Comparison of Genetic Diversity in Naturally Regenerated Norway Spruce Stands and Seed Orchard Progeny Trials

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Abstract: Forest ecosystems in Europe are expected to experience changes in temperature and water regimes associated with increased risks of extreme environmental events and disasters. Genetic diversity and relatedness has been linked to resilience of forest stands and landscapes. Genetic diversity indicators were compared between a Norway spruce population naturally regenerated after extensive windthrow and Norway spruce progeny populations derived from two seed orchards. In addition, genetic diversity in an undisturbed stand in a long established national park and a spruce genetic resource stand were analyzed. Populations were genotyped at 11 simple sequence repeat (SSR) loci. Average genetic diversity indicators were similar across populations. However, the total number of alleles, average number of alleles over all loci, effective number of alleles, average gene diversity, and average allelic richness were highest in the naturally regenerated population and lowest in one of the seed orchard progeny populations. The genetic diversity in progeny from seed orchards used for stand renewal is comparable to the genetic diversity in naturally regenerated stands. However, fluctuations in seed production between years can have a large impact on genetic diversity in seed orchard progeny. The use of improved Norway spruce germplasm deployed via clonal seed orchards for forest renewal can maintain similar levels of genetic diversity compared to naturally regenerated stands, while also increasing production and timber quality.

Keywords: adaptation; regeneration; forest management; tree breeding; microsatellite markers; genotyping

1. Introduction

Forest ecosystems in Europe are expected to experience changes in temperature and water regimes associated with increased risks of forest disturbances [1]. Projected future climate scenarios pose uncertainties related to increased risks of extreme events and disasters [2]. The predictions for vegetation cover (climatic envelope) models suggest that forest composition is already expected to shift by the end of the 21st century due to climatic changes [3]. Furthermore, under most scenarios, Norway spruce (Picea abies (L.) Karst.) would experience difficulties in growth, and particularly regeneration (caused by droughts, pests, storm damages) [4–6]. Recovery of ecosystems and their further development also depends on management decisions regarding natural succession or planting of improved germplasm, which may have impacts on the genetic diversity of forest stands [7,8].

There are many interactions and often evolutionary processes (evolutionary adaptation) that occur as a result of natural or anthropogenic disturbances, which play essential roles in forest resilience [9].
The environmental alterations may affect ecosystem responses via tree species migration, phenotypic plasticity, or evolutionary adaptation [10]. In particular, evolutionary changes related to natural regulation have a major effect on the ability of populations to survive in their environment [11]. Potentially, one effect of large scale disturbances (or clear-cuts by humans) on tree populations could be a decrease in genetic diversity due to considerable tree mortality [12]. Consequently, regeneration influences the ability of tree populations to maintain sustainable levels of genetic diversity. Pollen and seed dispersal mechanisms also impact tree populations. Post-disturbance genetic structure is influenced by the fitness of individuals already present at a site and by genotypes migrating from elsewhere [13]. Naturally regenerated genotypes become less genetically similar with increasing geographical distance between them, and these are not distributed randomly [14]. Understanding the factors impacting the genetic diversity of naturally regenerating populations will provide a basis for forest management decisions.

Simple sequence repeat (SSR) markers are widely used for population genetic studies as they exhibit codominance and are usually highly polymorphic. Prior to the increasing availability of sequence data, their development required a significant investment, and their cross-species transferability was limited because of absence of the repeat region or degeneration of the primer binding sites. Expressed sequence tag SSRs (EST-SSRs) are developed from expressed RNA sequences and can be identified from sequence databases. One advantage of EST-SSRs is that they are directly associated with a coding gene, and so may be useful for association with phenotypic traits. Also, because expressed sequences are more likely to be evolutionary conserved, cross-species PCR amplification of EST-SSRs is expected to be more successful compared to SSRs developed from genomic DNA. However, their levels of variability may not be as great because of selective constraints [15].

The forest management practices that aim to sustain diversity for future generations have encountered new challenges (e.g., ensuring a low level of co-ancestry to avoid inbreeding [16]). The combination of breeding and silvicultural activities may have major consequences related to the existing natural genetic variation in forest tree populations [17]. In particular, genetic diversity and relatedness has often been the main subject in scientific and public discussions related to maintenance of valuable genetic resources and intensive forest management, with increased demand for superior improved germplasm for reforestation purposes [18]. Specifically, it is necessary to capture the majority of alleles that are present in natural populations in seed orchards at similar frequencies [19].

Long-term Norway spruce breeding programs have been developed to improve adaptation to climatic conditions and resistance to the effects of biotic and abiotic factors [20]. Thus, there is an ongoing need for assessment of possible changes in genetic diversity and relatedness among and within breeding populations of Norway spruce and the progeny derived from these populations. In this study, microsatellite markers were used to analyze genetic diversity in forest stands with differing structures and regeneration history: a population in Slitere naturally regenerated after significant windthrow damage, an undisturbed population in a long-established national park, a Norway spruce genetic resource stand, and two Norway spruce seed orchard progeny trials (established from two different seed orchards). The aim of the study was to assess the genetic diversity of regenerating populations of Norway spruce after large-scale disturbance in comparison to managed genetic resource stands and seed orchard progeny trials.

2. Materials and Methods

2.1. Analyzed Populations

The Slitere Nature Reserve (SNR) is located in the north-western part of Latvia (Figure 1), with a total area of 1100 ha, and is the oldest part of the Slitere National Park, which was established in 1923. Since then, no silvicultural activities have been undertaken in this area. The SNR is dominated by forest stands specific to hemiboreal forests [21]. The most abundant tree species in this area are Norway spruce (Picea abies L. Karst.) and Scots pine (Pinus sylvestris L.) (44% and 31%, respectively). A major
In addition, two “natural” spruce stands were analyzed—the Rezekne spruce forest genetic resource (FGR) stand (coordinates: 56.599, 27.414) (96 samples), and the Moricsala nature reserve (coordinates: 57.195, 22.147) (48 samples) (Figure 1). Samples from these stands were collected from individuals separated by a minimum of 50 m. Both of these stands are considered to be autochthonous and naturally established. The mean age of the Rezekne FGR stand is over 100 years, and it is a source-identified seed stand. The Moricsala nature reserve is the oldest nature reserve in Latvia, established in 1912, and is a strictly protected area. Two progeny trials established using seeds from two clonal Norway spruce seed orchards were also analyzed. The Remte seed orchard is located in the south-western part of Latvia (coordinates: 56.735, 22.795; area: 27.72 ha) and was established in 1965, and the Katvari seed orchard is located in the northern part of Latvia (coordinates: 57.530, 24.745; area: 15.5 ha) and was established in 1975. The Remte seed orchard consists of 50 clones in total, and the Katvari plantation of 20 clones in total. The distance to the nearest stand of the same species for the Remte seed orchard is 1000 m and for Katvari is 500 m. The trees are distributed in a 4 m × 6 m grid in Remte and a 5 m × 5 m grid in Katvari. A random sample of bulk seed from the Remte and Katvari seed orchards (seeds from the 2001 and 1998 harvest years, respectively) were used to establish the progeny trials. These progeny trials (Remte, 72 individuals; Katvari, 90 individuals) were sampled for genetic analysis (Figure 1). Details about the analyzed populations are summarized in Table 1.
Table 1. Summary of analyzed populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Type</th>
<th>Establishment Year</th>
<th>Age (Years)</th>
<th>Number of Clones</th>
<th>Nearest Con-Specific Stand</th>
<th>Location (N; E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slitere progeny</td>
<td>Autochthonous—naturally regenerated</td>
<td>&gt;1969</td>
<td>&lt;50</td>
<td>-</td>
<td>-</td>
<td>57.63; 22.28</td>
</tr>
<tr>
<td>Slitere parents</td>
<td>Autochthonous</td>
<td>&lt;1969</td>
<td>&gt;50</td>
<td>-</td>
<td>-</td>
<td>57.63; 22.28</td>
</tr>
<tr>
<td>Rezekne</td>
<td>Autochthonous</td>
<td>~1912</td>
<td>≥100</td>
<td>-</td>
<td>100 m</td>
<td>56.60; 27.41</td>
</tr>
<tr>
<td>Moricsala</td>
<td>Autochthonous</td>
<td>1910-1930</td>
<td>~90</td>
<td>-</td>
<td>2400 m</td>
<td>57.20; 22.15</td>
</tr>
<tr>
<td>Remte</td>
<td>Seed orchard progeny trial</td>
<td>1965</td>
<td>54</td>
<td>50</td>
<td>1000 m</td>
<td>57.53; 24.75</td>
</tr>
<tr>
<td>Katvari</td>
<td>Seed orchard progeny trial</td>
<td>1975</td>
<td>44</td>
<td>20</td>
<td>500 m</td>
<td>57.33; 24.75</td>
</tr>
</tbody>
</table>

2.2. Genetic Analysis

DNA samples from spruce needles were isolated using a CTAB-based method [23]. Genotyping was done using 10 nuclear SSR markers (Table 2). Each forward primer was labelled with a different fluorophore (6-FAM, HEX, or TMR) to facilitate visualization using capillary electrophoresis. The PCR reactions for the nuclear SSR markers were carried out in a 20 µL solution containing a final concentration of 0.2 mM dNTPs, 2 mM MgCl2, 0.2 µM of each primer, 1.5 µL DNA solution, 1× Taq buffer, and 1 U of recombinant Taq DNA polymerase (Thermo Scientific, Waltham, MA, USA).

Table 2. Microsatellite loci utilized for genotyping spruce individuals.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer Sequences (5′-3′)</th>
<th>Label (F Primer)</th>
<th>Repeat Motif</th>
<th>Fixation Index (Over All Progeny Populations) (SE)</th>
<th>Estimated null Allele Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpAGC1</td>
<td>F:TTCACCTTACCGCGAGAACC  R:ACTGAGGAGTTGTCTGCTGA</td>
<td>6-FAM</td>
<td>(TC)_{10}</td>
<td>0.091 (0.032)</td>
<td>0.022</td>
</tr>
<tr>
<td>SpAGC2</td>
<td>F:TACCTATTACCCAAAGGA  R:GTGTATGGTTTTTCTTTGCC</td>
<td>HEX</td>
<td>(GA)_{20}</td>
<td>0.563 (0.054)</td>
<td>0.301</td>
</tr>
<tr>
<td>SpAGG3</td>
<td>F:CTCAACTTCCCATGTAGCT  R:AGCGAGGACCTTAAATATAC</td>
<td>TMR</td>
<td>(GA)_{23}</td>
<td>0.026 (0.012)</td>
<td>0.018</td>
</tr>
<tr>
<td>UAPgTG25</td>
<td>F:TCAGCTTCTTCCCCCCAGAG  R:GTGTATGGTTTTTCTTTGCC</td>
<td>HEX</td>
<td>(TG)_{27}</td>
<td>0.629 (0.040)</td>
<td>0.280</td>
</tr>
<tr>
<td>UAPgAG150</td>
<td>F:ACCAATGCTTTTACCAAACGT  R:GTGTATGGTTTTTCTTTGCC</td>
<td>TMR</td>
<td>(AG)_{19}</td>
<td>0.172 (0.082)/0.260 (0.038)</td>
<td>0.043/0.106</td>
</tr>
<tr>
<td>WS0033.A18</td>
<td>F:GGGCTCTCTCTCATCGGTTTT  R:GTGTATGGTTTTTCTTTGCC</td>
<td>6-FAM</td>
<td>(TA)_{26}</td>
<td>0.689 (0.031)</td>
<td>0.300</td>
</tr>
<tr>
<td>WS0022.B15</td>
<td>F:TGTGAGGCTGCTGAGAGTA  R:TGGCTTTTATTCACGCAAGA</td>
<td>HEX</td>
<td>(AG)_{12}</td>
<td>0.086 (0.049)</td>
<td>0.087</td>
</tr>
<tr>
<td>WS0073.H08</td>
<td>F:GTGCTCTCTTATATTCTCCAG  R:GGTCAGGAGAAGATATCTAGG</td>
<td>TMR</td>
<td>(AT)_{14}</td>
<td>0.066 (0.018)</td>
<td>0.042</td>
</tr>
<tr>
<td>WS0073cG10</td>
<td>F:AGCATGAGGATTTGCTACCT  R:CGCTGGAGAAGAAATTCAGG</td>
<td>6-FAM</td>
<td>(GGC)_{9}</td>
<td>0.194 (0.028)</td>
<td>0.096</td>
</tr>
<tr>
<td>pSGB3</td>
<td>F:AGTGATTTAAACTCTGACCC  R:ACGTGAAATCTTACATCC</td>
<td>HEX</td>
<td>(AT)_{11}</td>
<td>0.062 (0.018)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Note: 1 Pfeiffer et al., 1997 [24]; 2 Hodgetts et al., 2001 [25]; 3 Rungis et al., 2004 [15]; 4 Besnard et al., 2003 [26]; 5 Rungis, unpublished.

PCR cycling conditions consisted of an initial denaturation step of 95 °C for 4 min; 35 cycles of 94 °C for 30 s, 52 °C for 45 s, and 72 °C for 60 s; followed by a final extension step of 72 °C for 10 min. All PCR reactions were carried out in an Eppendorf Mastercycler gradient thermal cycler. Amplification fragments were separated on an ABI Prism 3130xl Genetic Analyzer (Life Technologies, Foster City, CA, USA) and genotyped with GeneMapper 3.5. Genotype data was checked using the Micro-checker software [27] to identify errors caused by the presence of null alleles and other
factors the confidence interval was set at 95%, and 1000 randomizations were performed. Null allele frequencies were estimated using the Van Oosterhout estimator. Analysis of nuclear SSR data was done using GenAlEx 6.5 [28], including calculation of pairwise relatedness using the Queller and Goodnight [29] estimator. Pairwise linear genetic distances and geographic distances of the Slitere progeny population were compared using a Mantel test (999 permutations). Allelic richness and gene diversity were calculated using Fstat v.2.9.3.2 [30]. Effective population size (Ne) was estimated using the LD method of Waples and Do (2010) [31], as implemented in NeEstimator V2, using a lowest allele frequency threshold of 0.05.

3. Results

A total of 10 SSR markers were utilized to genotype the Norway spruce samples. As reported previously [24], marker UAPgAG150 amplified two loci, which were independently genotyped, therefore the stands were genotyped at a total of 11 loci. The number of alleles amplified at each locus ranged from 8 (UAPgAG150A and WS0073.H08) to 31 (SpAGC1), with a mean of 10.73 ± 0.64. The number of effective alleles ranged from 1.79 (UAPgAG150A) to 11.75 (SpAGG3), with a mean of 4.83 ± 0.42. Shannon’s information index (I) ranged from 0.83 (UAPgAG150A) to 2.62 (SpAGG3) with a mean of 1.65 ± 0.13. The mean observed heterozygosity (0.53 ± 0.04) was lower than the expected heterozygosity (0.72 ± 0.07), and the fixation index was above zero for all analyzed loci, ranging from 0.01 (SpAGG3) to 0.71 (WS0033.A18), with a mean of 0.26 ± 0.03. This indicates that there was an excess of homozygotes compared to the expected values, assuming that the populations are in Hardy–Weinberg equilibrium. Potential null alleles were detected at all analyzed loci, except for SpAGC1, SpAGG3, and paGB3. At four loci, the estimated null allele frequency was over 0.1 (SpAGC2, UAPgTG25, UAPgAG150A, WS0033.A18). The null alleles were present in similar frequencies in all analyzed populations, and genetic distances between populations were not calculated (which can be influenced by the presence of null alleles); therefore, all 11 loci were retained for genetic diversity and within population pairwise relatedness analyses.

Average genetic diversity indicators—allele number, allele number with a frequency of over 0.05, effective allele number, Shannon’s information index, expected and observed heterozygosity (calculated over all loci)—in the analyzed populations were similar. Mean allelic richness (based on a minimum sample size of 45 individuals) ranged from 8.99 in the Remte progeny to 10.22 in the Slitere progeny (Table 3).

Table 3. Genetic diversity indicators in the analyzed Norway spruce stands.

<table>
<thead>
<tr>
<th></th>
<th>Slitere Progeny</th>
<th>Slitere Parents</th>
<th>Rezekne</th>
<th>Moricsala</th>
<th>Remte</th>
<th>Katvari</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>95</td>
<td>58</td>
<td>96</td>
<td>48</td>
<td>72</td>
<td>90</td>
</tr>
<tr>
<td>Total Na</td>
<td>134</td>
<td>112</td>
<td>122</td>
<td>110</td>
<td>106</td>
<td>123</td>
</tr>
<tr>
<td>Average Na</td>
<td>12.18 (1.75)</td>
<td>10.18 (1.48)</td>
<td>11.09 (1.86)</td>
<td>10.00 (1.61)</td>
<td>9.64 (1.28)</td>
<td>11.18 (1.57)</td>
</tr>
<tr>
<td>Total Na Frequency ≤ 5%</td>
<td>85.0 (0.59)</td>
<td>5.09 (0.89)</td>
<td>4.82 (0.64)</td>
<td>5.00 (0.65)</td>
<td>4.00 (0.40)</td>
<td>4.82 (0.70)</td>
</tr>
<tr>
<td>Average Na Frequency ≥ 5%</td>
<td>5.14 (1.26)</td>
<td>5.18 (1.31)</td>
<td>5.09 (1.10)</td>
<td>4.87 (0.96)</td>
<td>4.02 (0.59)</td>
<td>5.03 (1.03)</td>
</tr>
<tr>
<td>Neff</td>
<td>1.67 (0.19)</td>
<td>1.65 (0.18)</td>
<td>1.66 (0.18)</td>
<td>1.67 (0.16)</td>
<td>1.57 (0.14)</td>
<td>1.70 (0.17)</td>
</tr>
<tr>
<td>I</td>
<td>0.55 (0.07)</td>
<td>0.52 (0.18)</td>
<td>0.55 (0.07)</td>
<td>0.52 (0.08)</td>
<td>0.52 (0.07)</td>
<td>0.53 (0.07)</td>
</tr>
<tr>
<td>Ho</td>
<td>0.71 (0.05)</td>
<td>0.71 (0.04)</td>
<td>0.71 (0.05)</td>
<td>0.72 (0.05)</td>
<td>0.69 (0.05)</td>
<td>0.72 (0.05)</td>
</tr>
<tr>
<td>He</td>
<td>0.22 (0.08)</td>
<td>0.28 (0.08)</td>
<td>0.24 (0.07)</td>
<td>0.27 (0.09)</td>
<td>0.27 (0.08)</td>
<td>0.27 (0.08)</td>
</tr>
<tr>
<td>No. of unique alleles</td>
<td>15</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Average gene diversity</td>
<td>0.71 (0.05)</td>
<td>0.72 (0.04)</td>
<td>0.72 (0.05)</td>
<td>0.73 (0.05)</td>
<td>0.70 (0.05)</td>
<td>0.73 (0.05)</td>
</tr>
<tr>
<td>Average Allelic richness</td>
<td>10.22 (1.52)</td>
<td>9.65 (1.43)</td>
<td>9.79 (1.49)</td>
<td>9.87 (1.57)</td>
<td>8.99 (1.12)</td>
<td>10.01 (1.41)</td>
</tr>
</tbody>
</table>

Standard errors shown in brackets. Abbreviations: N—number of individuals; Na—number of alleles; Neff—effective number of alleles; I—Shannon’s diversity index; Ho—observed heterozygosity; He—expected heterozygosity; F—fixation index.
The total number of alleles identified in each population ranged from 134 (Slitere progeny) to 106 (Remte). The number of low frequency alleles (<0.05) ranged from 85 (Slitere progeny) to 55 (Moricsala). The number of alleles unique to one population ranged from 15 (Slitere progeny) to three (Remte). The total number of alleles unique to only one population was 53 (out of a total of 193 alleles identified over all populations). The majority of these unique alleles were low frequency, with only one with a frequency above 0.05 (Moricsala, f = 0.054).

Genetic differentiation between populations (F_{st}) was low. The average pairwise F_{st} value between all analyzed populations was 0.012, with the lowest value between Slitere offspring and Rezekne (0.005), and the highest between Moricsala and Remte (0.023). The average pairwise F_{st} value between the mature populations (Slitere parents, Rezekne, Moricsala) was 0.013, reflecting the low genetic differentiation of spruce populations within Latvia. The F_{st} value between the Slitere parent and progeny populations was 0.006. The F_{st} values between the Remte and Katvari population was 0.010, which were similar to those between the Slitere progeny population and the Remte (0.012) and Katvari (0.009) populations. The Remte and Katvari seed orchard progeny populations were not highly differentiated from the mature populations (Slitere parents, Rezekne, Moricsala) (average pairwise F_{st} was 0.013). Pairwise relatedness was also calculated in the three progeny populations (Slitere, Remte, and Katvari progeny). The mean relatedness values in the Slitere progeny and Remte progeny populations were positive (0.011 and 0.027, respectively), while in the Katvari population, mean relatedness was -0.008 (Figure 2). There was no evidence for spatial genetic structure in the Slitere progeny population, with no significant difference between the pairwise geographic and genetic distance matrices (Mantel test, 999 permutations).

![Figure 2](image_url)  
**Figure 2.** Average within population pairwise relatedness [29] values (r). Error bars indicate the 95% confidence interval of the mean values, as determined by resampling (999 bootstraps); red bars indicate the 95% confidence interval of the null hypothesis of “no difference” between the populations.

The effective population size (Ne) was estimated with the linkage disequilibrium (LD) method. The estimated effective population sizes were smallest in the seed orchard progeny populations, and were proportional to the number of clones in each plantation. The effective population size of the Slitere progeny was smaller than that of the older trees, and was probably influenced by the relatively smaller number of individuals contributing to the regeneration of the Slitere stand after the extensive windthrow damage. The Rezekne and Moricsala stands have large estimated effective population sizes, reflecting the large size of these populations and the high gene flow into the sub-populations sampled from the surrounding stands (Table 4).
Table 4. Effective population size (Ne) of populations calculated using the linkage disequilibrium (LD) method, with upper and lower 95% confidence intervals (CI).

<table>
<thead>
<tr>
<th>Population</th>
<th>Ne</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slitere progeny</td>
<td>167.3</td>
<td>103.3</td>
<td>361.7</td>
</tr>
<tr>
<td>Slitere parents</td>
<td>251.4</td>
<td>114.2</td>
<td>Infinite</td>
</tr>
<tr>
<td>Rezekne</td>
<td>685.5</td>
<td>220.7</td>
<td>Infinite</td>
</tr>
<tr>
<td>Moricsala</td>
<td>714.6</td>
<td>141.3</td>
<td>Infinite</td>
</tr>
<tr>
<td>Remte</td>
<td>68.2</td>
<td>47.5</td>
<td>108.4</td>
</tr>
<tr>
<td>Katvari</td>
<td>48.5</td>
<td>38.5</td>
<td>62.7</td>
</tr>
</tbody>
</table>

4. Discussion

Forest tree breeding programs strive to balance genetic gain and selection intensity while maintaining sufficient genetic diversity within the deployed material. Seed orchards are the mechanism used to deliver the results of forest breeding programs for use in planting and stand renewal. Particularly for long rotation species such as Norway spruce, which are planted in regions where this species is autochthonous, the deployment of improved germplasm should be monitored to ensure that levels of genetic diversity are maintained, and that they are comparable to naturally renewed stands. Most clonal orchards contain 30–50 genotypes, with varying numbers of ramets [32]. This number of clones seems to ensure high levels of genetic diversity comparable to natural populations. The high levels of genetic diversity are a result of the diversity present within the clones, which in many cases is supplemented by high levels of pollen flow from outside the seed orchard. Previous studies comparing the genetic diversity of seed orchards with that of natural populations have reported comparable levels of genetic diversity, with the exception of the reduction of rare frequency alleles [32]. The levels of genetic diversity in seed orchards and their progeny can in fact be higher than those of surrounding populations, as the clones present in a seed orchard may be sourced from a wide geographic distribution [33].

The values of most genetic diversity indicators in the seed orchard progeny trials (particularly the Katvari progeny trial) were similar to the naturally regenerated Norway spruce stand in Slitere, as well as the natural stands at Rezekne and Moricsala. Effective population sizes were larger in the mature natural stands, as well as in the naturally renewed Slitere stand, compared to the seed orchard progeny trials. The effective population sizes in the seed orchard progeny were relative to the number of clones in each seed orchard (50 in Remte and 20 in Katvari). Pollen flow from outside of the main producing blocks in these seed orchards resulted in larger Ne estimates than the clone number in each plantation. All 11 loci were utilized for the calculation of Ne, despite the probable presence of null alleles, which can reduce Ne estimates of populations [34]. However, the Ne estimates do not seem to be unusually low, and are similar to those reported previously in a comparison of Norway spruce seed lots from seed orchards and natural stands [35]. The estimation of effective population sizes with the LD method is more accurate for populations with smaller Ne, and the Ne of the Rezekne and Moricsala stands are probably overestimated due to high immigration rates [36]. The higher LD, and subsequently lower Ne sizes in the Remte and Katvari progeny, may also be caused by the relative isolation of the seed orchards (in comparison to the other analyzed stands), resulting in decreased pollen contamination and more structured progeny populations. The Remte and Katvari seed orchards are not particularly isolated from surrounding spruce stands (1000 m and 500 m, respectively), but the majority of pollen is dispersed at short distances. However, this does not exclude the influence of long-distance pollen dispersal on the introduction of additional genetic diversity [37]. In this study, the diversity of the progeny was estimated from established progeny trials or naturally renewed stands rather than directly from seed lots. In the case of the progeny trials from seed orchards, the sampling of mature progeny trees may reduce the levels of identified genetic diversity compared to direct sampling of seed lots because of the selection and retention of vigorous genotypes within the progeny trials. In addition, each
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of the sampled progeny trials were established from a single year seed lot. In contrast, the naturally
renewed spruce stands at Slitere contained individuals of differing ages, established at various times
after the storm that devastated the stand. The presence of large areas of Norway spruce adjacent to this
stand ensured a large amount of pollen and seed flow from these surrounding areas. A similar increase
in genetic diversity in naturally regenerated Norway spruce populations in comparison to putative
maternal populations has also been reported in Lithuania [38]. The low population differentiation (Fst)
values between all the analyzed populations is a reflection of the low level of population structure in
Norway spruce, particularly within Latvia, which does not have notable gene flow barriers or strong
environmental gradients. The seed orchard progeny populations were not differentiated from the
mature populations (Slitere parents, Rezekne, Moricsala), indicating that the breeding process has
not greatly altered gene frequencies compared to natural populations. The selection criteria in the
Latvian Norway spruce breeding program are based on quantitative traits, such as growth rate and
stem quality, which are controlled by multiple genes (QTLs), and moreover, the number of markers
utilized in this study is not sufficient to effectively identify any signs of selection. The genetic similarity
of seed orchard progeny and natural stands is also maintained by pollen flow from surrounding stands
into seed orchards. Differentiation due to genetic drift as a consequence of the lower populations sizes
(clone numbers) in seed orchards may be of higher concern.

Surprisingly, the genetic diversity indicators in the Katvari seed orchard (20 clones) progeny
trials were higher than in the Remte seed orchard (50 clones) progeny trials, particularly the average
pairwise relatedness. While this may be due to a higher level of isolation from pollen contamination at
the Remte seed orchard, seed year can also have a significant effect on the genetic diversity of seed
crops, particularly for Norway spruce, which has pronounced mast years [39]. The available data
from seed orchard managers indicate that seed production in the year when seeds were collected from
the Remte plantation was very low. Seed production from Remte in 2000–2001 was 162 hl or 156 kg
(5.6 kg ha⁻¹), compared to Katvari in 1998, which was 780 hl or 738 kg (46.6 kg ha⁻¹), and potentially
only a few clones were significantly contributing to the average seed sample lot that was used to
establish the Remte progeny trial (data from JSC “Latvia’s State Forests”, Seeds and Plants division).
The lower values in the Remte progeny trial for some genetic diversity indicators (e.g., number of
unique alleles, average gene diversity, and allelic richness) is probably due to the low seed harvest
in that year. Additional progeny trials, established from different seed year lots from the Remte
plantation are available, and analysis of these can determine if these lower levels of genetic diversity
are a result of a particular seed year. The higher genetic diversity in the Katvari plantation progeny
trials indicates that despite the relatively small number of clones from which seeds were harvested
(20), there is sufficient pollen flow to maintain diversity levels comparable to natural populations.
In fact, the average pairwise relatedness was lower in the Katvari population compared to the naturally
regenerated Slitere progeny population. The comparatively lower diversity in the Remte progeny
population, despite the seeds being collected from a larger number of clones, suggests that fluctuations
in seed production between years can have a large impact on genetic diversity. While no direct data
is available, the actual number of seed producing clones in the Remte plantation in the year that the
seeds were collected is probably lower than the number of clones in the seed orchard. Therefore, seeds
collected in non-mast years need to be deployed with caution to prevent a loss of genetic diversity in
the progeny populations.

5. Conclusions

The genetic diversity in progeny from seed orchards used for stand renewal is comparable to
the genetic diversity in naturally regenerated stands. One of the main concerns when using seed
orchard progeny is the loss of rare alleles found in natural populations. However, the germplasm in
the Latvian spruce breeding program is essentially derived from Latvian populations, and natural
spruce stands are found throughout Latvia, with no barriers for pollen dispersal. Therefore, pollen
flow into seed orchards can ensure the introduction of these rare alleles into the progeny from these
orchards. The use of improved Norway spruce germplasm deployed via clonal seed orchards for forest renewal can maintain similar levels of genetic diversity compared to naturally regenerated stands, while also increasing production and timber quality.


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