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Natural Regeneration, Genetic Diversity, and Provenance of Introduced *Fagus sylvatica* L. Stands in Latvia

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Abstract: European beech (*Fagus sylvatica* L.) has a wide distribution range through Central and Western Europe, and is tolerant to a range of environmental factors and shade. Due to the high wood quality and growth rate, there is increasing interest in the cultivation of this species in Northern Europe, and European beech is appropriate for closer-to-nature forestry management practices, where shade-tolerant species are essential. Latvia is located to the north of the natural range of European beech, but stands have been successfully established in the 19th century, using reproductive material of unknown origin. This study investigated the natural regeneration, genetic diversity, population structure, and provenance of Latvian *F. sylvatica* populations from two areas in western Latvia—Skede and Kaleti. Parent–offspring analyses did not identify a decrease in genetic diversity parameters in the naturally regenerated offspring, indicating that natural regeneration has not decreased the genetic viability of these populations. The three stands located in Skede were genetically very similar (pairwise Fst values ranged from 0.004 to 0.007). The Kaleti stand was more differentiated from the Skede stands (pairwise Fst values with the Skede stands ranged from 0.047 to 0.051), and the genetic diversity was low (H_e = 0.638). This is probably a result of the use of reproductive material collected from a very limited number of individuals to establish this stand, which also prevented the determination of the origin of this material. The Skede stand was compared to German, Polish, and Swedish *F. sylvatica* populations, and a Bayesian clustering analysis indicated that the most likely provenance of the Skede stand in Latvia was from southern Germany.

Keywords: European beech; parental assignment; adaptation; microsatellite markers; assisted migration; climate change

1. Introduction

Rapid global climate changes are already affecting the distribution and composition of forest tree species. The potential distribution ranges of species are constrained by the climate and are expected to shift toward higher latitudes and elevations as global warming intensifies [1,2]. Trees have long life spans and delayed reproduction, and thus are not able to swiftly react to changing environmental conditions, as adaptation, migration, and related mutation accumulation are long-term events [3]. Intraspecific genetic variation and

local adaptation are essential for the survival of populations growing at species distribution range limits [4], and research concerning local genetic diversity and species potential under current conditions is essential, while also considering predicted changes in the future [5]. Assisted migration is the deliberate movement of species or populations to accelerate natural range expansion as a part of climate change mitigation management processes [6]. It is considered as one way to maintain forest productivity and health under changing climactic conditions [7], and the assessment of the potential benefits of the assisted migration of forest tree species is becoming more relevant with regard to sustainable forest management and future planning [8]. The global scientific community is assessing potential risks and benefits of such actions. In this study, an existing case of the assisted migration of European beech (*Fagus sylvatica* L.) in Latvia is investigated.

F. sylvatica is a deciduous, monoecious broad-leaved tree species, that reaches reproductive age after 40–50 years [9]. It is mainly outcrossing, with self-pollination mostly resulting in empty seeds, and selfing rates are estimated to be less than 10% [10,11]. *F. sylvatica* has a wide distribution range through Central and Western Europe [12], and is considered as a successful forest tree species because of its tolerance to variable environmental factors and shade [13,14], and due to its ability to form both large single-species stands in suitable areas as well as productive admixed stands [15]. However, currently, a large proportion of areas suitable for *F. sylvatica* are used for agriculture or are reforested with Scots pine (*Pinus sylvestris* L.) [16]. The potential use of selective cutting forestry management practices for *F. sylvatica* and the high quality of beech wood as well as good growth characteristics has led to growing interest in expanding the area of *F. sylvatica* stands in Northern Europe [17]. The interest to use this species is further boosted by the intent to implement closer-to-nature forestry management practices, where shade tolerant species are essential [18].

Latvia is located beyond the northern natural range limit of *F. sylvatica*, and no natural introductions or migration of this species have been identified. During the 19th century, German foresters successfully introduced *F. sylvatica* in western Latvia, using germplasm of unknown origin [19], and in the last decades, natural regeneration in these stands has been observed [20]. In 1969, the *F. sylvatica* forest at Skede covered 11 ha, with the oldest trees ranging from 60 to 80 years old (the seedling count after a mast seed year was approximately 360,000 ha^{-1}) [20]. Tree ring widths of *F. sylvatica* in Latvia were not supressed, indicating adequate growth conditions under current climactic parameters [21]. The distribution of *F. sylvatica* is constrained by winter temperatures and summer precipitation [22,23]. However, the potential distribution range may be larger than currently delineated, and forest management practices in the past may have contributed to a reduction in the area where European beech is currently distributed [24]. Climate change projections predict the northward migration of European beech [25], and an increase in its competitiveness at northern range margins [26].

F. sylvatica populations have been intensively studied in Central Europe, using various marker types [9,11,27–35]. In general, isozymes and nuclear markers revealed high levels of diversity and a low differentiation between populations. Chloroplast marker analyses have revealed higher levels of population differentiation, indicating that gene flow by seed dispersal is relatively low [36,37]. SSR markers have been the most widely used markers system in studies of *F. sylvatica* [31]. They are also widely used in population genetics studies as they are codominant, highly polymorphic, and technically relatively easy to implement [38]. Studies utilising DNA markers have indicated that forest management techniques such as shelter wood systems have little or no impact on the genetic diversity of beech stands [39,40]. Climate change can be expected to have negative effects on beech stands, mainly in the southern part of its distribution range, due to temperature and water regime changes [41]. However, it has been suggested that intra-population genetic diversity,

gene flow, and phenotypic plasticity can mitigate these threats [42] and can be utilised to assist the shift in the distribution range of European beech northwards.

Studies of *F. sylvatica* have shown that it has a strong family structure of up to a 30 m radius [43], but the spatial genetic structure can also be influenced by wind and gravity or bird and animal activity, and a 27% seed immigration rate has been reported in *F. sylvatica* stands in France [11]. Research has shown that up to 90% of *F. sylvatica* seeds are dispersed in a 25 m radius [20,44], but some studies have reported an even more limited dispersal [11]. As seed dispersal distances are short, pollen flow can ensure longer-distance gene transfer, but these distances can also be limited in comparison with other broad-leaved tree species, with the pollen dispersal reported to be approximately 42 m [11].

The overall genetic diversity of *F. sylvatica* in Europe is reduced due to intensive forestry practices over several centuries, and as a high genetic diversity is vital for ensuring long term evolutionary and adaptive changes [45], an assessment of the current situation and conservation of existing genetic resources is important for the future [46]. A sufficient level of genetic diversity in the parent population is also important for the wide-scale use of locally adapted reproductive material for propagation at the fringes of the current distribution range [21]. A recent study in Lithuania revealed a significant association between the phenology and stem quality of introduced European beech populations, which has significant implications for forest management practices, as the planting of stands can disrupt the spatial genetic structure and grouping of related individuals, thus enhancing genetic diversity [47].

The objectives of this study were to investigate the genetic structure and diversity of existing *F. sylvatica* stands in Latvia and to determine the proportion of mature *F. sylvatica* trees that are involved in regeneration, as well as to determine the provenance of the reproductive material used to establish these stands. The results can assist in the evaluation of future perspectives and management options for *F. sylvatica* stands in Latvia that are propagated with locally adapted material. Parent–offspring analyses were conducted in two stands to assess the genetic aspects of natural regeneration processes. The genetic diversity and population structure were assessed in four Latvian *F. sylvatica* stands planted with imported reproductive material in the 19th century and the beginning of the 20th century. In addition, the potential provenance of the reproductive material introduced to Latvia was investigated by comparing them to *F. sylvatica* stands in Southern Germany, Poland, and Sweden. Historical records indicate that the material used for establishing the *F. sylvatica* stands in Latvia was sourced from unknown areas in Germany [19]. Poland and Sweden are the closest countries to Latvia that are at least partially within the natural distribution range of *F. sylvatica*. A previous study from Lithuania, also outside of the natural distribution range, and immediately to the south of Latvia, indicated that reproductive material from southern Germany was used to establish some of the Lithuanian stands, and at least one stand was established material from Poland [48]. The results obtained in the current study will assist in the management of Latvian *F. sylvatica* stands, determining if natural regeneration processes are maintaining genetic diversity in subsequent generations. The assessment of the genetic diversity and population structure of mature *F. sylvatica* stands provides information for the efficient management of forest genetic resources. Identifying the origin of the reproductive material used to establish these Latvian stands forms the basis for an ecogeographical assessment of the long-range transfer of *F. sylvatica* to areas outside its current natural distribution and makes a valuable contribution to research on assisted migration.

2. Materials and Methods

2.1. Analysed F. sylvatica Stands and Sample Collection

The samples for the parent–offspring analysis were collected from two stands (Skede 20 and Kaleti), located in the western part of Latvia. The sampling design was adapted from Vranckx et al. [49]. In the centre of each stand, one circular sample plot with an area of 0.78 ha was established. All mature trees (potential parents/mothers) in the circular sample plot were sampled. Young trees (offspring) were sampled from a 20 × 20 m subplot in the centre of the circular plot (Figure 1). Leaves from young trees and woody tissue from mature trees were collected for DNA extraction. *F. sylvatica* was the dominant species in the Skede stand (57°15′ N, 22°44′ E), with admixture of several conifer and deciduous tree species, and the age of the trees was 112–118 years, area was 2.44 ha, and basal area was 42 m² ha⁻¹. It is situated on flat terrain on fertile, well-drained clay soil, and the elevation is approximately 90 metres. The Kaleti stand (56°36′ N, 21°48′ E) is dominated by *F. sylvatica* with some admixture of deciduous tree species. The age of the mature trees in the Kaleti stand was 112 years, stand area was 5.8 ha, and basal area was 36 m² ha⁻¹. It is situated on flat terrain, well-drained clay soil, with an elevation of approximately 30 metres.
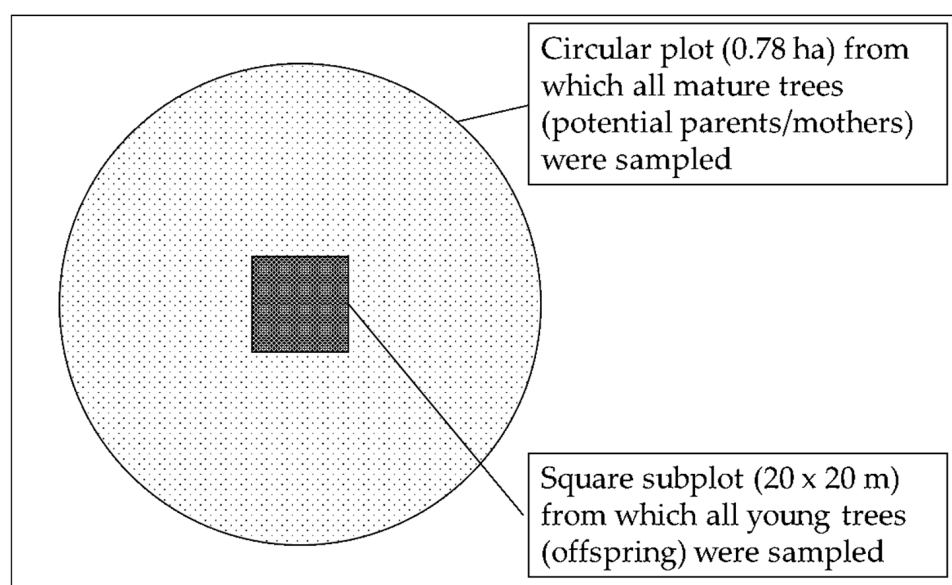

Figure 1. Design of sampling plots where mature trees (potential parents/mothers) and young trees (offspring) were sampled.

For the genetic diversity and population structure analysis, samples from mature individuals only were collected from two additional stands within the larger Skede population—Skede 21 (43 individuals) and Skede 23 (42 individuals) (121–125 years old).

For the provenance analysis, fifty mature individuals from the Skede 23 stand were analysed with the same SSR markers as used previously to compare introduced European beech stands in Lithuania with potential source and reference populations in Germany, Poland, and Sweden [48].

The locations of the analysed *F. sylvatica* stands and populations are shown in Figure 2, and details are provided in Supplementary File S1.
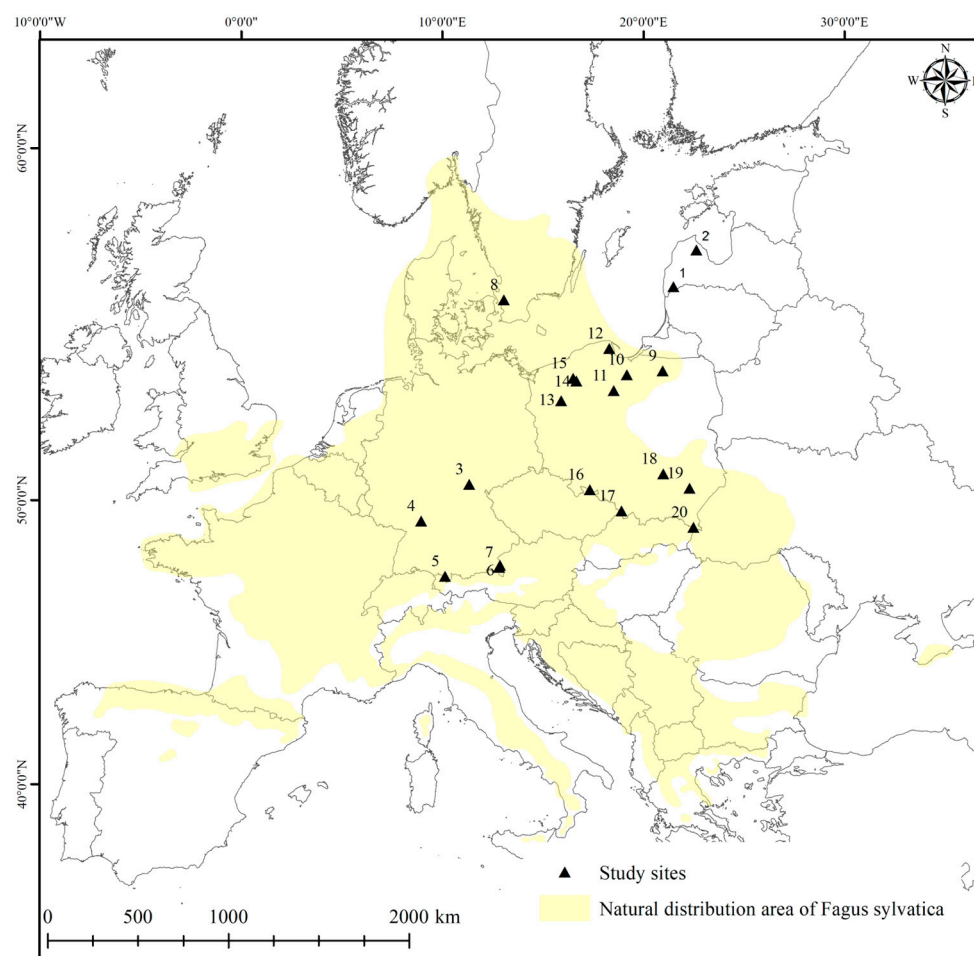
Figure 2. Locations of analysed *F. sylvatica* stands and populations.

2.2. DNA Extraction and Genotyping

DNA was extracted from leaves and woody tissue using a CTAB buffer-based method [50]. For the parent–offspring and diversity and structure analysis of the Skede and Kaleti stands, genotyping was done using 18 nuclear SSR markers. Each forward primer was fluorescently labelled for detection using capillary electrophoresis. PCR reactions for the eleven SSR markers described by Lefèvre et al. [30] (csolfagus_31, sfc_0036, csolfagus_06, csolfagus_19, csolfagus_29, EEU75_A_0, EJV8T_A_0, EMILY_A_0, ERHBI_A_0, DZ447_A_0, and DE576_A_0) were carried out according to the published protocol. The PCR reactions for the seven SSR markers described in Pastorelli et al. [28] (FS1-15, FS1-25, FS1-03, FS1-11, FS3-04, FS4-46, and FCM5) were carried out in 20 μL reactions consisting of 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 1.5 μL DNA solution, $1\times$ Taq buffer, and 1U of recombinant Taq DNA polymerase (Thermo Scientific, Waltham, MA, USA). The PCR conditions were as follows: initial denaturation—95 °C for 5 min, 30 cycles of 95 °C for 30 s, 52 °C for 45 s, and 72 °C for 60 s with a final extension step of 72 °C for 10 min. The PCR products were separated by capillary electrophoresis using an ABI 3100XL capillary sequencer (Applied Biosystems, Waltham, MA, USA) and genotyped using GeneMapper 4.0 software (Applied Biosystems, Waltham, MA, USA).

For the provenance and phylogeographic analysis, samples from the Skede 23 stand in Latvia were genotyped with the same ten SSR markers (FS1-15, FS3-04, csolfagus_31, csolfagus_19, DE576_A_0, MFC11, MFC5, MFC7, sfc_0036, and MFS11) as in a previous phylogeographic study [48]. Genotype data from this previous study of the German, Polish, and

Swedish populations were compared to the genotypes of the Skede 23 stand. The sequences of the primers used for the PCR amplifications are provided in Supplementary File S2.

2.3. Data Analysis

The possible presence of null alleles was determined using the program MICRO-CHECKER version 2.2 [51]. Null alleles are genetic variants where PCR amplification fails due to mutations, deletions in primer binding sites, or large indels, reducing the apparent genetic diversity and complicating the parent–offspring analyses.

Genetic diversity parameters, as well as the pairwise relatedness within each candidate parental and offspring population (using the Ritland estimator [52]), were calculated using GenAlEx 6.501 [53]. A principal coordinates analysis (PCoA) of pairwise Nei genetic distances was also done using GenAlEx 6.501. The genetic diversity parameters calculated were the number of alleles (Na), effective number of alleles (Ne), information index (I), observed heterozygosity (Ho), expected heterozygosity (He), and fixation index (F). Na is the total number of alleles, and Ne is the number of equally frequent alleles needed for the same expected heterozygosity. The information index, similar to the Shannon–Weaver index, is an unbounded alternative to the polymorphism information content (PIC) or expected heterozygosity. Observed heterozygosity is calculated from codominant markers, while expected heterozygosity measures genetic variation. The fixation index (-1 to $+1$) indicates inbreeding or null alleles (positive) and excess heterozygosity (negative).

Offspring and mother tree groups were analysed separately in each population, and loci with potential null alleles in both offspring and mother tree groups were excluded from the pedigree analysis. The assignment of parentage was done using the Cervus software package version 3.0.3 [54], which uses likelihood ratios to assign parentage, and simulations to determine the level of confidence in parental assignments. As *F. sylvatica* is a monoecious species, the candidate mothers and fathers were the same individuals. The parental assignment was done for one parent (i.e., maternity or paternity), as well as for parent pairs, with a confidence level of >0.95. The identification of full- and half-sib families was done using the COLONY 2.0.5 software package [55], which implements a maximum likelihood to estimate full- and half-sib relationships. The allelic dropout rate and the rate of other kinds of genotyping errors (e.g., false alleles, miscalling) of each marker were both set at 0.005.

Bayesian analysis can be used to infer the genetic clustering of individuals by grouping individuals with similar patterns of genetic variation [56]. No prior grouping or population information about the individuals to be analysed is required, but can be provided (e.g., sampling location, ecology, or other characteristics) to shape the clustering analysis. STRUC-TURE 2.3.4 was used to determine the clustering of the populations using a 150,000 burn-in period, followed by 300,000 MCMC steps, and K was determined for 1 to 15 with 10 runs for each value of K. The optimal value of K was determined using the ΔK method [57], using StructureSelector [58]. In addition, to check for correlations between pairwise genetic and geographic distances, or isolation by distance (IBD), the pairwise genetic and geographic distance matrices of individuals from the populations within the natural distribution range of *F. sylvatica* (i.e., the German, Polish, and Swedish populations) were compared using a Mantel test with 999 permutations in the GenAlEx 6.501 software package.

3. Results

3.1. Screening of SSR Loci

To increase sensitivity and accuracy, samples with missing genotypes at more than three loci were discarded from further analysis. Possible null alleles were identified in each population and group. Loci with potential null alleles that were identified in both

mother and offspring groups were not utilised for further pedigree analysis to avoid the false exclusion of potential kin relationships. In the Skede 20 population, three loci were excluded (FS1-15, FS4-46, and FSM5), and two loci were excluded in the Kaleti population (FS4-46 and FSM5). In addition, four pairs of individuals in the Kaleti offspring group had identical multilocus genotypes; therefore, one of the individuals in each pair was excluded from further analysis. The sibling probability of identity (PIsibs), which provides an estimation of the average probability that two siblings will by chance have the same multilocus genotype, was 2.4×10^{-5} (using all analysed loci). The more likely explanation of these identical multilocus genotypes is that these individuals were clones from root suckers, which was also observed while assessing regeneration in other sites in Latvia [20].

After the exclusion of individuals and loci as described above, a pedigree analysis in the Skede population was done with 15 loci and 63 potential parent trees and 101 offspring. In the Kaleti population, 16 loci were retained, alongside 45 potential parent trees and 106 offspring.

3.2. Parent–Offspring Analysis in the Skede 20 Stand

Of the 101 offspring in the Skede stand, 54 were assigned to single parent trees from the collected mature individuals. A total of 20 parent trees were identified from the 63 candidate parent individuals. For the parent pair analysis, 15 offspring individuals were assigned to parent pairs from the sampled candidate parent individuals. In total, combining the assigned parents from the parental assignment analysis, 22 parent trees were identified from the 63 candidate parental individuals, indicating that approximately 30% of the parents contributed to the regeneration of the sampled offspring plot. Of the 20 parent trees assigned with a confidence level of >0.95, 8 were assigned to only 1 offspring individual, while the remaining 12 parent trees were assigned to between 2–13 offspring individuals (Figure 3).
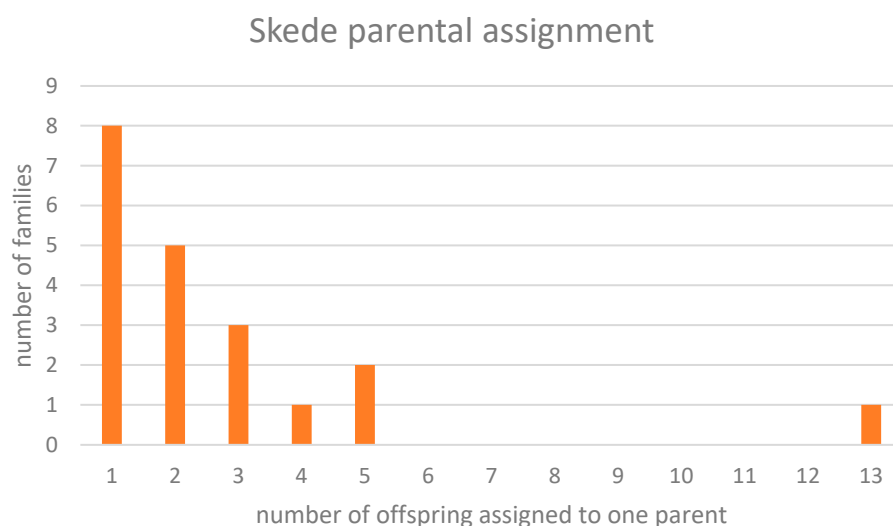
Figure 3. Assignment of offspring to parents in the Skede stand.

The COLONY 2.0.5 software analysis identified 8 full sib families ($p > 0.90$), most with two members, but two families were identified with 4 and one with 5 individuals. An additional 4 potential full-sib families were identified, with $p < 0.90$.

The origin of the reproductive material utilised to establish the two stands was unknown; therefore, a kinship analysis of the parent trees was also done. In the Skede stand, of the 63 parent trees analysed, only two pairs of individuals were inferred as being full-sibs ($p > 0.95$). In addition, the most likely number of both paternal and maternal half-sib fami-

lies was between 58–63, with the majority of likely half-sib configurations falling within that range (>0.95 of both inferred paternal and maternal half-sib families).

The pairwise relatedness in the Skede population candidate parent group and the offspring group were calculated, and there was no significant difference in the average pairwise relatedness (−0.001 in both groups).

3.3. Parent–Offspring Analysis in the Kaleti Stand

Of the 106 offspring in the Kaleti stand, 44 were assigned to single parent trees from the collected mature individuals. A total of 18 parent trees were identified from the 45 candidate parent individuals. For the parent pair analysis, 6 offspring individuals were assigned to parent pairs from the sampled mature individuals. In total, combining the assigned parents from the parental assignment analysis, 22 parent trees were identified, indicating that approximately 50% of the parents contributed to the regeneration of the sampled offspring plot. Of the 18 parent trees assigned with a confidence level of >0.95, 10 were assigned to only one offspring individual, while the remaining 8 parent trees were assigned to between 2–11 offspring individuals (Figure 4).
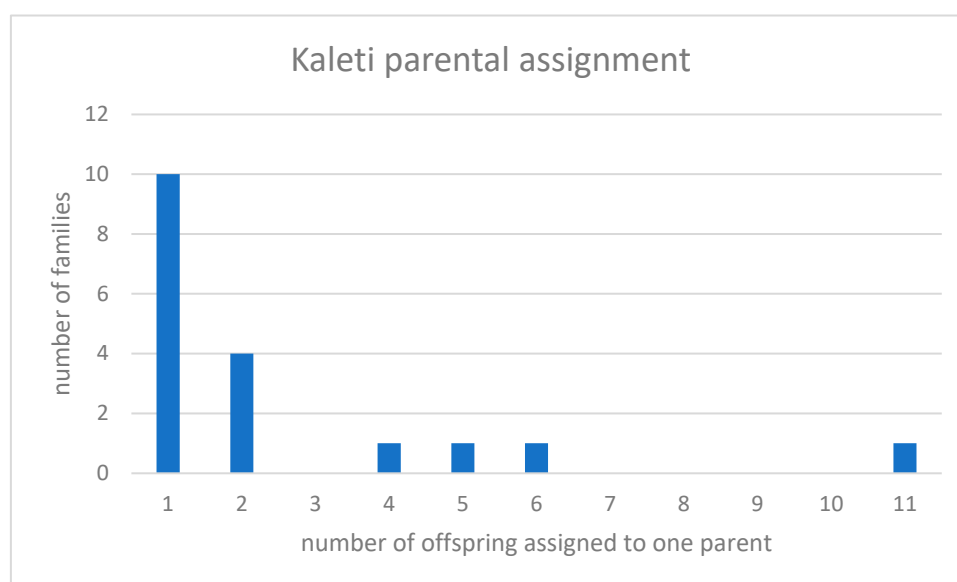
Figure 4. Assignment of offspring to parents in the Kaleti stand.

The COLONY 2.0.5 software analysis identified 4 full sib families ($p > 0.90$), containing 2, 3, 6, and 14 individuals. An additional 6 potential full-sib families were identified, with $p < 0.90$.

The kinship analysis of the parent trees in the Kaleti stand revealed a higher level of relatedness among them. Two full-sib families with more than two members ($p > 0.95$) were identified, consisting of two and seven members. However, a large full-sib family of 15 members was inferred with a p-value of 0.931. The most likely number of full-sib families (including families with only one inferred member) was 15–19 (0.985 of inferred full sib families). In addition, most likely number of both paternal and maternal half-sib families was between 12–19, with the majority of the likely half-sib configurations falling within that range (>0.95 of both inferred paternal and maternal half-sib families).

The pairwise relatedness in the Kaleti population candidate parent group and the offspring group was calculated, and the average pairwise relatedness in the candidate parent group (0.012) was significantly higher than in the offspring group (0.000).

3.4. Comparison of Genetic Diversity Between the Parent and Offspring Groups

Genetic diversity indicators (number of alleles, effective number of alleles, and expected heterozygosity) were similar between the parental and offspring groups in each analysed stand. Unique (private) alleles were found in each parent and offspring group in both stands. The unique alleles in the offspring group are probably due to pollen flow from outside the sample plots. The genetic diversity was lower in the Kaleti population, in comparison to the Skede 20 population (Table 1, Figure 5). While the origin of the reproductive material utilised to establish these stands is not known, the kinship and pairwise relatedness analysis indicated a higher degree of relatedness between the parental trees sampled from the Kaleti stand.

Table 1. Genetic diversity parameters in the Kaleti and Skede 20 parental and offspring populations.

Pop	Na (SE)	Ne (SE)	I (SE)	Ho (SE)	He (SE)	F (SE)
Kaleti parents	4.400 (0.273)	2.912 (0.184)	1.172 (0.059)	0.697 (0.041)	0.633 (0.028)	−0.100 (0.036)
Kaleti offspring	4.933 (0.396)	2.524 (0.197)	1.059 (0.065)	0.588 (0.035)	0.569 (0.033)	−0.040 (0.031)
Skede 20 parents	8.933 (1.007)	3.881 (0.389)	1.516 (0.114)	0.672 (0.037)	0.695 (0.036)	0.030 (0.027)
Skede 20 offspring	7.867 (0.920)	3.983 (0.378)	1.523 (0.104)	0.718 (0.040)	0.712 (0.032)	−0.012 (0.038)

Na—mean number of alleles, Na Freq. ≥ 5%—mean number of alleles with a frequency over 0.05, Ne—mean number of effective alleles, I—mean Shannon's information index, Ho—mean observed heterozygosity, He—mean expected heterozygosity, and SE—standard error.
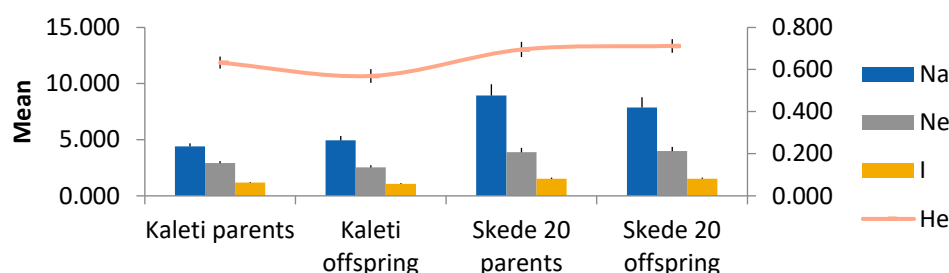
Figure 5. Genetic diversity parameters in the Kaleti and Skede 20 parental and offspring populations. Na—mean number of alleles, Ne—mean number of effective alleles, I—mean Shannon's information index, and He—mean expected heterozygosity.

3.5. Genetic Diversity and Structure of Mature Stands

The comparison of the mature individuals from four Latvian stands (Skede_20, Skede_21, Skede_23 and Kaleti) revealed that the number of alleles, effective number of alleles, and information index were lower in the Kaleti stand. However, the observed heterozygosity was highest in the Kaleti stand, and the fixation (inbreeding) index was negative for the Kaleti stand, but was positive for the three Skede stands analysed (Table 2).

Table 2. Genetic diversity parameters of mature individuals in the Kaleti and Skede stands.

Stand	Na (SE)	Ne (SE)	I (SE)	Ho (SE)	He (SE)	F (SE)
Skede_20	8.500 (0.976)	3.722 (0.382)	1.474 (0.114)	0.669 (0.040)	0.685 (0.037)	0.021 (0.027)
Skede_21	7.286 (0.952)	3.713 (0.386)	1.456 (0.124)	0.649 (0.043)	0.676 (0.044)	0.031 (0.029)
Skede_23	7.000 (0.864)	3.553 (0.413)	1.396 (0.125)	0.618 (0.047)	0.658 (0.045)	0.058 (0.03)
Kaleti	4.286 (0.266)	2.956 (0.192)	1.177 (0.063)	0.707 (0.042)	0.638 (0.030)	−0.107 (0.038)

Na—mean number of alleles, Ne—mean number of effective alleles, I—mean Shannon's information index, Ho—mean observed heterozygosity, He—mean expected heterozygosity, F—mean fixation index, and SE—standard error.

The Kaleti stand was genetically differentiated from the three Skede stands. The principal coordinates analysis (PCoA) of the pairwise Nei genetic distances is shown in Figure 6. The pairwise Nei genetic distance values between the three Skede stands were 0.028 (Skede_20−Skede_21), 0.017 (Skede_20−Skede_23), and 0.031 (Skede_21−Skede_23). The pairwise Nei genetic distance values between the Kaleti stand and the Skede_20, Skede_21, and Skede_23 stands were 0.213, 0.231, and 0.220, respectively. The pairwise Fst values between the three Skede stands were 0.006 (Skede_20−Skede_21), 0.004 (Skede_20−Skede_23), and 0.007 (Skede_21−Skede_23). The pairwise Fst values between the Kaleti stand and the Skede_20, Skede_21, and Skede_23 stands were 0.047, 0.052, and 0.051, respectively.

Figure 6. Principal coordinates analysis (PCoA) of the pairwise Nei genetic distances between the four analysed Latvian European beech stands. Percentage of variation explained by axis 1: 98.36%, and axis 2: 1.31%.

3.6. Provenance of the Skede Stand

The Bayesian clustering analysis of the Skede 23 stand and *F. sylvatica* stands from Southern Germany, Poland, and Sweden was done using the STRUCTURE 2.3.4 software program. The most likely number of genetic clusters determined by the deltaK method was eight, but there was also a high deltaK value for K = 4 (Figure 7).
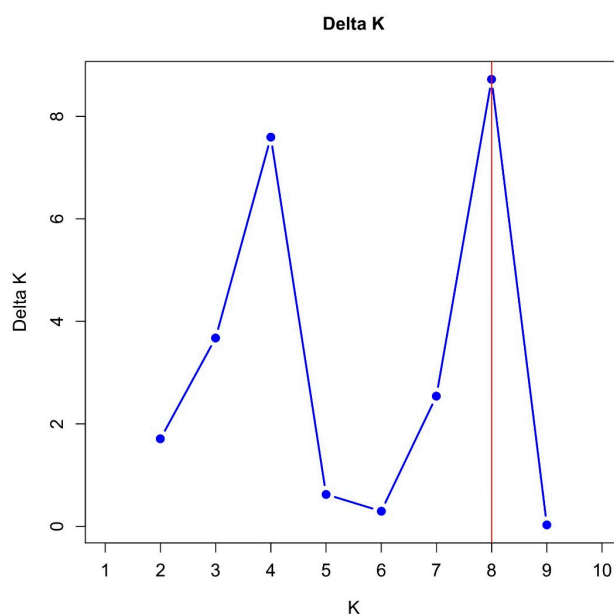
Figure 7. DeltaK plot for the analysed individuals from southern Germany, Poland, Sweden, and Latvia.

The coefficients of membership of each of the predefined populations for K = 8 and K = 4 are shown in Figure 8. The K = 4 clustering broadly divided the analysed populations according to geography, with the populations from southern Germany in one group, together with the Latvian population. The populations from Poland were more heterogeneously clustered into three groups, with the exception of the southeast populations, which were clustered in one group. The populations with the highest similarity to the Latvian population are the populations from southern Germany, in particular the DE03_BA, DE_K_BA, and DE09_OR populations for the K = 8 clustering. The comparison of pairwise genetic and geographic distance matrices at both the individual and population level of the populations within the natural distribution range of *F. sylvatica* (i.e., the German, Polish, and Swedish populations) indicated a significant positive correlation (*p* = 0.001 for both individuals and population comparisons). This genetic isolation by distance is not surprising, given the large geographic scale of the analysed populations. However, long-range gene dispersal is probably effective in *F. sylvatica*, as the analysed Polish population is less structured. The results of this clustering analysis clearly indicate that the analysed Skede stand was established with germplasm originating from southern Germany, rather than the geographically closer Polish *F. sylvatica* populations.
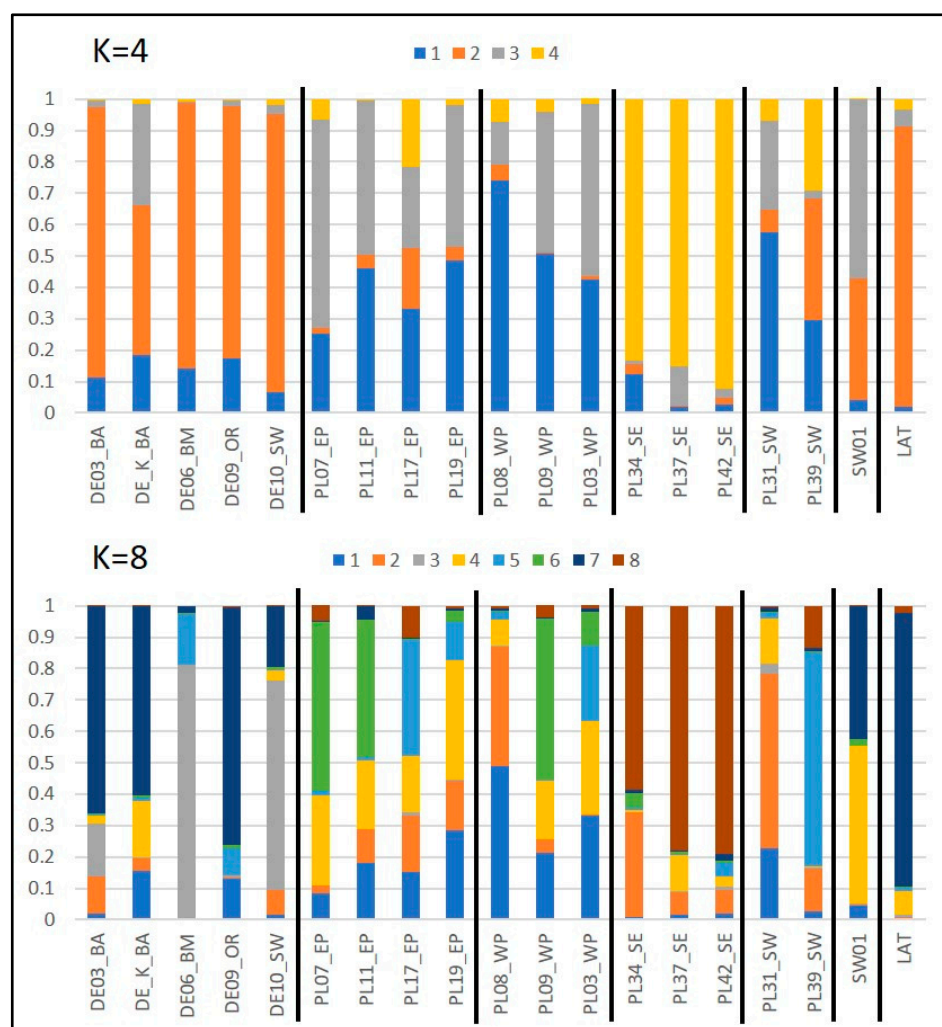

Figure 8. Coefficients of membership (Y axis) of each population for K = 4 and K = 8 for the 19 predefined *F. sylvatica* populations.

This clustering was confirmed by running a STRUCTURE 2.3.4 analysis on a subset containing only the Skede (LAT) and the southern German populations (allele frequencies correlated, no admixture model, burn-in period of 15,000 repetitions, 30,000 MCMC repetitions after burn-in, K limited to 2 groups, and 10 iterations). When the number of clusters is set to two, the similarity of the Skede (LAT) population with the DE03_BA, DE_K_BA, and DE09_OR populations is clearly defined (Figure 9).
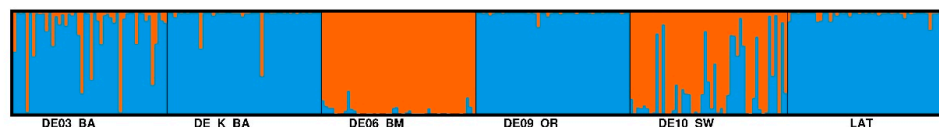
Figure 9. Results of the STRUCTURE 2.3.4 clustering on a data subset containing the five southern German populations and the Skede (LAT) population for K = 2. Each vertical bar represents one individual; the Y axis indicates the membership coefficients to the two clusters.

4. Discussion

Based on the experimental design, and previous reports on seed dispersal distance in *Fagus*, it could be expected that a high proportion the sampled candidate parent group would be the parents of the sampled offspring group in each stand. In the Skede stand, 54 of a total of 101 sampled offspring were assigned to at least one parent, while in the Kaleti stand, 44 of 106 offspring were assigned to at least one parent. These assignments were at a high confidence level (>0.95), and with less stringent conditions, a majority of the offspring from each stand were assigned to sampled parents. The lower level of assigned parents in the Kaleti stand was possibly also due to the lower level of genetic diversity in this stand, which meant that the parental assignments were conducted with a lower confidence level compared to the Skede stand [59]. At a confidence level of >0.80, 73 of 101 Skede offspring were assigned to parents, and 79 of 106 Kaleti offspring. The unassigned offspring could be attributed to seed dispersal from outside the parental sample plots.

As the parental stands were established using imported reproductive material, no spatial genetic structure (SGS) would be expected in the mature individuals. However, further investigation of the SGS in the offspring generation could provide additional information about regeneration processes in these introduced stands. Significant SGS has been reported to develop in beech as soon as after one generation, but that pollen from outside of the local forest stand can decrease the degree of SGS in subsequent generations [60]. Given that there are no *F. sylvatica* stands surrounding these stands, which were established in Latvia using imported reproductive material, and that the genetic base of the seeds used for stand establishment is narrow, a high SGS could be expected in the naturally regenerated offspring generations [61]. This information would be useful for the management of these *F. sylvatica* stands in Latvia.

The Skede parent population did not exhibit a high degree of relatedness, and the offspring were assigned to approximately 30% of the candidate parents sampled. This could be due to seed dispersal or the exclusion of parentage due the presence of null alleles (even though loci with potential null alleles were excluded). The Kaleti parent population was structured, and showed extensive relatedness. This is probably due to the reproductive material utilised [62]. Nevertheless, the offspring group did not show higher levels of relatedness or a lower genetic diversity than the parent group. The seed source and details of the establishment of the Kaleti stand are not known. One possibility is that seeds from a small number of individuals were used, leading to the higher relatedness between the analysed mature individuals. The higher observed heterozygosity could be a result of the low selfing rate in *F. sylvatica* [11,63], among the limited number of individuals from which the seeds were sampled [64].

In the Skede stand, approximately 30% of the sampled parents contributed to the regeneration of the sampled offspring plot, while in the Kaleti stand, approximately 50% of the sampled parents were identified as parents of the sampled offspring. However, the genetic diversity parameters of the offspring were similar (in Skede) or higher (in Kaleti) than those of the sampled mature trees. Further investigations are required to determine if the parents who contribute to the next generation vary between years due to various factors, e.g., flowering time, seed set, etc. However, from a forest management perspective, these results indicate that genetic diversity is being maintained via natural regeneration processes.

The differences in the population structure and diversity between the parental populations in the Kaleti and Skede stands are probably due to the methods used to collect seeds (or seedlings) for stand establishment [65]. A previous study investigated the provenance and population structure of mature *F. sylvatica* stands in Lithuania [48]. Lithuania is also outside of the northern limit of the *F. sylvatica* distribution in Europe, and similarly to the situation in Latvia, beech stands have been established in the previous centuries using imported seed material, but their origin and methodologies are not known [66]. The Lithuanian study found a high allelic diversity in the mature beech stands, but also high inbreeding levels, and the authors suggested that this could be due to the collection of seeds from a number of different, and genetically differentiated beech stands, but from only a few, high-quality individuals from each stand [48]. These results are similar to the results from the Skede stand, which had high allelic diversity parameters, but had higher inbreeding levels compared to the Kaleti stand and the Skede offspring. The information about genetic diversity and population structure can be used to improve forest genetic resource management strategies and is required for the development of climate-smart forestry indicators [67]. The three analysed Skede stands are genetically very similar, and were probably established with the same reproductive material. Further investigation of the Kaleti stand is needed to determine the quality and other parameters. While the genetic diversity is low in this stand, which was probably established using a narrow range or germplasm, the criteria used for the selection of this material are not known, and perhaps this stand contains trees with unique traits. This can inform the selection of genetic resource stands, either for in situ or ex situ conservation approaches.

The Bayesian clustering analysis indicated that the most likely provenance of the Skede stand in Latvia was from southern Germany. This is in agreement with results from a study in Lithuania [48], where Lithuanian *F. sylvatica* populations were compared to the same southern German, Polish, and Swedish populations as in this study. Five of the ten analysed Lithuanian populations also clustered with the DE03_BA, DE_K_BA, and DE09_OR populations. Of these five Lithuanian populations, three were forest stands, and two were manor parks. These results suggest that the reproductive material introduced into the Baltic region in the 19th century was largely of southern German origin, and possibly collected from the same or similar sources for introduction into Latvia and Lithuania. Some of the other analysed Lithuanian populations also clustered with the Polish populations, indicating that, in some cases, reproductive material from Poland was also in introduced to Lithuania. In Latvia, the Skede population is the only large and well-established *F. sylvatica* population in Latvia. The Kaleti population, located in south-western Latvia close to the Lithuanian border, is small, and as shown in this study, likely to have been established with reproductive material from a very limited number of individuals. Probably due to this founder effect, the Kaleti population was genetically differentiated from all other *F. sylvatica* populations analysed in this study, including the Skede population, and identifying a provenance region for the Kaleti population was not possible. The determination of the provenance of the *F. sylvatica* material introduced into Latvia approximately 100 years

ago enables an assessment of the long-range transfer of germplasm outside of the natural distribution of this species. The successful survival and reproduction of this material indicates that these long-range transfers can result in the establishment of viable forest stands in the long-term.

5. Conclusions

This study provides the first genetic data about European beech stands in Latvia, which is outside the distribution area of this species, and makes a significant contribution to understanding the genetic diversity, population structure, and provenance of *these stands*. The analysis of naturally regenerated young *F. sylvatica* individuals in two stands indicates that there is no decrease in genetic diversity in the offspring. The Kaleti stand was probably established with germplasm which was obtained from a limited number of mother trees, as a number of full and half sib families could be identified, and the level of genetic diversity was lower than that of the Skede stand. Nevertheless, there was no decrease in genetic diversity parameters in the sampled offspring, and the average pairwise relatedness in the offspring was lower than that of the parents. This indicates that the introduced *F. sylvatica* populations have adapted to Latvian environmental and climactic conditions, and natural regeneration has not decreased the genetic viability of these introduced populations. The most probable provenance of the reproductive material used to establish the Skede stand is from southern Germany, similar to that of some European beech stands that were established at a similar time in neighbouring Lithuania [48]. The management of European beech genetic resources in Latvia should be focussed on the stands located at Skede, as the Kaleti stand is small and has a lower genetic diversity. Further phenotypic analysis of the Kaleti stand is needed to determine if this stand contains individuals with unique or silviculturally valuable traits.

In terms of climate change predictions for north-eastern Europe, beech is sensitive to drought [68], but the response of European beech provenances to environmental constraints varies, and the phenotypic plasticity and evolutionary adaptability of this species may not be fully appreciated [24]. However, genetic diversity is the foundation of adaptation in populations, and so, this information is crucial for the long-term management of tree genetic resources. More detailed analyses of growth rates (e.g., by using dendrochronological (tree ring) analysis), can provide additional information on the responses of this introduced germplasm to long-term local climactic and environmental conditions. This can contribute data for modelling and other analyses to inform decisions about assisted migration in the future. The findings lay a crucial foundation for future research into fine-scale spatial genetic structure, which will enhance strategies for managing these introduced stands. Additionally, integrating growth and quality assessments with genetic analyses offers valuable insights into the genetic architecture and control of silviculturally important traits. This approach also opens new opportunities for selective breeding, utilising the germplasm introduced to Latvia over a century ago, while advancing the potential for selective genomics and assisted migration strategies.

Data Availability Statement: Genotyping data are provided in Supplementary File S3.

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