Brasier 2001). This disease spread to large areas of Europe, South America, and Asia, causing very high mortality rates, and contributing to the current very dispersed distribution. Genetic changes associated with small, fragmented populations, and increased isolation may limit the evolutionary potential of a species and affect its ability to adapt to new challenges related to climate change as a consequence of lost genetic diversity via random drift (Schaberg et al. 2008). Small, fragmented populations are more prone to adverse effects due to random processes such as the “founder” and “bottleneck” effects (Sork and Smouse 2006).

Recently, special attention has been given to the protection of remaining natural or seminatural riparian forest communities. In many countries, there are places where it has become important to re-naturalize river valleys to restore their natural, economic, and recreational value. The white elm is an extremely important element of these communities. The first step to develop a species protection strategy is to document the level and pattern of its genetic variation. Microsatellite markers of nuclear DNA are widely used in this respect (e.g., Litkowiec et al. 2018; Scotti-Saintagne et al. 2021). Previous research on the genetic structure of U. laevis in Europe using different marker systems demonstrated a low level of overall genetic variation and significant differentiation between populations, especially in peripheral populations of the species (Vakkari et al. 2009; Nielsen and Kjær 2010; Fuentes-Utrilla et al. 2014).

Microsatellite markers have been developed and successfully used for many elm species (Whiteley et al. 2003; Collada et al. 2004; Zalapa et al. 2008). However, the degree of U. laevis genetic diversity in Poland has not been determined thus far. This study was initiated to shed light on the genetic diversity and structure of many elm populations throughout their central range in Poland. We aimed to answer several questions: (1) Is the level of genetic diversity and genetic differentiation within and among examined populations comparable to other populations from entire natural distribution? (2) Is the observed genetic diversity the result of recent population decline or severe, past population bottlenecks? (3) Is the genetic structure of examined populations as a consequence of postglacial history?

2 Materials and methods
2.1 Plant sampling
This study examined 41 U. laevis populations from the entire species range in Poland (Fig. 1). The number of specimens sampled from each population ranged from 11 to 50 individuals; a total of 1672 individuals were analyzed (Table 1). Most trees were located near watercourses and lakes in fertile or moderately fertile, moist areas. Almost half of the collected material came from nature reserves. Additionally, most of the areas from which the research material was collected were located in nature protection areas (Natura 2000).

2.2 DNA extraction, amplification, and genotyping
The total genomic DNA was isolated from approximately 20 mg of leaf tissue using an ISOLATE II PLANT DNA kit (Bioline, London, UK). Suitable markers originally described for Ulmus species were tested for their ability to provide repeatable, high-quality results, sufficient polymorphism and unambiguous allele binding. Finally, six polymorphic markers: Ulm19, Ulm2, Ulm3, Ulm6, Ulm9 (Whiteley et al. 2003), and UR188a (Zalapa et al. 2008) were simultaneously amplified in a multiplex reaction using Multiplex Master Mix (Qiagen, Hilden, Germany). The polymerase chain reaction (PCR) program was as follows: 3 min at 94 °C; 30 cycles of 15 s at 94 °C, 90 s at 53 °C, and 2 min 72 °C, and 20 min at 72 °C. The fluorescently labeled PCR products, along with a size standard (GeneScan 600 LIZ, Thermo Fisher Scientific, Waltham, Massachusetts, USA), were separated on an ABI 3500 capillary sequencer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Alleles were identified based on their size using GeneMapper software (ver. 5.0; Thermo Fisher Scientific, Waltham, Massachusetts, USA), and all
variants were checked and approved manually (Litkowiec 2022).

2.3 Genetic diversity and differentiation
The following genetic diversity estimators were computed using FSTAT v 2.9.3 software (Goudet 2001): the number of alleles (A), allelic richness (AR), estimated for the minimum sample size of 11 individuals, observed heterozygosity (H_0), and unbiased expected heterozygosity (H_e). The number of private alleles (P_p) and effective number of alleles (A_e) were calculated using GenAlEx 6 (Peakall and Smouse 2006). An allele was declared “private” when it was detected only in a particular population and was absent in the other populations.

Microsatellite markers are susceptible to genotyping errors, such as null alleles (Guichoux et al. 2011), and can overestimate the F-statistic on account of false homozygotes in populations (e.g., Litkowiec et al. 2018). Therefore, the loci were also tested for the presence of null alleles (N0) using INEST 2.0 software (Chybicki and Burczyk 2009). The multiple sample score test (U test, Raymond and Rousset 1995), implemented in GENEPOP ver. 4.3 (Rousset 2008), was used to determine the significant deviation from Hardy-Weinberg equilibrium (HWE).

The genetic differentiation among populations was assessed using F_st values with FSTAT v. 2.9.3. Considering the presence of null alleles at all loci, FreeNA software was used to estimate the F_st values based on the Cavalli-Sforza and Edwards (1967) genetic distance using the Excluding Null Alleles (ENA, F_ena) correction method (Chapuis and Estoup 2007). The bootstrap 95% confidence intervals (CI) for the global F_st values were calculated using 10,000 replicates over the analyzed loci. We also compared the pairwise F_st and R_st values to indicate the phylogeographic structure using the SpaGeDi 1.3d program (Hardy and Vekemans 2002; Hardy et al. 2003). R-statistics are analogous to F-statistics but are based on allele sizes instead of allele identity; R_st assumes the diversity resulting from genetic drift and mutation processes according to a stepwise mutation model (SMM). The R_ST values were compared after the allele sizes were permuted using the within loci pR_st (permuted R_st, corresponding to F_st). The statistical significance of the alternative hypothesis of R_st > pR_st, which suggests that allele size mutations contributed to the population differentiation, was estimated by a permutation (10 000 permutations) test implemented in the SpaGeDi 1.3d program (Hardy and Vekemans 2002).

The genetic structure of the white elm populations was evaluated using the Bayesian clustering method implemented in STRUCTURE ver. 2.3.4 (Pritchard et al. 2000). The assumed parameter sets were admixture allele models with correlated allele frequencies and no prior information about the location of the analyzed population. The Monte Carlo Markov Chain (MCMC) sampling
The scheme was run for 200,000 iterations with a 100,000 iteration burn-in period; the $K$ values ranged from 1 to 41, and 10 independent replications were performed for each $K$ value. The optimal $K$ value was estimated using the StructureSelector (Li and Liu 2018) which implements the Evanno’s method (Evanno et al. 2005), as well as four alternative statistical measures (Puechmaille 2016). To check for the presence of isolation by distance

Table 1  Site locations, codes, sample sizes, and LBG code of genetic resources of the U. laevis Pall. populations studied

| No | Code | Name | Sample size | Locality | LBG Code |
|----|------|------|-------------|----------|----------|
|    |      |      |             | Latitude | Longitude |
| 1  | WIR  | Wiązy Reskie a | 50        | 15.3528  | 53.7844  | WZS/10/09   |
| 2  | GRZ  | Grądowe Zbocz a | 15        | 15.5707  | 53.2712  | WZS/10/03   |
| 3  | POJ  | Podjuchy      | 50        | 14.5656  | 53.3171  | WZS/10/02   |
| 4  | TRB  | Trawiasta Buczyna a | 50        | 14.7401  | 53.2798  | WZS/10/05   |
| 5  | ZRB  | Żródliskowa Buczyna a | 37        | 14.6939  | 53.2978  | WZS/10/04   |
| 6  | OLO  | Olszyn Ostrowskie a | 46        | 14.3515  | 52.9332  | WZS/10/06   |
| 7  | LEM  | Lemierzycy a | 50        | 14.9078  | 52.5691  | WZS/10/07   |
| 8  | SLO  | Słońsk          | 31        | 14.8254  | 52.5708  | WZS/10/08   |
| 9  | URA  | Urad           | 50        | 14.7028  | 52.2515  | WZS/14/02   |
| 10 | KLE  | Klenica        | 48        | 15.6799  | 51.9903  | WZS/14/03   |
| 11 | BIE  | Bieniów        | 50        | 15.2548  | 51.7142  | WZS/14/01   |
| 12 | BRZ  | Brzeźnik       | 50        | 15.5366  | 51.1965  | WZS/13/03   |
| 13 | WC   | Wilczków       | 49        | 16.5163  | 51.2327  | WZS/13/01   |
| 14 | URW  | Uroczysto Wrosy a | 50        | 16.5528  | 51.3654  | WZS/13/05   |
| 15 | CZL  | Czerszewski Las a | 46        | 17.5223  | 52.1342  | WZS/09/01   |
| 16 | DWU  | Dwunastak a | 40        | 17.5197  | 52.1641  | WZS/09/02b  |
| 17 | SOK  | Sokółki a     | 50        | 18.2017  | 52.2816  | WZS/09/05   |
| 18 | BIL  | Bielawy a     | 50        | 17.4672  | 52.4577  | WZS/09/03   |
| 19 | WIL  | Wielki Las a  | 43        | 16.2538  | 52.4479  | WZS/09/07   |
| 20 | BIL  | Buki nad Jeziorem Lutomskim a | 50        | 16.1305  | 52.6154  | WZS/09/06   |
| 21 | WIW  | Wielka Wieś   | 50        | 17.3474  | 54.5976  | WZS/11/01   |
| 22 | LAM  | Las Mątawski a | 31        | 18.8803  | 53.9602  | WZS/15/01   |
| 23 | ROZ  | Rogóźno Zamek a | 28        | 18.9521  | 53.5191  | WZS/12/04   |
| 24 | LOP  | Łęki na Ostrowiu Panieńskim a | 22        | 18.4078  | 53.3563  | WZS/12/03   |
| 25 | WIK  | Wielka Kępa a | 30        | 18.1880  | 53.1435  | WZS/12/02   |
| 26 | OLR  | Olszyny Rakowickie a | 50        | 19.2396  | 52.5186  | WZS/12/01   |
| 27 | LUS  | Luszyn         | 34        | 19.7379  | 52.2654  | WZS/06/02   |
| 28 | KRZ  | Krzętów        | 50        | 19.1957  | 51.0863  | WZS/06/01   |
| 29 | MEL  | Melchów       | 30        | 19.6289  | 50.7626  | WZS/02/02   |
| 30 | BAB  | Baborów       | 40        | 18.0320  | 50.1159  | WZS/02/01   |
| 31 | KLC  | Kleczanów     | 11        | 21.5265  | 50.7528  | WZS/16/02   |
| 32 | SKU  | Skuły         | 50        | 20.6729  | 51.9997  | WZS/16/03   |
| 33 | POD  | Podleż a     | 50        | 21.4821  | 51.7391  | WZS/17/02   |
| 34 | SZC  | Szczypiorno   | 50        | 20.6476  | 52.4860  | WZS/17/01   |
| 35 | TUM  | Tumiany       | 40        | 20.9637  | 53.9839  | WZS/07/04   |
| 36 | GRA  | Graniczne     | 24        | 21.0787  | 54.2841  | WZS/07/01   |
| 37 | BOB  | Bobry         | 34        | 21.3484  | 54.2873  | WZS/07/02   |
| 38 | MAX  | Mały Kamień   | 33        | 21.4564  | 54.1786  | WZS/07/03   |
| 39 | PRZ  | Przekop a    | 42        | 22.6159  | 52.3843  | WZS/05/02   |
| 40 | KAL  | Kalinik a    | 49        | 22.5761  | 52.3630  | WZS/05/01   |
| 41 | STU  | Stubno        | 19        | 23.0316  | 49.8933  | WZS/04/01   |

a Nature reserve
(IBD, Rousset 1997), a Mantel correlation test (Man-
tel 1967) was used. The significance of the correlations
between the pairwise geographic distances and pairwise
genetic distances, measured as \( F_{st}/(1-F_{st}) \), was tested
using 9,999 permutations implemented in GenAlEx 6.

2.4 Demographic history
With NeEstimator 2.01 (Do et al. 2014), the effective pop-
ulation size (\( N_e \)) of each population was estimated via the
linkage disequilibrium method (Waples and Do 2008),
assuming a random mating model and a critical allele fre-
quency (\( P_{cr} = 0.02 \)). The 95% confidence intervals (CI\( N_e \))
were determined with the jackknife method described by
Waples and Do (2008).

The examined Ulmus populations were tested for evi-
dence of genetic bottlenecks using two methods. For
each population, the \( M \)-ratios (Garza and Williamson
2001) defined as the ratio of \( k \) (number of microsatel-
pite alleles) to \( r \) (overall range in allele size, i.e., \( M=k/r \))
and Wilcoxon test for heterozygosity excess (Cornuet and
Luikart 1996) were calculated using INEST 2.2 soft-
ware (Chybicki and Burczyk 2009). This analysis was
performed using the two-phase mutation (TPM) model
with two parameters: the proportion of multistep muta-
tions (\( p_g \)) and the mean size of multistep mutations (\( \delta_g \)).
The parameters \( p_g = 0.22 \) and \( \delta_g = 0.31 \) were used as recom-
manded (Peery et al. 2012). The significance of a
potential bottleneck was tested using Wilcoxon’s signed-
rank test \( P \) values based on 1,000,000 permutations to
obtain approximate values. Also, we used microsatellite
data with the approximate Bayesian computation (ABC)
method, implemented in DIYABC v 2.0.1 (Cornuet et al.
2014) to analyze the population demographic history.
We used three different scenarios to test the changes in
population size. Scenario 1 is a constant size population
of white elm (\( N_e \) constant from past to present); scenario
2 is a population expanded recently, \( Na \) (\( N_e \) during the
expansion, \( Ne < Na \)); and scenario 3 consist of a popula-
tion that still experiencing a bottleneck, \( Nb \) (\( N_e \) during
the bottleneck, \( Ne > Nb \)). We pooled all populations into
a single sample, and for scenario construction, a total
10,000 simulations were performed to generate the ref-
ence table, and all summary statistics included in the
DIYABC were used (Cornuet et al. 2014). The posterior
probability of each scenario was assessed using logistic
approaches (Cornuet et al. 2014). The scenario with the
highest posterior probability was selected, and the asso-
ciated parameters were determined.

3 Results
3.1 Genetic diversity and differentiation
All nSSRs were polymorphic, and only 59 alleles were
detected in the studied populations, among which 14
were private alleles. The smallest number of alleles (6)
was found in the Ulm6 locus, and the highest number
(15) was found in both the Ulm3 and Ulm9 loci. Low fre-
quencies of null alleles were found, with an average fre-
quency of 0.014. As the frequency of null alleles did not
exceed the threshold (0.2) over which null alleles can
result in a significant underestimation of \( H_e \) (Chapuis and
Estoup 2007), all loci were used in the further analyses.

In general, the studied populations were characterized
by a moderate level of genetic variation (Table 2). The
mean number of alleles \( (A) \) was 4.2, ranging from 3.7 in
the LAM population to 5.7 in the KLE population. The
effective number of allele \( (A_e) \) values was much lower,
ranging from 2.1 in the LAM population to 2.6 in the
WIR population, with an average value of 2.4. Due to the
unequal sizes of the studied populations, the allelic rich-
ness (AR) values were calculated by reducing the sizes
of all populations to 11 individuals (the size of the KLC
population). The mean AR value was 4.0, and the popula-
tions were quite homogeneous, with AR values ranging
from 3.0 in ZRB and BAB to 3.9 in KLE and PRZ. Private
alleles \( (P_p) \) were found in nine populations at very low
frequencies (below 10%), with an average frequency of
5%. The largest \( P_p \) number was detected in the BOB pop-
ulation \( (P_p = 4) \), and the POD and KLE populations each
had two private alleles. The observed \( (H_o) \) and expected
\( (H_e) \) heterozygosity values ranged from 0.595 (KLE) to
0.675 (BIE) and from 0.529 (LAM) to 0.585 (BIE), re-
spectively. The mean \( H_o \) value (0.641) was higher than the
mean \( H_e \) value (0.553), indicating an excess of heterozy-
gotes \( (F_{is} = −0.149) \). The deviation of genotypic frequen-
cies from Hardy–Weinberg equilibrium (HWE) in all
studied populations was not statistically significant in all
cases. The pairwise \( F_{st} \) values ranged from \(-0.0033 \) to
0.227, with an overall \( F_{st} \) of 0.076 (CI95\% = 0.053–0.092;
\( p < 0.001 \)). The differentiation value was somewhat lower
when the null alleles were included, with an \( F_{st} \) value of
0.074 at a 95% confidence interval (CI95\% = 0.055–
0.090). This result suggests that null alleles have a non-
significant influence on the differentiation pattern among
populations.

The global genetic differentiation based on allele size
\( (R_a = 0.061; CI95\% = 0.046–0.124) \) was not signifi-
cantly different from the differentiation that accounted for
allele identities \( (pR_{st} = 0.077, p = 0.481) \), indicating the
absence of a geographic structure and that gene flow is
high compared with the mutation rate.

3.2 Genetic structure
Grouping and thus finding the optimal number of clusters
\( (K) \) is difficult because the current genetic structure of
natural species populations is multifaceted and complex,
as a consequence of the demographic, environmental,
Table 2 Parameters used to estimate the genetic structure of the *Ulmus laevis* populations studied, averaged across six nuclear microsatellite loci

| CODE | A Average number of alleles, | Ae Effective number of alleles, | AR Average allelic richness (after rarefaction), | Pa Number of private alleles, | Ho Unbiased expected heterozygosity, | He Observed heterozygosity, | N0 (%) Null allele frequency, | Fis Wright's fixation index (all not significantly differ from zero). |
|------|-----------------------------|-------------------------------|---------------------------------|-----------------------------|-------------------------------|-----------------------------|-----------------------------|--------------------------------|
| WIR  | 4.0                         | 2.6                           | 3.8                             | 0                           | 0.635                         | 0.540                       | −0.139                     | 0.18                           |
| GRZ  | 4.0                         | 2.3                           | 3.6                             | 0                           | 0.639                         | 0.542                       | −0.186                     | 0.00                           |
| POJ  | 4.0                         | 2.3                           | 3.1                             | 0                           | 0.638                         | 0.540                       | −0.214                     | 0.00                           |
| TRB  | 4.0                         | 2.3                           | 3.4                             | 0                           | 0.636                         | 0.539                       | −0.118                     | 1.02                           |
| ZRB  | 4.0                         | 2.3                           | 3.0                             | 0                           | 0.635                         | 0.538                       | −0.169                     | 0.80                           |
| OLO  | 4.0                         | 2.3                           | 3.3                             | 0                           | 0.638                         | 0.541                       | −0.135                     | 0.48                           |
| LEM  | 4.0                         | 2.3                           | 3.2                             | 0                           | 0.635                         | 0.538                       | −0.146                     | 0.33                           |
| SLO  | 4.0                         | 2.3                           | 3.5                             | 0                           | 0.634                         | 0.539                       | −0.078                     | 1.53                           |
| URA  | 4.2                         | 2.4                           | 3.3                             | 0                           | 0.637                         | 0.573                       | −0.101                     | 1.12                           |
| KLE  | 5.7                         | 2.5                           | 3.9                             | 2                           | 0.595                         | 0.573                       | −0.030                     | 2.90                           |
| BIE  | 4.8                         | 2.5                           | 3.5                             | 1                           | 0.675                         | 0.585                       | −0.144                     | 2.48                           |
| BRZ  | 4.1                         | 2.4                           | 3.6                             | 0                           | 0.640                         | 0.548                       | −0.167                     | 2.86                           |
| WIC  | 4.1                         | 2.4                           | 3.8                             | 0                           | 0.640                         | 0.549                       | −0.035                     | 2.25                           |
| URW  | 4.1                         | 2.4                           | 3.7                             | 0                           | 0.641                         | 0.550                       | −0.161                     | 0.06                           |
| CZL  | 4.0                         | 2.3                           | 3.8                             | 0                           | 0.648                         | 0.549                       | −0.069                     | 0.69                           |
| DWU  | 4.0                         | 2.3                           | 3.6                             | 0                           | 0.646                         | 0.549                       | −0.102                     | 1.91                           |
| SOK  | 4.0                         | 2.3                           | 3.1                             | 0                           | 0.645                         | 0.546                       | −0.253                     | 0.0                            |
| BIL  | 4.0                         | 2.3                           | 3.3                             | 1                           | 0.650                         | 0.547                       | −0.178                     | 0.30                           |
| WIL  | 4.0                         | 2.3                           | 3.6                             | 1                           | 0.645                         | 0.548                       | −0.114                     | 0.91                           |
| BJL  | 4.0                         | 2.3                           | 2.2                             | 1                           | 0.643                         | 0.542                       | −0.362                     | 0.00                           |
| WW2  | 4.0                         | 2.3                           | 3.6                             | 0                           | 0.635                         | 0.540                       | −0.164                     | 0.00                           |
| LAM  | 4.0                         | 2.3                           | 3.1                             | 0                           | 0.608                         | 0.529                       | −0.133                     | 1.39                           |
| ROZ  | 4.0                         | 2.3                           | 3.8                             | 0                           | 0.636                         | 0.541                       | −0.123                     | 0.56                           |
| LOP  | 4.0                         | 2.3                           | 3.3                             | 0                           | 0.634                         | 0.539                       | −0.173                     | 0.00                           |
| WIK  | 4.0                         | 2.3                           | 3.8                             | 0                           | 0.638                         | 0.544                       | −0.118                     | 0.74                           |
| OLR  | 4.0                         | 2.3                           | 3.5                             | 0                           | 0.639                         | 0.545                       | −0.111                     | 2.91                           |
| LUS  | 4.0                         | 2.3                           | 2.8                             | 0                           | 0.650                         | 0.547                       | −0.321                     | 1.61                           |
| KRZ  | 3.9                         | 2.3                           | 2.6                             | 0                           | 0.643                         | 0.539                       | −0.244                     | 0.02                           |
| MEL  | 4.0                         | 2.4                           | 3.7                             | 0                           | 0.645                         | 0.567                       | −0.110                     | 2.05                           |
| BAB  | 4.0                         | 2.4                           | 3.0                             | 0                           | 0.643                         | 0.552                       | −0.219                     | 7.40                           |
| KLC  | 4.0                         | 2.3                           | 3.7                             | 0                           | 0.639                         | 0.542                       | −0.033                     | 2.48                           |
| SKU  | 4.0                         | 2.3                           | 3.6                             | 1                           | 0.642                         | 0.542                       | −0.166                     | 1.32                           |
| POD  | 4.0                         | 2.3                           | 3.7                             | 2                           | 0.639                         | 0.547                       | −0.028                     | 0.80                           |
| SZC  | 4.1                         | 2.3                           | 3.4                             | 0                           | 0.638                         | 0.546                       | −0.177                     | 0.97                           |
| TUM  | 3.9                         | 2.3                           | 3.2                             | 1                           | 0.645                         | 0.543                       | −0.114                     | 0.51                           |
| GRA  | 3.9                         | 2.3                           | 3.4                             | 0                           | 0.646                         | 0.541                       | −0.171                     | 0.82                           |
| BOB  | 3.9                         | 2.3                           | 3.5                             | 4                           | 0.645                         | 0.540                       | −0.181                     | 0.31                           |
| MAK  | 3.9                         | 2.3                           | 3.2                             | 0                           | 0.647                         | 0.543                       | −0.157                     | 2.28                           |
| PRZ  | 4.1                         | 2.3                           | 3.9                             | 0                           | 0.647                         | 0.554                       | −0.117                     | 1.33                           |
| KAL  | 4.0                         | 2.3                           | 3.5                             | 0                           | 0.640                         | 0.544                       | −0.166                     | 0.04                           |
| STU  | 4.0                         | 2.3                           | 3.2                             | 0                           | 0.640                         | 0.544                       | −0.198                     | 2.20                           |

**Mean** 4.2 2.4 4.0 0.05 0.641 0.553 −0.149 1.21

*A* average number of alleles, *Ae* effective number of alleles, *AR* average allelic richness (after rarefaction), *Pa* number of private alleles, *Ho* unbiased expected heterozygosity, *He* observed heterozygosity, *N0 (%)* null allele frequency, *Fis* Wright’s fixation index (all not significantly differ from zero). The population codes are shown in Table 1.
and historical processes influence (Meirmans 2015). As the Evanno method (delta $K$) did not lead to a biological interpretation for our dataset, we used alternative measures (MedMean and MaxMean) proposed by Puechmaille (2016) to find optimal $K$ for our populations. Puechmaille methods were found to be more accurate than delta $K$ or mean $\ln P(K)$ with unevenly sampled populations, which is our case. The optimal number of clusters was $K = 5$ for all 41 $U. laevis$ populations (Fig. 2). However, the proportions of each cluster in the gene pools of the examined populations were comparable and homogenous, except for six populations for which the frequency of one of the four clusters was slightly higher than 0.55 (Fig. 1). Mantel tests of isolation by distance found nonsignificant correlation between the geographical and genetic distance matrices ($R = 0.002, p = 0.328$).

### 3.3 Demographic history and effective population size

The effective population size based on linkage disequilibrium ($N_{e,LD}$) varied widely among the white elm populations and ranged from 2.7 (STU) to 360.9 (LEM), an overall harmonic mean of 16.8 (Table 3). The $N_{e,LD}$ in the 11 white elm populations was lower than the overall harmonic mean $N_{e,LD}$. Most Ne confidence intervals overlapped (not a surprise when using few loci) and their interpretation was done with caution. The $M$-ratios (MRs) were significantly reduced according to the mean MRs derived under mutation-drift equilibrium ($M_{eq}$) for all examined white elm populations; this result is strong evidence of a past bottleneck. On the other hand, Wilcoxon’s test for heterozygosity excess ($H_e$), performed under the TPM model, indicated recent population reductions in only ten of the analyzed populations (Table 3). Moreover, in the inference of population demographic of Polish populations $U. laevis$, the scenario 2 "recent expansion" was highly favored (posterior probability = 0.481 [CI95 % = 0.4230 - 0.538]) over the scenario 1 "stable population" (posterior probability = 0.325 [CI95 % = 0.2717 - 0.3790]) and the scenario 3 "bottleneck population" (posterior probability = 0.1935 [CI95 % = 0.1449 - 0.2421]). This result revealed that the $N_e$ population of $U. laevis$ has undergone population size reduction in the postglacial refugia or shortly after the postglacial recolonization. The DIYABC result was in accordance with the result from $M$-ratio which revealed an ancestral and extended declines of population size.

### 4 Discussion

#### 4.1 Genetic variation

Overall, the populations examined in our study showed a moderate level of genetic diversity. This is contrary to the premise that outcrossed, wind-pollinated, widespread temperate trees typically exhibit high levels of within-population genetic diversity and low to moderate levels of among-population genetic differentiation resulting from large populations, extended gene flow, and phenotypic plasticity (e.g., Hamrick et al. 1992; Nybom 2004). Although the Polish populations of European white elm are in the core of the natural range of the species, their level of genetic variation is slightly higher compared to the level of genetic variation maintained by the peripheral populations of this species (Nielsen and Kjær 2010; Venturas et al. 2013; Fuentes-Utrilla et al. 2014; Tamošaitis et al. 2021).

Some of the analyzed nuclear loci were previously used in the study of European white elm in other part of Europe. Out of the set of six loci, four (Ulm2, Ulm3, Ulm9, and UR188a) were also analyzed in Danish (Nielsen and Kjær 2010) and five (Ulm2, Ulm3, Ulm9,
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Ulm19, and UR188a) in Spanish populations (Ven-turas et al. 2013). Overall, much more alleles have been found in Poland than in the Spanish and Danish popu-lations. For example, the most variable Ulm3 and Ulm9 loci in Poland each had 15 alleles. In the material from Denmark and Spain, there were only 4 and 3 alleles for the Ulm3 locus and 7 and 6 alleles for the Ulm9 locus, respectively. Presented data suggest that selected nuclear

| CODE | He TPM | He p value | MR | MR p value | NeLD | CINe |
|------|--------|------------|----|------------|------|------|
| WIR  | 0.591  | 0.046      | 0.534 | <0.0001    | 45.1 | 21–408 |
| GRZ  | 0.562  | 0.218      | 0.486 | <0.0001    | NA   | -    |
| POJ  | 0.489  | 0.421      | 0.425 | <0.0001    | 24.4 | 12–65 |
| TRB  | 0.530  | 0.499      | 0.469 | <0.0001    | 191.1 | 41–∞  |
| ZRB  | 0.513  | 0.340      | 0.514 | <0.0001    | 25.1 | 88–187 |
| OLO  | 0.540  | 0.156      | 0.460 | <0.0001    | 32.1 | 15–98 |
| LEM  | 0.556  | 0.031      | 0.512 | <0.0001    | 360.9 | 43–∞  |
| SLO  | 0.533  | 0.219      | 0.475 | <0.0001    | 17.3 | 7–51  |
| URA  | 0.578  | 0.219      | 0.425 | <0.0001    | 10.2 | 4–18  |
| KLE  | 0.579  | 0.719      | 0.492 | <0.0001    | 72.5 | 32–900 |
| BIE  | 0.591  | 0.218      | 0.487 | <0.0001    | 8.9  | 4–15  |
| BRZ  | 0.620  | 0.015      | 0.514 | <0.0001    | 35.7 | 17–122 |
| WIC  | 0.615  | 0.156      | 0.522 | <0.0001    | 32.9 | 18–73 |
| URW  | 0.589  | 0.156      | 0.518 | <0.0001    | 47.9 | 24–164 |
| CZL  | 0.579  | 0.500      | 0.532 | <0.0001    | 19.3 | 11–34 |
| DWJ  | 0.556  | 0.422      | 0.543 | <0.0001    | 12.5 | 7–23  |
| SOK  | 0.503  | 0.218      | 0.513 | <0.0001    | 18.9 | 10–41 |
| BIL  | 0.611  | 0.015      | 0.464 | <0.0001    | 13.1 | 6–26  |
| WIL  | 0.581  | 0.577      | 0.484 | <0.0001    | 24.8 | 13–57 |
| BJL  | 0.520  | 0.062      | 0.402 | <0.0001    | 60.5 | 10–∞  |
| WAW  | 0.539  | 0.281      | 0.486 | <0.0001    | 19.0 | 11–35 |
| LAM  | 0.536  | 0.109      | 0.522 | 0.016      | 7.3  | 3–7   |
| ROZ  | 0.636  | 0.015      | 0.543 | 0.017      | 5.4  | 3–11  |
| LOP  | 0.521  | 0.109      | 0.505 | <0.0001    | 8.7  | 3–30  |
| WIK  | 0.636  | 0.031      | 0.493 | <0.0001    | 16.9 | 7–54  |
| OLR  | 0.609  | 0.109      | 0.518 | <0.0001    | 209.5 | 47–∞  |
| LUS  | 0.508  | 0.031      | 0.473 | <0.0001    | 22.5 | 7–263 |
| MEL  | 0.469  | 0.078      | 0.469 | <0.0001    | 38.5 | 12–∞  |
| MEL  | 0.621  | 0.046      | 0.457 | <0.0001    | 25.8 | 11–146 |
| BAB  | 0.523  | 0.156      | 0.507 | <0.0001    | 9.0  | 3–27  |
| KLC  | 0.572  | 0.218      | 0.480 | <0.0001    | NA   | -     |
| SKU  | 0.532  | 0.499      | 0.543 | <0.0001    | 51.1 | 24–239 |
| POD  | 0.603  | 0.344      | 0.541 | 0.016      | 12.9 | 8–20  |
| SZC  | 0.524  | 0.421      | 0.470 | <0.0001    | 9.2  | 4–16  |
| TUM  | 0.554  | 0.218      | 0.477 | <0.0001    | 262.9 | 30–∞  |
| GRA  | 0.575  | 0.077      | 0.562 | <0.0001    | 26.2 | 8–∞   |
| BOB  | 0.535  | 0.001      | 0.524 | <0.0001    | 16.8 | 8–39  |
| MAK  | 0.585  | 0.015      | 0.503 | <0.0001    | 16.8 | 6–59  |
| PRZ  | 0.617  | 0.155      | 0.474 | <0.0001    | 42.5 | 19–248 |
| KAL  | 0.549  | 0.577      | 0.510 | <0.0001    | 186.5 | 40–∞  |
| STU  | 0.527  | 0.218      | 0.467 | <0.0001    | 2.7  | 2–8   |

Mean 16.8
microsatellite loci are polymorphic and thus accurate to infer for level of genetic diversity. In Polish populations of *U. laevis*, genetic diversity parameters, such as the mean number of alleles and observed heterozygosity (*A* = 4.2; *H*<sub>a</sub> = 0.641), were slightly higher than those in Spanish populations (*A* = 2.6; *H*<sub>a</sub> = 0.490), Danish populations (*A* = 3.8; *H*<sub>a</sub> = 0.530), and Norwegian group of trees (*A* = 2.8; *H*<sub>a</sub> = 0.560) (Nielsen and Kjær 2010; Fuentes-Utrilla et al. 2014). However, as each of these studies analyzed a different number of trees and utilized different microsatellite marker sets, one should exercise caution when comparing results. Notably, in Poland, more than 1,500 *U. laevis* individuals were analyzed, in contrast to the 91 and 110 trees analyzed in Denmark and Spain, respectively. Moreover, differences in genetic variation levels may result from the locations of the studied populations. The Danish and Spanish *U. laevis* populations are located along the edge of the natural range of this species. Another study based on different system markers such as isozymes pointed out a low level of genetic diversity in marginal Finnish *U. laevis* populations (Vakkari et al. 2009). Our findings indicate that *U. laevis* shows lower genetic diversity at nSSRs compared to congeneric species such as *U. glabra* (Martín del Puerto et al. 2017; Chudzińska et al. 2018; Tamošaitis et al. 2021) and *U. minor* (Buitoveld et al. 2016; Zebec et al. 2016; Chudzińska et al. 2019; Tamošaitis et al. 2021).

In our study, a low to moderate level of genetic differentiation was found among the studied *U. laevis* populations, with and without adjusting for null alleles (*F*<sub>st</sub> = 0.076 versus *F*<sub>st</sub>Null = 0.074, *p* < 0.01). Similarly, Whiteley (2004) found low differentiation levels among five Central and Northeastern European populations. The overall population differentiation was lower than that observed among Spanish populations (*F*<sub>st</sub> = 0.155; Fuentes-Utrilla et al. 2014). These differences may have resulted from greater gene flow between the Polish elm populations located in a smaller area than the Spanish populations, which may have prevented increased genetic differentiation among populations. Moreover, in this study, the global genetic differentiation estimated based on allele sizes (*R*<sub>st</sub>) was lower than that of the *pR*<sub>st</sub> analog to *F*<sub>st</sub>, indicating that random genetic drift was more important than mutation in causing the observed differences among the studied *U. laevis* populations in Poland.

The Bayesian clustering methods indicated that five clusters (*K* = 5) most likely provided representations of the overall genetic structure of the analyzed *U. laevis* populations. Generally, the gene pools of the analyzed populations were genetically homogeneous. The studied populations had comparable gene pool compositions except for a few populations, where one of five clusters was dominant. The non-geographically structured gene pool of Polish *U. laevis* populations was confirmed by the non-significant Mantel test (*R* = 0.002, *p* = 0.328). The current genetic divergence pattern of *U. laevis* in Europe, including in Poland, is the result of Quaternary climate change leading to a reduction in population sizes and the long-term isolation of populations during glacial-interglacial cycles and postglacial migration (Hewitt 2000). The observed genetic structure pattern implies the occurrence of free gene exchange among populations and that they probably share a common postglacial history. The investigation of postglacial history of *U. laevis* in Europe, using chloroplast DNA markers (cpDNA) identified three cpDNA haplotypes (A, B, and C) which are characteristic for potential glacial refugia for white elm (Whiteley 2004). The haplotype A was found with high frequency from France to Northwest Russia of the natural range distribution of *U. laevis*. The other two haplotypes are very rare. The haplotype B was found in southern France while the haplotype C was found in the Balkans and Southwest Russia. The presence of both haplotypes A and C in Russia indicates a core Russian glacial refuge from which current white elm populations have originated by postglacial expansion. However, the southern distributions of haplotypes B and C could indicate additional refugia for white elm, but probably postglacial recolonization from these areas was limited (Whiteley 2004). It can be speculated that the *U. laevis* entered to the territory of Poland from the Russian or the Balkan refuge, or Poland, was under the range of both refugial areas. The hypothesis that *U. laevis* migrated to Poland from two refugia Russian and Balkan is probably true because we observed the heterozygosity excess in all of the studied populations as a consequence of the mixing of two previously isolated populations (“isolate-breaking” effect) (Wahlund 1928). These hypotheses should be verified using cpDNA markers. Moreover, Bayesian analysis of population structure of *U. laevis* performed by Fuentes-Utrilla et al. (2014) showed the differentiation between Iberian/SW France and Central Europe core distribution of this species. The pattern of genetic diversity of Spanish populations is not consistent with the pattern of presence in Central Europe. The authors concluded their results stating that Spanish populations of *U. laevis* may represent relict populations of an Iberian glacial refugia.

### 4.2 Demographic history

A bottleneck is a factor that can negatively influence the genetic structure of natural populations due to a decline in population sizes increasing the level of inbreeding and reducing the level of genetic diversity; thus, bottlenecks threaten the sustainability of populations in the short and long terms. On the other hand, the life history traits that are common to long-lived forest trees, such as
long-distance pollen and seed dispersal, overlapping generations and longevity, may protect populations against the effects of sharp population declines over the short term. In this study, the specific tests used to examine the bottleneck effect with microsatellites yielded interesting results, with the calculated $M$-ratios test suggesting bottlenecks in all populations; however, the heterozygosity excess test performed with the TPM model showed evidence of bottlenecks in only ten of the analyzed populations. Tests that are based on heterozygosity using a given number of alleles are better able to identify recent, less-severe bottlenecks. Recently, bottlenecked populations show excess heterozygosity relative to that expected based on the number of alleles. In contrast, the $M$-ratio is smaller in a bottlenecked population than in an equilibrium population. Moreover, the $M$-ratio test is more powerful at detecting ancestral and extended declines than the heterozygosity excess test (Williamson-Natesan 2005). As the recovery time of the $M$-ratio is longer than that of the heterozygosity excess test, the low $M$ value obtained reflects older and more severe reductions in the sizes of the studied populations (Garza and Williamson 2001; Williamson-Natesan 2005). Thus, the low $M$-ratio values obtained for all studied Ulmus populations are likely to be a consequence of genetic decline during postglacial recolonization. Our results indicated that the loss of Ulmus genetic diversity probably occurred in their refugia or shortly after their postglacial recolonization. This assumption was confirmed by the result of analysis DIYABC, where the best fit scenario to our data probably indicted the reduction size of the ancestral population. Another investigation also showed a signature of historical bottlenecks after Holocene migration in Spanish Ulmus populations (Whiteley 2004; Fuentes-Utrilla et al. 2014). Similarly, relict Ulmus glabra populations from the Iberian Peninsula experienced historical reductions in their population sizes (Martín del Puerto et al. 2017).

The genetic bottleneck phenomenon is largely related to the $N_e$ and serves as a warning sign for the conservation status of a given population (Frankham 2005; Luikart et al. 2010). The assumed value of 50/500 proposed by Franklin (1980) has become an essential indicator in conservation genetics. Based on this theory, $N_e = 50$ is sufficient to prevent inbreeding depression in the short term (over five generations), whereas $N_e \geq 500$ is appropriate for securing long-term viability because the population can maintain a balance between genetic drift and mutation, thereby retaining its evolutionary potential. The Polish Ulmus laevis populations studied herein are characterized by low population sizes with a mean $N_e$ value of 16.8. Moreover, only eight out of forty-one (20%) examined populations exhibited $N_e$ values over 50. Based on these assumptions, the studied populations probably maintain a low evolutionary potential. However, field observations of Polish Ulmus laevis populations indicated that they are in good condition despite their moderate level of genetic diversity and low effective population size.

### 4.3 Loss of genetic variation and Dutch elm disease

In Poland, the maximum spread of Ulmus in the Holocene started at approximately 6000 B.P. from the southeast direction, before the advent of the Neolithic people, and the proportion of Ulmus in the overall forest stand composition was higher than 10% (Ralska-Jasiewiczowa et al. 2003). Then, approximately 5000 B.P., a rapid decrease down to 2% occurred in the proportion of Ulmus in the forest stands. Undoubtedly, settlement activities and regional climate change contributed to reduced forest cover in areas where Ulmus occurred (Ralska-Jasiewiczowa et al. 2003). However, according to many authors, the rapid reduction rate of Ulmus corresponds better with the pathogenic hypothesis caused by cyclical Dutch elm disease pandemics (Girling and Greig 1985; Ralska-Jasiewiczowa et al. 2003; Caseldine and Fyfe 2006). In Poland, the last pandemic was first recorded in Katowice in 1927 and then in northern Poland and Warsaw in 1932 and 1935, respectively. In the 1950s and 1960s, the disease was reported in all parts of the country and caused substantial losses in urban green areas, roadsides, and forests (Mańka 2005). Compared to other tree species with similar life history, the Ulmus laevis populations maintained a strongly reduced level of genetic variation and low genetic differentiation. For example, black poplar, which occupies habitats similar to those of Ulmus laevis, still maintains a high level of genetic variation and a low level of genetic differentiation (Lewandowski and Litkowiec 2017; Wójkiewicz et al. 2019; Wójkiewicz et al. 2021).

In the case of a species having a high level of genetic variation, the random loss of various alleles as a result of the recent pandemic should increase the level of interpopulation variation; however, we did not observe this in our study. Moreover, only a few populations displayed “private” alleles, with very low frequencies. Thus, it appears that despite the high mortality rate of white elm during the last pandemic, the species lost a small number of alleles. This may indicate that the Polish elm populations that existed before the last pandemic were homogeneous with a low level of genetic variation, similar to that expressed by the species today. Additionally, some other studies have suggested that there is no evidence of genetic diversity losses in elm populations as a consequence of the last pandemic (Nielsen and Kjær 2010; Brunet et al. 2016; Buiteveld et al. 2016). It is possible that elm populations lost most of their genetic variation during previous pandemics. As our research shows, all
analyzed populations have experienced severe reductions in their \( N_e \). It cannot be ruled out that the loss of genetic variation in white elm already took place in their refugia; therefore, more extensive research is needed involving populations from other parts of Europe.

5 Conclusion
The *U. laevis* populations examined appeared to maintain a moderate level of genetic variation and low genetic differentiation and no evidence of genetic population structuring. Our results indicated demographic processes such as reduced population sizes via past bottlenecks. We speculated that the loss of genetic variation in *U. laevis* probably occurred in their refugia or shortly after their postglacial recolonization. This hypothesis should be confirmed by additional investigation using cpDNA and nSSR markers. Also, a much more detailed sampling of the Russian would be extremely valuable.

Despite the pandemic and moderate genetic diversity, white elm individuals are still quite numerous in Poland. However, in white elm populations, very significant reductions in the numbers of individuals have taken place, and most populations have low effective population sizes. In Poland, a twofold increase has occurred in the area of forest stands dominated by elms in the last 50 years (Napieral-Filipiak et al. 2016), mostly consisting of areas with white elm individuals that are least prone to the disease. *U. laevis* often occurs in legally protected areas, such as nature reserves, or in areas covered by the Natura 2000 network. Therefore, we currently do not see any urgent need for ex situ or in situ conservation action. We propose that the most valuable populations with high effective population sizes, such as TRB, LEM, OLR, TUM, and KAL, be considered candidates for dynamic conservation units (DCUs) in the European information system on forest genetic resources (EUFGIS; http://www.eu fgis.org/) conservation network and subjected to continuous monitoring.

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Authors’ contributions
Conceptualization: A Lewandowski, M Litkowiec, and Cz Kozioł; Methodology: M Litkowiec, A Pasławska, M Chudzińska, and M Pałucka; Formal analysis and investigation: M Chudzińska, A Pasławska, M Litkowiec, and M Pałucka; Data visualization: M Litkowiec and M Chudzińska; Writing—original draft preparation: M Litkowiec and A Lewandowski; Writing—review and editing: A Lewandowski and M Litkowiec; Funding acquisition: Cz Kozioł and A Lewandowski; Supervision: A Lewandowski. The authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated and analyzed during the current study are available in the RepOD repository, https://doi.org/10.18150/LLHKEX.

Declarations

Ethics approval and consent to participate
The authors declare that they follow the rules of good scientific practice.

Consent for publication
All authors gave their informed consent to this publication and its content.

Competing interests
The authors declare that they have no competing interests.

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