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# Changes in the gene pool composition of Scots pine depending on the mode of regeneration

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**Abstract:** Silvicultural practices can alter forest genetic resources in unpredictable ways, thereby influencing the adaptive and evolutionary potential of forest populations. This is especially alarming in the case of species with more northern distribution centers, due to the lack of area that can be colonized further north. In this article, we studied the genetic diversity of the Scots pine seed tree stand in Poland, its natural regeneration, and two artificially established progeny plantations. The research aimed to determine whether the regeneration mode had affected the efficiency of the gene pool transmission from the maternal seed stand to its offspring. Using nuclear microsatellite markers we compared the parameters of genetic variation and allelic composition among the studied stands. The results showed that all stands represent a common genetic pool with slightly higher values of observed heterozygosity in the case of progeny plantations. Inbreeding was significant only in natural regeneration. All stands have gained and lost rare alleles compared to the maternal seed stand. Nevertheless, the analysis of population differentiation showed that the gene pool of the maternal stand had been transmitted more efficiently to the natural regeneration, though the difference was only minimal. Possible reasons for the differences in transmission efficiency between natural regeneration and artificially established progeny plantations mainly include variations in the number of mother trees and crossing patterns in different reproductive seasons. Furthermore, some individuals that grow in the studied progeny plantations may be natural regeneration of the neighboring stands. In light of the obtained results, we discuss the genetic considerations for establishing and using seeds from progeny plantations in Poland.

**Keywords:** genetic diversity, microsatellites, natural and artificial regeneration, progeny plantations

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## Introduction

The genetic diversity of forest trees ensures the stability and sustainability of forest ecosystems (Schaberg et al., 2008). High genetic variability, including the presence of rare alleles, allows natural selection to result in adaptation (Savolainen et al., 2007). As the pressure posed by various abiotic and biotic stresses is constantly increasing, all organisms need to find strategies to adjust to these changes. This is especially difficult for forest trees because they cannot change their location and are likely to encounter numerous environmental changes throughout their long life spans. There is a hypothesis that adaptation from pre-existing genetic variation happens faster because beneficial alleles are immediately available and at higher frequencies than in the case of adaptation from new mutations (Innan & Kim, 2004). Therefore, the maintenance of genetic variation through generations gives more chances that at least some individuals in a population are capable to adapt to new environmental conditions (Ivetić et al., 2016).

The composition and richness of the gene pools of forest tree populations result from many factors including their evolutionary history, mutation rate, the effect of genetic drift, migration patterns, environmental pressure, and human activity. Silvicultural techniques associated with the mass production of forest reproductive material (FRM) and reforestation are considered to be the driving force of alterations in the forest genetic resources (Hosius et al., 2006; Schaberg et al., 2008; Ratnam et al., 2014; Ivetić et al., 2016). Although there is little evidence that the artificial regeneration of forest stands leads to a strong reduction of their genetic diversity (Koski, 2000; El-Kassaby et al., 2003; Dzialuk & Burczyk, 2006; Fageria & Rajora, 2013), some changes in the gene pool composition of managed forest populations concerning natural stands are obvious (Hawley et al., 2005; Kosińska et al., 2007; Marquardt et al., 2007; Dering & Chybicki, 2012). The number and frequency of rare alleles are usually decreased, thereby lowering the future adaptive potential of particular stands (see Ivetić et al., 2016 and references therein). It has to be emphasized, however, that these conclusions were drawn based on neutral markers. Therefore, it is hard to predict how silvicultural practices will affect productivity, if at all. Regardless of this, artificial regeneration can result in genetic changes that apply not only to the planted stands but also to the neighboring forests via gene flow (Finkeldey & Ziehe, 2004). Moreover, some breeding practices, like phenotype selection or thinning operations, prefer specific genotypes. Seed processing and storage, as well as nursery conditions and operations, can also favor certain families and discard others (see Ivetić et al., 2016 and references therein; Konecka et al., 2018). The transfer of FRM also involves some risks of spreading pests and diseases as well as introducing invasive tree species (Koskela et al., 2014). The extent of genetic impacts depends on the management system applied, stand structure as well as species' distribution, demography, ecology, and biological attributes (see Ratnam et al., 2014 and references therein; Gautam et al., 2021). Considering the fundamental importance of genetic diversity to the continued adaptation, health, and long-term productivity of tree populations (Hughes et al., 2008), the modifications of the gene pool due to anthropogenic influence can be a serious threat to the stability of forest tree populations and entire forest ecosystems, particularly in the face of ongoing global environmental changes. Therefore, the impact of routine forest management practices on the composition of genetic pools of natural populations must be carefully investigated.

In Poland, forests are dominated by Scots pine (*Pinus sylvestris* L.), which covers 58% of their area (Statistics Poland, 2021). The current species composition results mainly from the afforestation and restoration with pine monocultures that have been carried out since the end of the 18$^{th}$ century. Scots pine, as a pioneering species, very easily took the place previously occupied by oak-hornbeam and oak forests. At present, forest tree breeding in Poland is carried out using two methods to improve a given trait in a progeny population, as compared to the maternal stand. The first method can be referred to as population selection, which is applied in Poland most frequently (due to the richest genetic pool). Seeds are used from the selected forest stands: production seed stands (PSSs) and reserved seed stands (RSSs). Currently, PSSs are the basic seed source for renewals. Reserved seed stands are used much less often, but they constitute the main source of seeds for establishing progeny plantations (PPs, see below). The registry of PSSs and RSSs is constantly updated – new stands are still being selected, with others being removed from the register due to damage or disease.

By applying population selection the genetic gain, defined as the amount of increase in performance that is achieved annually through artificial selection (Xu et al., 2017), is small, but the genetic diversity of the stand is not disturbed. The second method involves individual selection by choosing plus trees (PTs) and establishing first-generation vegetative and generative seed orchards (SOs) designated for the mass production of seeds. Individual selection is stronger and therefore the genetic gain is higher. However, both selection methods may cause some losses in the original genetic diversity observed in Scots pine stands. Seeds from RSSs and SOs are further used to establish PPs to maintain the selected genotypes ensuring the formation of stands of high quality and breeding value. They are supposed to increase quantitative production and, above all, constitute the basic future source of seeds with improved genetic value for establishing fast-growing tree plantations providing large amounts of wood in a short time. As of the 31st December 2021, there were 68,108 ha of PPs in Poland, out of which 47,251 ha (69.38%) were occupied by Scots pine (Statistics Poland, 2021).

In this article, we focused on the genetic diversity of the Scots pine RSS and its naturally and artificially regenerated progeny. Nuclear microsatellite markers were used to describe and compare the composition of the genetic pools of the studied forest stands. We aimed to find out whether the gene pool of the maternal population had been efficiently transmitted to its progeny or if there are some significant changes depending on the mode of regeneration (natural vs. artificial). We also evaluated the composition of alleles within two PPs that were artificially established at different times and using different batches of seeds. Finally, in light of the obtained results, we discuss the genetic considerations for establishing and using seeds from PPs in Poland.

# Materials and Methods

## Plant material and DNA extraction

The research included four stands (which we also refer to as populations) of Scots pine located in the Syców Forest District in Poland (51.19N, 17.98E) (Table 1). This area is occupied by the Rychtal Scots pine, which is one of the most valuable ecotypes of this species in Poland with great stock density and wood quality as well as high adaptability to changing climatic and soil conditions (Giertych, 1980; Matras, 1989). We analyzed randomly chosen trees occupying one RSS and its naturally regenerated progeny (NR) as well as two PPs at different ages. The age of NR was estimated based on the seedlings' height and circumference of the main shoot. The PPs were established artificially by planting the seedlings grown from the seeds collected in the studied RSS. In total, our research included 416 individuals of Scots pine. We collected fresh needle samples that were subsequently stored at −20 °C until DNA extraction. Genomic DNA was extracted from 50–100 mg of needle tissue using a modified CTAB protocol (Dumolin et al., 1995). The DNA concentration and quality were assessed with the use of a BioPhotometer (Eppendorf AG, Germany).

## Molecular analysis

The initial set of nuclear microsatellite markers described for Scots pine by Soranzo et al. (1998), Elsik et al. (2000), and Chagné et al. (2004) was screened to choose markers that provide repeatable results of high quality with sufficient polymorphism and unambiguous allele banding. The final set of nuclear loci used in our study included four nSSRs (SSrPtctg4363, PtTx8446, PtTx4001, and Spag7.14; Supplementary Table S1). The selected loci were amplified simultaneously in a multiplex reaction using the Qiagen Multiplex PCR Kit (Qiagen, Germany). A specific fluorescent dye was attached to the forward primer in each primer pair (Supplementary Table S1). Each reaction was performed in a total volume of 10 µl composed of 5 µl of Qiagen Multiplex Master Mix (2X), 0.2 µl of primer mix (20 µM), 1 µl of Q-Solution (5X), 0.8 µl of RNase-free water and 3 µl of DNA template (approximately 10–20 ng/µl). The amplification procedure started with an initial denaturation step at 95 °C for 15 min, followed by 10 touchdown cycles at 94 °C for 30 s, 1 min 30 s at 63 °C (−1 °C/cycle), 1 min at 72 °C, and then by 28 cycles at 94 °C for 30 s, 1 min 30 s at 55 °C, 1 min at 72 °C. The final extension was carried out at 72 °C for 10 min. The fluorescently labeled PCR products, along with GeneScan™ 600 LIZ™ Size Standard (Life Technologies, USA), were separated on the Applied Biosystems® 3130 Genetic Analyzer (Life Technologies, USA). The identification of alleles based on their sizes was determined using the GeneMapper™

Table 1. Description of the sampled Scots pine stands

| Stand | Forest division | Location (WGS 84) | Management type | Age of trees (yrs) | Area (ha) |
|---|---|---|---|---|---|
| RSS | 68a-b, 69a, 70a, 71b-c | N: 51.1956; E: 17.9275 | natural reserved seed stand | 130–135 | 57.71 |
| NR | 68a-b, 69a | N: 51.1956; E: 17.9275 | natural regeneration | 5–17 | – |
| PP1 | 74c | N: 51.1899; E: 17.9690 | artificial progeny plantation | 8 | 3.16 |
| PP2 | 60g | N: 51.1933; E: 17.9726 | artificial progeny plantation | 26 | 1.1 |

software ver. 4.0 (Life Technologies, USA). The raw data were converted into discrete allele sizes using the program R2G (https://www.ukw.edu.pl/pracownicy/strona/igor_chybicki/software_ukw/). All variants were also checked manually.

## Data analysis

The score test (U test for heterozygote deficiency; Raymond & Rousset, 1995), implemented in Genepop ver. 4.7.5, was used to assess deviations from Hardy-Weinberg equilibrium (HWE) in each pine stand with the complete enumeration method, as described by Louis & Dempster (1987). The multi-sample score test was carried out additionally to test each locus across all populations. Basic genetic parameters, including the number of alleles (A), the effective number of alleles ($A_E$), rare alleles (with a frequency below 5%; $A_{<5\%}$), private alleles ($A_P$), observed ($H_O$), and expected heterozygosity ($H_E$) were computed for each locus and population in GenAlEx ver. 6.5 (Peakall & Smouse, 2012). FSTAT ver. 2.9.4 (Goudet, 2003) was used to estimate the inbreeding coefficients ($F_{IS}$), and allelic richness was calculated based on 103 individuals ($A_{R103}$). As we observed departures from HWE, nuclear microsatellites were also tested for the presence of null alleles with the use of the FreeNA software (Chapuis & Estoup, 2007) following the Expectation Maximization (EM) algorithm of Dempster et al. (1977). The inbreeding coefficients were then recalculated with the correction for null alleles ($F_{ISnull}$) using the INEst software ver. 2.2 (Chybicki & Burczyk, 2009). The following parameters were set: 200,000 cycles, 10,000 thinning, and 20,000 burning. To verify the significance of inbreeding, we compared two models: the full model incorporating the presence of null alleles, inbreeding, and genotyping failures, and the random mating model assuming no inbreeding. In the final step, we performed the Bayesian procedure of model comparison by computing the deviance information criterion (DIC) for each model as described in the INEst manual. Effective population size ($N_e$) was calculated for each stand using the sibship assignment method (Wang, 2009) in the program COLONY ver. 2.0.6.6 (Jones & Wang, 2010). The Full-Likelihood analysis was chosen with medium precision and medium run length. We set the parameters for a monoecious species with inbreeding and with female and male polygamy. We had no prior sibship as well as no excluded maternity, paternity, or sibship. A 0.01 genotyping error was set per each locus with updating allele frequencies and sibship scaling.

Genetic differentiation was assessed by computing the global and pairwise differentiation index ($F_{ST}$). The calculations were done with 10,000 permutations in Arlequin ver. 3.5.2.2 (Excoffier & Lischer, 2010). We subsequently used $F_{ST}$ in the Principal Coordinates Analysis (PCoA) implemented in GenAlEx to determine the genetic relationships among the studied stands. In the final step, INEst was used to calculate the possible bottleneck effect. The test implemented in this software was developed by Cornuet & Luikart (1996). It is based on the excess of heterozygosity that can be observed as a consequence of a bottleneck, as compared to a population with constant size. We chose a two-phase model (TPM) that enables both single-step and multi-step mutations, as microsatellite markers do not mutate under a strict single-step mutation model (SMM) (Di Rienzo et al., 1994). The TPM option was set to allow for 22% of the multi-step changes with an average multi-step mutation size of 3.1 as recommended by Peery et al. (2012). The number of coalescent simulations was set to 10,000. We used the Wilcoxon signed-rank test to determine the p-values based on 1,000,000 permutations.

# Results

## Genetic polymorphism at the studied loci

The set of four microsatellite loci used in our study turned out highly polymorphic. The observed heterozygosity varied from $H_O = 0.49$ to $H_O = 0.73$, with an average of $H_O = 0.625$. We found significant deviations from HWE in all loci that resulted mainly from the presence of null alleles as shown by the results of the analysis carried out in INEst. The expected heterozygosity of the studied markers was, therefore, higher ($H_E = 0.71$–$0.95$; mean $H_E = 0.803$). The frequency of null alleles ranged between NAF = 0.05 for SSrPtctg4363 and PtTx8446 to NAF = 0.16 for Spag7.14. As it was lower than the threshold value of NAF = 0.19 (Chapuis et al., 2008), above which the value of $H_E$ is significantly overestimated, all markers were used in further analyses. We identified 85 different alleles, yielding a mean number of A = 21.25 alleles per locus. Locus Spag7.14 was the most polymorphic with A = 37, as compared to A = 16 detected in the remaining loci. The effective number of alleles ($A_E = 3.44$–$21.41$; mean $A_E = 8.57$) was significantly lower due to the presence of many alleles with low frequencies (Supplementary Table S1).

## Genetic variation within populations

The comparison among the studied pine stands revealed that they did not vary significantly in the values of the mean number of alleles ($\bar{A}$ = 16.25–17.50), the mean effective number of alleles ($\bar{A}_E$ = 8.18–8.98), and allelic richness based on 103

Table 2. Descriptive statistics of genetic variation within the studied Scots pine stands.

| Stand | N | $\bar{A}$ | $\bar{A}_E$ | $A_{R103}$ | $A_{<5\%}$ | $A_P$ | $H_O$ | $H_E$ | $F_{IS}$ | $F_{ISnull}$ (CI95%) | $N_e$ (CI95%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RSS | 104 | 17.00 | 8.18 | 16.87 | 45 | 7 | 0.57 | 0.81 | 0.30 | 0.13 (0.00–0.26) | 66 (48–96) |
| NR | 103 | 16.25 | 8.34 | 16.10 | 42 | 3 | 0.59 | 0.80 | 0.26 | 0.20 (0.04–0.29) | 71 (51–100) |
| PP1 | 104 | 17.50 | 8.98 | 17.40 | 52 | 1 | 0.65 | 0.80 | 0.20 | 0.05 (0.00–0.16) | 76 (55–107) |
| PP2 | 105 | 15.75 | 8.76 | 15.66 | 45 | 4 | 0.69 | 0.80 | 0.14 | 0.04 (0.00–0.07) | 71 (52–103) |
| Mean | 104 | 16.625 | 8.565 | 16.51 | 46 | 3.75 | 0.625 | 0.80 | 0.225 | 0.105 | 71 |

N – number of individuals; $\bar{A}$ – mean number of alleles; $\bar{A}_E$ – mean effective number of alleles; $A_{R103}$ – allelic richness based on 103 individuals; $A_{<5\%}$ – rare alleles (with a frequency below 5%); $A_P$ – private alleles; $H_O$ – mean observed heterozygosity; $H_E$ – mean expected heterozygosity; $F_{IS}$ – inbreeding coefficient; $F_{ISNull}$ – inbreeding coefficient with the correction for null alleles (CI95% – 95% confidence interval for $F_{ISNull}$); $N_e$ – effective population size (CI95% – 95% confidence interval for $N_e$).

Table 3. Rare, private, and gained/lost alleles, as compared to RSS

| Stand | Alleles | | | |
|---|---|---|---|---|
| | rare | private | gained | lost |
| RSS | 45 (53%) | 7 (8%) | – | – |
| NR | 42 (49%) | 3 (4%) | 10 (12%) | 13 (15%) |
| PP1 | 52 (61%) | 1 (1%) | 10 (12%) | 8 (9%) |
| PP2 | 45 (53%) | 4 (5%) | 8(9%) | 13 (15%) |
| Mean | 46 | 3.75 | 9.33 | 11.33 |

individuals ($A_{R103}$ = 15.66–17.40). All populations had a very high number of rare alleles that ranged from $A_{<5\%}$ = 42 for NR to $A_{<5\%}$ = 52 for PP1. Private alleles ($P_A$) were found in all stands with very low frequencies of 0.5–1.5%. The highest number of $P_A$ was detected in RSS ($P_A$ = 7). Three $P_A$ were found in NR, one in PP1, and four in PP2 (Table 2). The progeny stands gained an average of 9.33 new alleles (range: 8–10; total: 28) in comparison to RSS. They lost slightly more alleles, with a mean number of 11.33 (range: 8–13; total: 34) (Table 3).

The values of $H_O$ were below the mean $H_O$ = 0.625 in RSS and NR. On the contrary, PP1 and PP2 had higher $H_O$, whereas $H_E$ was almost identical in all populations ($H_E$ = 0.80–0.81). The deviations from HWE were also apparent considering the inbreeding coefficient values ranging from $F_{IS}$ = 0.14 in PP2 to $F_{IS}$ = 0.30 in RSS. The observed inbreeding was lower in both PPs, as compared to RSS and NR. The difference was even more evident when we corrected $F_{IS}$ for the presence of null alleles ($F_{ISNull}$). The mean $F_{ISNull}$ = 0.105 was more than two times smaller than the mean $F_{IS}$ = 0.225. Nevertheless, CI95% for $F_{ISNull}$ overlapped zero in RSS, PP1, and PP2. Indeed, the comparison of DIC for the models with and without inbreeding showed that inbreeding was significant only in NR. Effective population sizes ($N_e$) were similar ranging from $N_e$ = 66 in RSS to $N_e$ = 76 in PP1 (Table 2).

## Population differentiation

The studied stands represented a common genetic pool. The global $F_{ST}$ value was very small though significant ($F_{ST}$ = 0.0034; $p < 0.05$). Pairwise $F_{ST}$ among the studied pine stands was insignificant except for the value calculated between RSS and PP2 ($F_{ST}$ = 0.0056; $p < 0.05$) that exceeded global $F_{ST}$ (Table 4). This result was also confirmed by the PCoA, which revealed that RRS and NR are genetically different from both PPs, as they were separated along the first axis (65.80% of total variation). The second axis (32.74% of total variation) showed that PPs differ not only from RSS and NR but also from one another (Fig. 1). The Wilcoxon signed-rank test, performed under the TPM model, did not confirm recent bottlenecks in any of the analyzed stands.

Table 4. Pairwise differentiation index ($F_{ST}$) among the studied pine stands

| | RSS | NR | PP1 |
|---|---|---|---|
| NR | 0.0006 | | |
| PP1 | 0.0042 | 0.0039 | |
| PP2 | 0.0056* | 0.0032 | 0.0031 |

*$p < 0.05$.

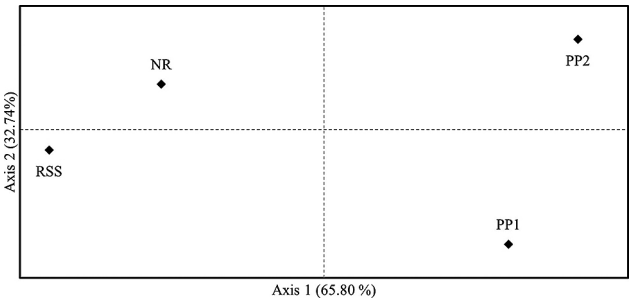


Fig. 1. Principal Coordinates Analysis (PCoA) showing the first two axes based on pairwise population $F_{ST}$. Acronyms as in Table 1

# Discussion

Silviculture is supposed not only to create and preserve existing forest stands by afforestation and renewals but also to enrich them in a way that ensures the sustainability of forest ecosystems. Breeding practices aiming at increasing forest productivity need to find a balance between selection and genetic variation. In this study, we wanted to find out

whether the mode of regeneration affects the genetic diversity of Scots pine progeny. We used nuclear microsatellites to describe and compare the genetic pools of the Scots pine RSS and its naturally and artificially regenerated progeny. We found out that most changes involve rare alleles that were gained and lost in all progeny populations. Nevertheless, some parameters of genetic variation seemed to depend on the mode of regeneration. There were also differences between the two PPs, which led us to the conclusion that more attention should be paid to the establishment and management of PPs in Poland.

All stands analyzed in our research showed a high and comparable level of genetic variation, which is typical for wind-pollinated, long-lived, and highly outcrossing tree species, such as Scots pine (see Tóth et al., 2017 and references therein). The values of $N_e$ were similar and exceeded 50 in each case, which is sufficient to maintain a balance between genetic drift and mutations (Franklin, 1980). Although we used only four microsatellites, they were highly polymorphic with frequencies of null alleles below the threshold above which the value of $H_E$ is significantly overestimated. Nowakowska et al. (2007) used three nuclear microsatellites to study the genetic variability of Scots pine and Norway spruce (*Picea abies* L. Karst) natural regeneration compared with their maternal stands. The results were very similar to the ones obtained in our research, considering the values of $\bar{A}$, $A_{R,}$ and $H_E$ with small differences regarding $\bar{A}_E$ and $H_O$, which most probably stem from the set of markers used, as the present study comprised one marker with lower variability. Similar results were also obtained by Kosińska et al. (2007) who studied two maternal populations of Scots pine in Poland and their naturally and artificially regenerated progeny using 13 enzyme systems encoded by 25 loci. Likewise, Dzialuk & Burczyk (2006) used eight isozyme gene loci to search for changes in genetic structure between parental and offspring populations of one Scots pine RSS in Poland, indicating only a small reduction of heterozygosity in progeny stands as well as increased inbreeding. A higher value of $F_{ISnull}$, which was significantly different from zero, was also observed in the natural regeneration analyzed in our study. This phenomenon seems to be a consequence of the mating of related individuals or self-fertilization. Nevertheless, inbred individuals are progressively eliminated throughout the lifespan of a population (Kosińska et al., 2007). In the case of naturally regenerating Scots pine stands, it happens at the age of 10–20 years (Yazdani et al., 1985), but in artificial stands, it starts already at the age of three years (Muona et al., 1987). Considering the age of the stands analyzed in the present work (5–17 for NR and 8 and 26 for PP1 and PP2, respectively), it is possible that the elimination of homozygotes indeed took place in both PPs at an early age but not in NR or it is still ongoing there.

The comparison of allelic patterns revealed that all populations had the same common alleles (see also: Wójkiewicz et al., 2019). A considerable percentage of rare alleles was detected in each stand, which exceeded 50% of all alleles in most populations (average value of 54%), except for NR with a value of 49%. All progeny stands gained and lost rare alleles, as compared to RSS. Differences in the distribution of rare alleles are typically observed in the studies comparing the genetic variation of progeny and maternal stands of forest trees (e.g. Kosińska et al., 2007; Ivetić et al., 2016). On one hand, such discrepancies come from the sampling strategy. On the other hand, it seems that they also result from the practice of seed collection in the maternal stands and its variation over years (Kosińska et al., 2007). We cannot also exclude the inflow of foreign pollen to the RSS analyzed in this work. Many researchers have confirmed that pollen of Scots pine can be transported over very long distances, even hundreds of km (e.g. Lindgren et al., 1995; Robledo-Arnuncio, 2011), although effective gene flow may be very limited (Robledo-Arnuncio et al., 2004). It should be emphasized that the analyzed NR occupied only part of RSS, which may also be an additional reason why we detected different sets of rare alleles when we compared RSS and NR. In the case of PPs, however, new rare alleles may also have appeared because of other reasons. Both PPs are surrounded by other Scots pine forest stands. It cannot be ruled out that some individuals that grow in the studied PPs are natural regeneration of the neighboring stands that have not been removed. Progeny plantations had higher values of $H_O$ in comparison to NR, which can also be explained by the presence of individuals that originated from foreign stands. Furthermore, some mistakes might have occurred during the seed and seedling production stage. Every step that includes further seed processing and storage or growing seedlings in nurseries, as well as FRM transfer, can lead to some changes in the genetic diversity of resulting individuals mainly by directional selection (Ivetić et al., 2016 and references therein; Konecka et al., 2018).

The slight differences in the genetic variation among the studied Scots pine stands were more evident in the PCoA result, which showed that NR differed from RSS to a lesser extent than both PPs. Progeny plantations also differed from one another, which might be also because they were established with different batches of seeds. In addition to this, pairwise $F_{ST}$ was significant only between RSS and PP1, which also had the highest $N_e$. Taking all these into account, we conclude that the gene pool of the maternal Scots pine population had been transmitted more efficiently in the case of naturally regenerated

progeny, but the difference is only minimal. It is impossible to predict whether it has any influence on the adaptive and evolutionary potential of the studied stands. Certainly, the dynamics of rare alleles in naturally vs. artificially regenerated offspring require more thorough research.

It should also be noted that our study did not take into account that RSSs as well as PPs are subjected to certain management practices that may also affect their genetic variation. These include cutting, thinning, and organizing of undergrowths and understories. Several review papers have shown that in general silviculture techniques do not influence parameters of genetic diversity typically reported in forest genetic population studies (Schaberg et al., 2008; Ratnam et al., 2014; Ivetić et al., 2016). Cutting and thinning have minor effects on heterozygosity and allelic diversity, but they can cause a significant loss of rare alleles which depends on the intensity of these practices (Schaberg et al., 2008; Danusevicius et al., 2016; Konecka et al., 2021). Selective thinning is based on the phenotypic measurement of trees, so we can expect some genetic changes regarding quantitative (e.g. height or diameter) and qualitative (e.g. stem form) traits which are at least partially under genetic control (Finkeldey & Ziehe, 2004). Simulations showed that selective thinning provides for a rather accurate replication of natural selective processes, but this conclusion was drawn based on the analysis of neutral markers (Konecka et al., 2021). Adverse effects on the genetic diversity of forest species are more prominent in the case of intensive thinning, fragmentation, and overexploitation. Aravanopoulos (2018) reviewed the consequences of various forest management practices, including coppicing, fragmentation, and exploitation as well as genetic resources of forest plantations. The observed changes were only subtle but regarded the genetic structure and adaptive potential of the analyzed tree stands. The same conclusions were drawn by Gautam et al. (2021) who reviewed 75 papers that included data on the genetic diversity of managed forest stands published from 1979 to 2020. No difference was identified in allelic richness or gene diversity among fragmented Scots pine stands in the Scottish Highlands and a remote, unmanaged stand using 12 nuclear microsatellite markers (González-Díaz et al., 2017). The authors concluded that although both historical and contemporary management had not impacted levels of genetic variation, they could have driven the spatial genetic structure in the studied stands.

## Conclusions

Reserved seed stands in Poland are selected and recognized to permanently maintain the valuable and unique features of particular ecotypes. At the same time, they should constitute a major source of seeds that will serve to establish new generations of forest stands with improved quality. The studied PPs are supposed to represent the genetic pool of their maternal RSS. In our research, this requirement seems to be fulfilled, but more stands need to be analyzed to address this issue. It should be noted that entirely natural regeneration is possible only in unmanaged protected areas, such as national parks or nature reserves. In commercially managed stands, human impact always has some effect on the genetic composition of even spontaneously regenerated individuals (Koski, 2000). Nevertheless, the studied NR was more representative and similar to the maternal RSS than both PPs, as shown by the analysis of population differentiation.

It appears that at least three factors have determined the genetic diversity of PPs: (1) a number of mother trees; (2) variation in crossing patterns in different reproductive seasons, and (3) possible natural regeneration from the neighboring stands. We, therefore, recommend paying more attention while harvesting seeds for artificial regeneration and establishing PPs. Seeds (cones) should be collected evenly, maintaining an equal proportion from individual mother trees. Regarding seed harvesting from PPs, they should be ideally collected from the center so that the surrounding trees constitute a buffer zone stopping the inflow of foreign seeds and pollen.

Silvicultural practices certainly have to take into account other factors, such as population size, reproductive biology, or growth rate of a species, to ensure the maintenance of genetic diversity and evolutionary potential of a particular stand. Allelic diversity measures are more suitable than $H_E$ in assessing the genetic consequences of FRM production and forest management because $H_E$ is only slightly sensitive to bottlenecks and perturbations in populations (Ratnam et al., 2014). Scots pine is a wind-pollinated, highly outcrossing tree species with a large effective population size. It is therefore relatively easy to maintain its genetic variation, but it may not be the case for species with different mating systems. Further studies are needed to elucidate how adaptive genetic diversity is affected by the regeneration mode and management of forest stands, but first, we need to identify genes directly involved in phenotypic traits. Currently, new genomic platforms comprising thousands of single nucleotide polymorphism markers may prove useful for such purposes (Perry et al., 2020).

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