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Assessment of Variability: Chloroplast Microsatellite DNA, Defoliation, and Regeneration Potential of Old Pine Stands of Different Origins in the Context of Assisted Genotype Migration

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Abstract: The development of transportation in the 19th century allowed for the transfer of large quantities of pine seeds between significant areas in Europe. This artificial migration usually had negative breeding consequences, so regulations were introduced to protect local gene pools. The current dynamically changing climate contributes to a reconsideration of the assisted migration of genotypes as a factor in the mitigation of breeding risks. However, the assisted migration of genotypes requires extensive research into safe geographic and genetic distances over which we can move gene pools. The analyses presented here demonstrate the differences in chloroplast microsatellite DNA variation (cpSSR) and the implications for the health and regeneration potential of old stands from introduced seeds and local seeds. Analyses of the genetic variation in chloroplast DNA, crown defoliation, and regeneration potential (number of cones and number of seedlings) were performed. The stand grown from introduced seeds (from France) had higher genetic variability than the local populations and differed genetically from the local genotypes (Fst from 4% to 12%). The high genetic variability in the studied period did not affect the lower defoliation of the stand. On the other hand, the stand grown from introduced seeds had a significantly lower yield, and there was no natural regeneration (seedlings) to ensure the transfer of genetic information to the progeny generation. The obtained results confirmed the mechanisms of natural selection acting on pine genotypes that are alien to local ecological conditions.

Keywords: scots pine; alleles; cpDNA; defoliation; seeds; seedlings; assisted migration; Tuchola Forests National Park

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1. Introduction

Forest trees, as a result of natural regeneration, have perpetuated themselves as autochthonous populations that, as a result of multigenerational selection, have adapted genetically to local climates and habitats and their slow changes [1]. Such adaptation to ecological conditions of occurrence has promoted forest stability and health. Unfortunately, the deformation of forest ecosystems that has occurred in the recent past, as a result of unsustainable forest management, and that focused exclusively on timber production has caused the weakened health of forest-tree species [2]. Scots pine (*Pinus sylvestris* L.) is also more susceptible to external biotic and abiotic stress factors [3]. Scots pine is a widespread species throughout the Eurasian region, and, due to its natural plasticity, it colonises a wide range of habitats from peat swamps (91D0 Natura 2000) to sand dunes (91T0 Natura 2000) (by Interpretation Manual—EUR28. Available online: https://ec.europa.eu/environment/nature/legislation/habitatsdirective/docs/IntManual_EU28.pdf (accessed on 2 August 2021)) [4]. According to Noss [5], the main causes of the weakened health of selected populations are the use of seeds of unknown origin and adaptation to local growing conditions. The damage caused by the importation of foreign and untested sources of forest reproductive material was first recognised in Sweden. In 1787, the first evidence of

negative observations of plants grown from seeds originating from distant regions was published by Urbanski [6]. Pines of foreign origin initially grew faster but later began to give way to pines that were native in growth and were also characterised by crooked, bent, and heavily branched trunks after only a dozen or so years. As a result of this negative experience, the Swedish Forestry Administration banned the use of foreign pine and spruce seeds in 1882. A second example of serious economic damage caused by the use of seeds of unknown origin can be found in 19th-century Germany. The high demand for seeds due to the regeneration of forests by full seeding and the simultaneous development of steam engines and railroad transportation led to the procurement of seeds on an industrial scale. In large factories (seed kilns), seeds were obtained from huge lots of cones from all over the country, as well as from abroad. In north-eastern Germany, as well as in the southwestern regions, a marked deterioration in the quality characteristics of progeny was subsequently observed. For this reason, a resolution to restrict seed importation was passed at a meeting of German foresters in 1906 [6]. In Poland, the work of Prus-Głowacki et al. [7], Nowakowska [8], and Przybylski et al. [9] has provided evidence on the introduction of seeds of foreign and untested origin into native pine stands. Consequently, long-term selection programmes have been implemented in the management of Polish forests to improve the breeding quality of pines, among other species [10].

The genetic variability manifested in a local gene pool by allelic polymorphism is a fundamental biological value that determines the plasticity of stands [11]. Maintaining a high level of genetic variation is beneficial for stand plasticity and sustainable forestry [12]. A study by Semerikov et al. [13] used cpDNA to describe the variation in pine stands. The study showed a variation between natural populations in Asia and Eastern Europe of 2.1% [13], and slightly lower results were found for populations from Estonia [14]. In Poland, a study of cpDNA diversity between the age classes of stocks was conducted [15], which showed similar levels of diversity between different populations. For pines, genetic diversity has a unique significance because the species is characterised by the diversity of the ecosystem in which it occurs, and a provenance-based study showed the adaptation of certain subpopulations to growth conditions [16]. For clones of Scots pine, a correlation between the starting date of flowering and the frequency at locus Sdh-A [17] was confirmed. Adaptation to local growing conditions as manifested in the promotion of adapted alleles was studied in wild pine stands and was regulated in natural cycles [18]. Studies conducted in the largest national forest park in Poland confirmed significant correlations between the values of observed allele numbers and effective allele numbers as well as the health statuses of the studied trees [3]. The role of genetic variation in pine stand evaluation was also described by Gulyaeva et al. [19], who explained the mathematical role and functionality of SSR markers. Currently, the issue of genetic variability and its importance for adaptation processes has mainly been studied in the context of climate change adaptation [5]. It is becoming increasingly important to maintain diversity within species as an "insurance policy" for populations, but it should be emphasised that the natural regeneration of pines contributes to the maintenance of in situ populations provided that these populations are adapted to a changing climate [20]. Because some local pine gene pools are likely unable to adapt to dynamically changing climatic conditions, it has often been considered that the assisted migration of genotypes is needed. In their work, O'Neill et al. [20] postulated the need for the translocation of genotypes within a species' natural range to maintain plasticity in the face of climate change. The postulate of O'Neill et al. [20] conflicts with the postulate of protecting local gene pools. Therefore, the main objective of the present study was to analyse the consequences of the transfer of a foreign seed pool after more than 100 years of tree growth, which is an example of the random assisted migration of genotypes postulated by O'Neill et al. [20]. Seeds were transported from France to Poland in the early 20th century as a part of post-war contributions. The study conducted here illustrated the plasticity of a (mature) stand grown from foreign seeds in contrast to native populations. Analyses were carried out in relation to the stability of the stands at the genetic and ecological levels from the point of view of an analysis of population yields

and yield efficiency measured by the number of seedlings. Stand vigour was also assessed by estimating the loss percentage of the assimilative apparatus relative to a reference tree (hereafter, defoliation). This study evaluated the stability traits of the stands as well as their reproductive potential, which determines the sustainability of an ecosystem over time and allows for the potential selection of unsuitable genotypes in the maternal generation and subsequent generations to be addressed.

2. Materials and Methods

2.1. Plant Material

The present study was conducted in the Tuchola Forests National Park in 4 pine stands (Figure 1). The stands are under legal protection and managed exclusion. The plots were optimally selected considering the oldest stands and the total park area (Table 1). Of the forest stands studied, the population of Kociol (KOC) originated artificially from seedlings derived from seeds transported to Poland from France in the early 20th century as part of economic activities related to silviculture (Supplementary Materials, Figure S1); the exact place of origin of the seeds in France could not be determined. Unpublished documents from the Tuchola Forests National Park hypothesise that they were a mixture of pine seeds from stands that originated in France (Supplementary Materials, Figure S1). In the present study, it was hypothesised that the KOC stand was an example of a gene pool non-native to the region. During 1990–2000, numerous insect invasions (especially *Panolis flammea*) were observed in all the stands studied.

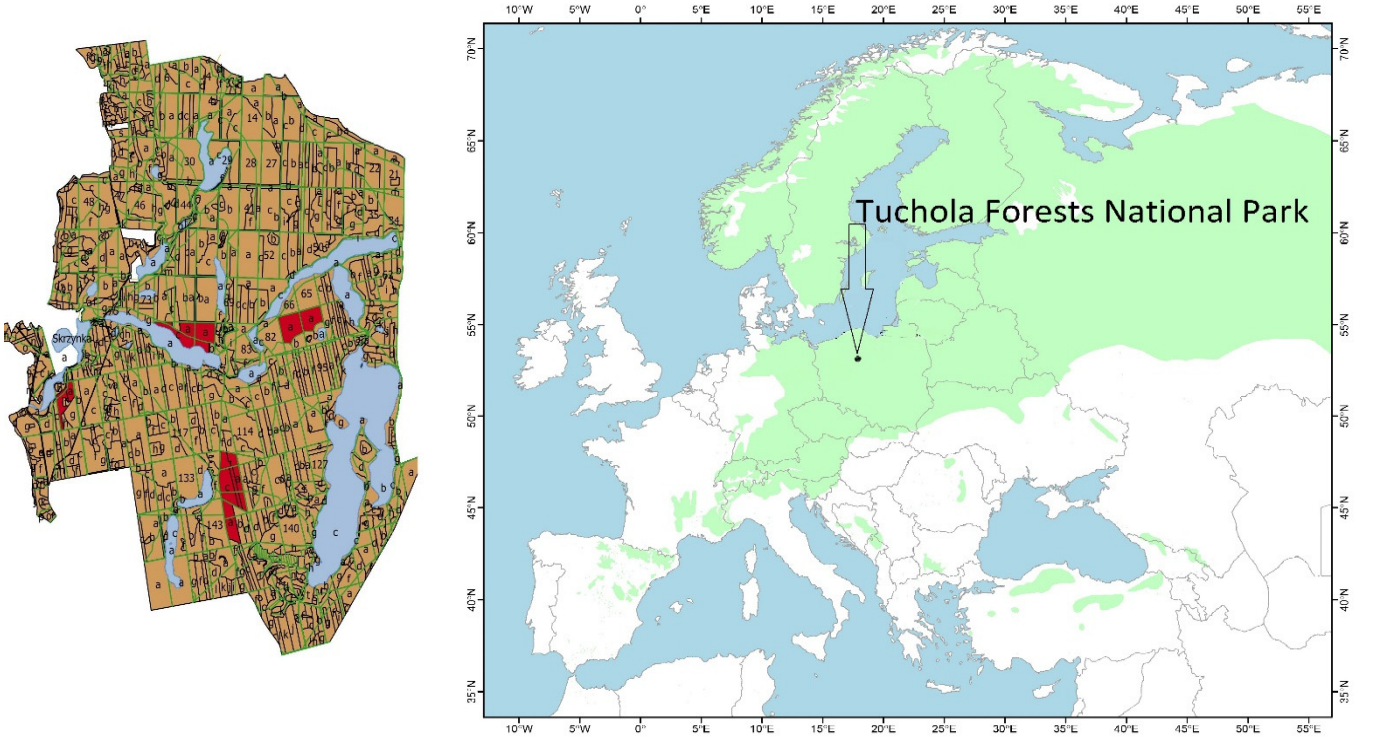

Figure 1. Location of the studied Tuchola Forests National Park against the background of the pine area (EUFORGEN) and European borders (right side). On the left is an administrative map of Bory Tucholskie with the forest administrative division; the red colour indicates the stands selected for the analyses.

Table 1. The characteristics of the studied stands.

Location	Gacno	Plesno	Mielnica	Kociol
Acronyms	GAC	PLE	MIE	KOC
Coordinates	N53.792663 E17.569418	N53.814655 E17.554436	N53.800613 E17.518334	N53.824116 E17.591752
Age * of the dominant *P. sylvestris*	130–140 (avg.: 135)	119–132 (avg.: 125)	150–190 (avg.: 170)	110–120 (avg.: 115)
Forest habitat type	fresh coniferous forest	fresh coniferous forest	fresh mixed coniferous	fresh coniferous forest

* Unpublished data from Tuchola Forests National Park.

In each stand, 50 randomly distributed sample trees were selected (Supplementary Materials, Figures S2–S5). Where possible, trees were selected along transects that covered the entirety of the selected study plots. Trees were marked using a GPS receiver (Garmin GPSMap 64st). Plant material (needles) was collected from selected sample trees, placed in sealed Eppendorf containers, and transported with refrigeration to the molecular biology laboratory.

2.2. DNA Extraction and Microsatellite Genotyping

The total genomic DNA was isolated from the collected material using a commercial kit (Macherey–Nagel Gmbh&Co Valencienner Str. 11; 52355, Duren, Germany). The quality of the DNA isolate was controlled using 2% agarose gel and a Quawell (LabX, 334 King Street, Midland, ON, Canada) spectrophotometer. All samples were diluted to 20–30 ng/µL using deionised water. Molecular analyses were performed using 6 (PCP26106, PCP30277, PCP36567, PCP450712, PCP719872, and PCP873142) chloroplast microsatellite markers selected on the bases of other research work [21–23]. The forward primers were fluorescently labelled with the fluorochromes VIC, PET, NED, and 6-FAM. Amplification was performed through two multiplex reactions. Each PCR reaction was performed in a volume of 10 µL with the following composition: 5 µL of Multiplex buffer (Qiagen, Poland), 0.2 µL (10 µM) of each primer, 1 µL of extracted DNA, and PCR-grade water up to a final volume of 10 µL. The PCR thermal profile was as follows: 95 °C for 15 min, followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, with a final extension of 60 °C for 30 min. Genotyping analysis was performed with an ABI 3500 Genetic Analyzer capillary sequencer (Applied Biosystems, Foster City, CA, USA), and allele length analysis was performed using GeneMapper® version 5 (Thermo Fisher Scientific Inc., Carlsbad, CA, USA).

2.3. CpSSR Data Analysis

Haplotypes were determined as a combination of different microsatellite variants across the cpDNA loci. The chloroplast haplotype variation within populations, i.e., the number of haplotypes (*A*), genetic diversity (*He*), haplotype richness (*Rh*), the number of private haplotypes (*P*), and mean genetic distance between individuals (D^2sh), was calculated using HAPLOTYPE version 1.05 [24].

Genetic structuring of the cpDNA between and within populations was assessed using analysis of molecular variance (AMOVA) implemented in ARLEQUIN version 3.0 [25], with significance tests based on 10,000 permutations. The distance matrix generated with pairwise F_{ST} values between populations was calculated using ARLEQUIN version 3.0 [25]. The statistical significance of F_{ST} values was assessed using 10,000 permutations. The analysed stands were grouped on the basis of the genetic distance of N_{ei} using a principal coordinate analysis (PCoA) implemented in GenALEx 6.5 [26].

2.4. Defoliation Level and Regeneration Potential of the Studied Stands

The level of damage to sample tree crowns was assessed in August 2019 based on the number of annual needle classes and the degree of crown defoliation. Defoliation and the number of annual needle classes were assessed from the ground simultaneously by two appraisers from opposite sides of the crown with the use of binoculars. Defoliation was

calculated as the arithmetic mean of the two evaluators' crown defoliation scores measured on a scale from 1% (no damage) to 100% (dead tree). The methodology is in accordance with international standards adopted in ICP Forests and ICP-Focus projects [27].

The cone yield was determined by visually counting the number of cones. The yield was determined in four locations (Table 1 and Figure 1) on 50 randomly selected trees (Figures S1–S4, Supplementary Materials) belonging to the dominant tree type per stand. The cones were counted around the crown, and then, in order to estimate the number of cones out of sight, the result was multiplied by two to obtain the number of cones for the entire crown. This procedure was previously described by Tyszkiewicz [28]. On account of the two-year life cycle of cones (from pollination to ripening), only closed cones of the study year were counted.

Four observation plots were designated in each of the study stands, where all pine seedlings were counted for further analysis. Details on the locations of the observation plots in the stands and the research conducted can be found in the research article by Przybylski et al. [29]

2.5. Statistic Stand Health Analysis

One-way analysis of variance (ANOVA) was used to compare population (stand) means for stand health traits (defoliation and number of annual needle classes). The analyses were carried out in R [30] according to the following model:

$$y_{ij} = P_i + e_{ij} \quad (1)$$

where y_{ij} is the jth observation of the trait in the ith population, P_i is the mean of the ith population, and e_{ij} is the error of the jth observation in the ith population. Tukey's post hoc test was carried out in R using the "laercio" statistical package [31] as well as a correlation analysis using "corrplot" [32] and a PCA analysis using "ggbiplot" [33].

3. Results

3.1. CpSSR Analyses

The stands showed an average of 38 haplotypes; individually, the highest number of haplotypes was in the KOC stand, and the least was in GAC (Table 2). The number of haplotypes shown concerned the number of private haplotypes (P_h), which was most abundant in the KOC stand (27 private haplotypes). The fewest private alleles were shown for the GAC population (18 private haplotypes). It should also be noted that all of the surveyed stands had private haplotypes. Private haplotypes, together with the total number of haplotypes, influenced the high haplotype richness (R_h) values which averaged at 35.98 for the stands in the study. Consistently, for haplotype richness values, the highest values were in the KOC stand, and the lowest were in GAC (Table 2). Genetic diversity in the analysed stands was above *He* (0.998), with the exception of the GAC stand. It was noted that there was a high genetic distance (D^2sh) between two populations: KOC and MIE (Table 2). The D^2sh parameter in the context of the AMOVA genetic diversity analysis (Table 3) generated more than 90% of the obtained biodiversity at the genetic level in the studied stands.

An analysis of the genetic differentiation (*Fst*) of the stands showed a general division of the study group into two subgroups with genetic similarity, as shown in Figure 2. The subgroup consisting of the MIE and PLE stands showed no statistical differences from each other; they had a low *Fst* value of 1.6% in the analysis (Table 4). The second subgroup consisting of the KOC and GAC stands had an *Fst* value of 4.2%, and this was a statistically significant diversity (p-value = 0.006). The mean *Fst* coefficient separating the two subgroups was 13.75% (Table 4), and this value was statistically significant (p-value = 0.000).

Table 2. Genetic diversity within populations of *P. sylvestris* L. from 6 cpSSR markers: number of individuals in a population (*N*), number of haplotypes (*A*), number of private haplotypes (*Ph*), haplotype richness (*Rh*), genetic diversity (*He*), and mean genetic distance between individuals (D^2sh).

Population	*N*	*A*	P_h	*Rh*	*He*	D^2sh
KOC	50	47	27	44.238	0.998	3.785
GAC	48	31	18	30.000	0.977	2.096
PLE	50	36	21	33.915	0.982	2.763
MIE	50	38	23	35.796	0.986	3.243
Mean	49.5	38	22.250	35.987	0.986	2.971

Table 3. AMOVA of stands analysed.

Source of Variation	Sum of Squares	Variance Components	Percent of Variation
Among populations	50.930	0.15009	9.43186
Among individuals within populations	530.357	1.44119	90.56814
Within individuals	0.000	0.00000	0.0000
Total	581.287	1.59127	

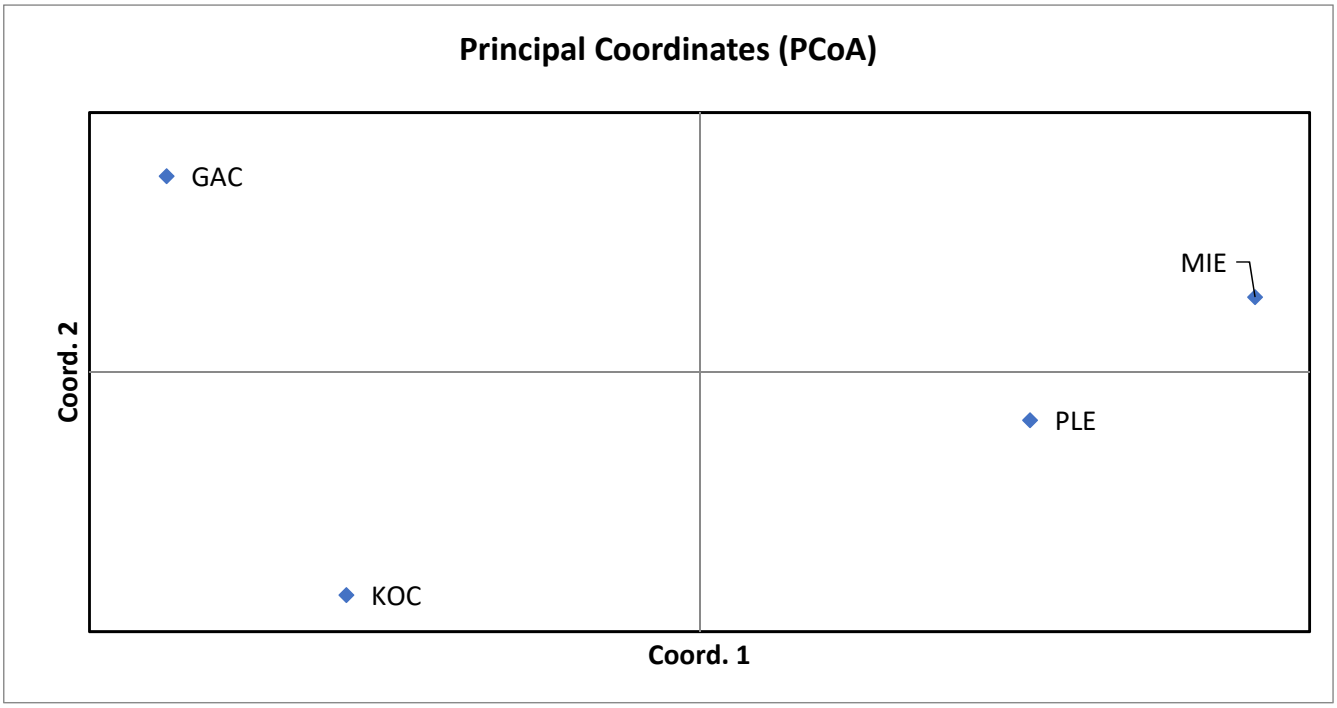

Figure 2. Nei (1978) genetic distance of analysed stands for selected cpSSR markers plotted with the PCoA method. Coord. 1 describes 87.7% of the genetic variation, and Coord. 2 describes 9.01% of the genetic variation.

Table 4. Proportion of the total genetic differentiation (*Fst*) between analysed stands in the bottom of the matrix and *p*-value results at the top of the matrix. Bold means not statistically significant.

	KOC	GAC	PLE	MIE
KOC	-	0.00594	0.00000	0.00000
GAC	0.04242	-	0.00000	0.00000
PLE	0.07938	0.15318	-	**0.21842**
MIE	0.12080	0.20449	0.01577	-

3.2. Estimated Number of Pine Seedlings and Health Statuses of Surveyed Stands

The stands were characterised by considerable diversity in reproductive potential, as determined by the number of seedlings in the understory. In the KOC stand, 2 pine seedlings were found, and in the PLE stand, there were slightly more seedlings (22 pine seedlings). On the other hand, in the MIE stand, 67 pine seedlings were found (Table 5). According to the numbers of pine seedlings found, the results divided the stands into two subgroups: the subgroup of GAC and MIE (with 85% of all the seedlings found) and the subgroup of PLE and KOC (Table 5). At the KOC site, only 1.2% of the total seedlings were found.

Table 5. Number of seedlings in study stands and estimated number of cones in crowns of sample trees.

Population	Number of Seedlings in the Stand	Number of Cones in the Stand
GAC	71	2120
PLE	22	1516
MIE	67	2328
KOC	2	1388

The number of seedlings was proportional to the number of cones estimated in the tree crowns. The highest cone yield was in MIE, with an estimated occurrence of 2328 cones. In MIE, the variation in cone abundance per tree ranged from 0 cones to 372 cones (Figure 2). On average, there were 116 cones per tree in MIE. A similar number of cones was found in the GAC stand, with 2120 pieces found on all the sample trees and an average of 106 cones/tree. In GAC, no sample trees were found without cones while a maximum of 308 cones per tree was estimated (Figure 3). In the populations of PLE and KOC, the total cone abundance was estimated at 1516 and 1388 cones, respectively. The fewest cones were found in the KOC stand. A total of 1388 cones was estimated for the sample trees, corresponding to an average of 69 cones/tree. On average, for the analysis, the MIE population accounted for 31.7% of the total cones, while the GAC population accounted for 28.8%. These two populations accounted for 60.5% of the total cones found. No statistically significant differences were found between the populations studied (Figure 3).

The analysis of the stand defoliation data identified the level of crown damage as medium, with more than 50% of the crowns of all the observed specimens having defoliation in the 26–60% range (Figure 4). It should be noted (due to the age of the trees) that most of the crowns had defoliation below 40% (Figure 4). The ANOVA results for significant components of defoliation were as follows: the number of vintages of needles (*p*-value = 0.000), the biosocial position (*p*-value = 0.034), and tree stands (*p*-value = 0.045) (Table 6). Among the study sites, populations were not diverse (Figure 4). However, a higher number of more severely damaged trees was observed in MIE (Figure 4).

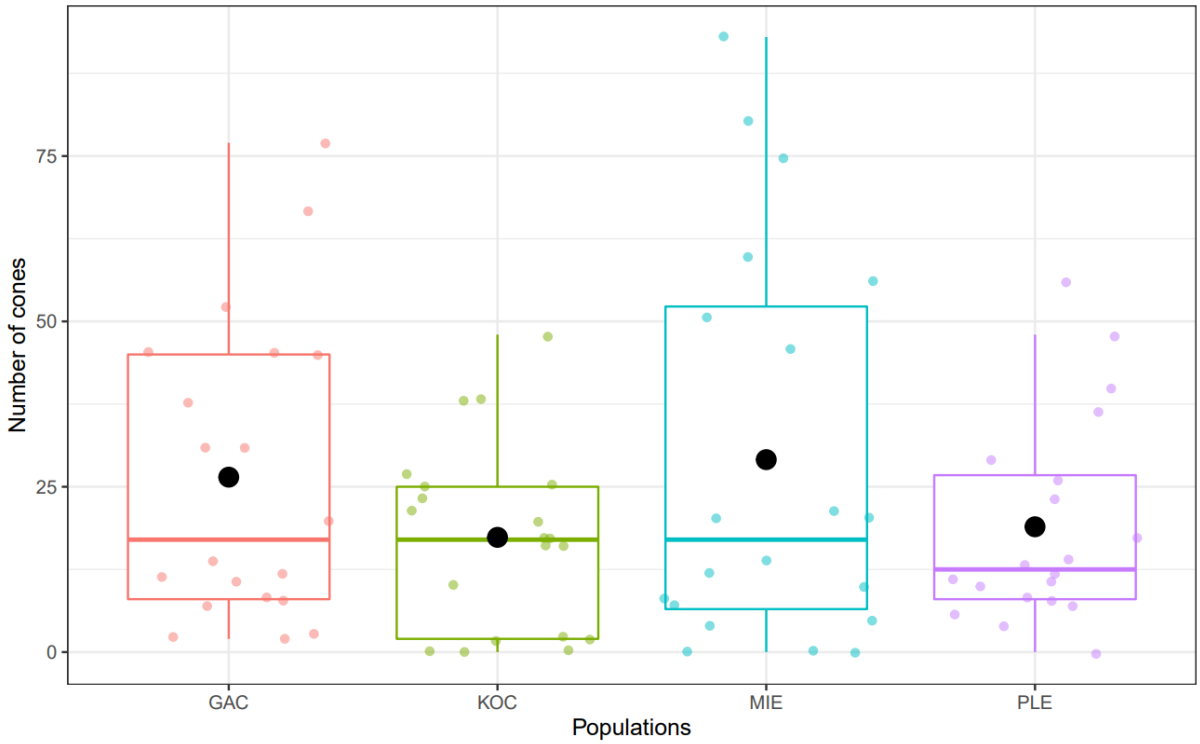

Figure 3. Analysis boxes and whisker plots of the number of cones in the crowns of the sample trees in the study stands. Coloured dots identify observed values; black dots denote mean value for each population. Boxes are 50% of data; whiskers are 99% of data.

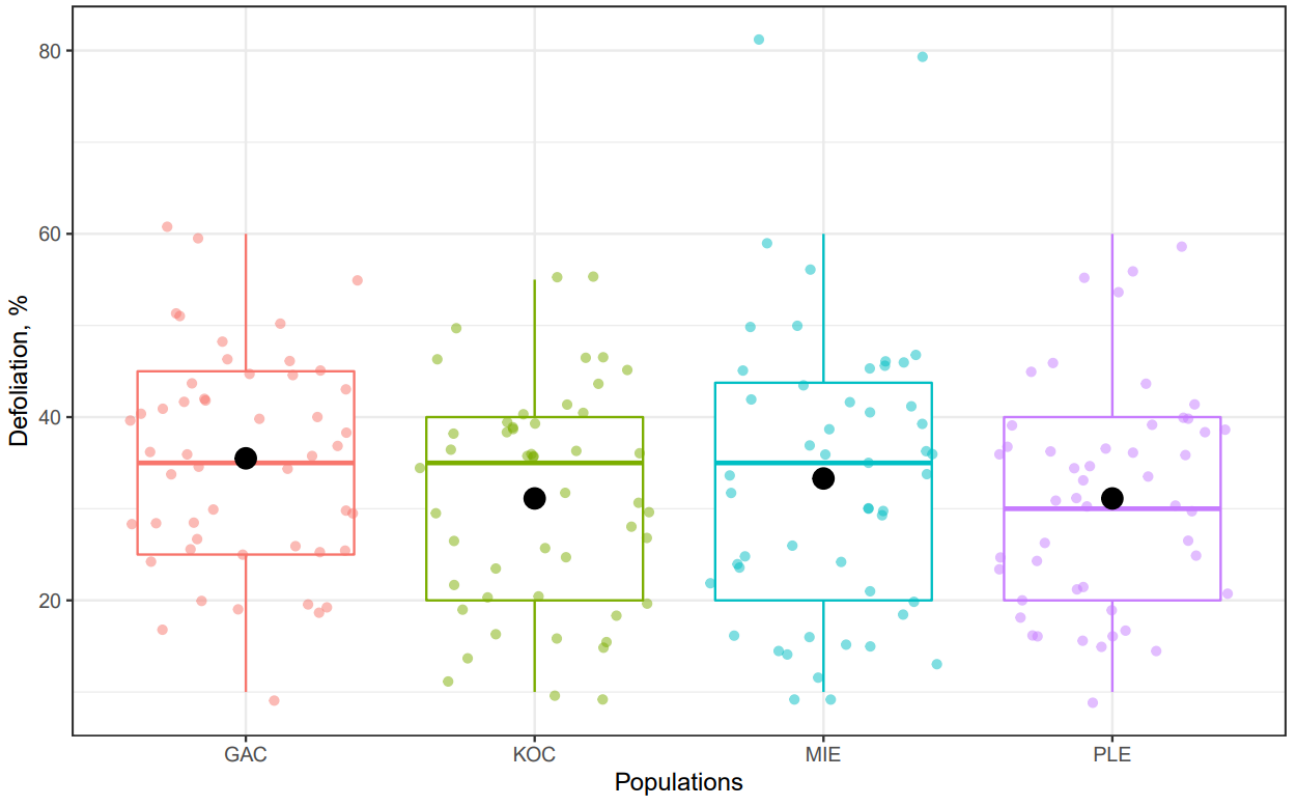

Figure 4. Analysis boxes and whisker plots of the degree of defoliation of the studied stands. Coloured dots identify observed values; black dots denote mean value for each population. Boxes are 50% of data; whiskers are 99% of data.

Table 6. ANOVA for the defoliation parameters of the analysed stands.

	Df	Sum Sq	Mean Sq	F_{value}	*p*-Value
Stands	3	653	218	2.725	0.045
Number of vintages of needles	2	17,132	17,132	214.609	0.000
Biosocial position	1	360	360	4.514	0.034
Residuals	192	15,327	80		

4. Discussion

Migration is an evolutionary mechanism of plants that helps them to adapt to changing growth conditions [34]. The natural migration of genotypes in response to a changing climate is a relatively slow process due to its association with the mechanisms of natural selection. Given the need to accelerate the adaptation processes of selected economically important forest-tree species to a dynamically changing climate, the possibility of the assisted migration of genotypes is being considered in forest management [20]. In the past, the transfer of seeds of foreign origin has led to negative breeding results [6], so the assisted migration of genotypes is controversial [35]. One of the issues that remains to be explained is the response of genetic variation in natural populations under foreign climatic conditions. In the present study, the KOC stand grown from seeds of foreign origin had the highest values for the number of haplotypes (*A*), haplotype richness (*Rh*), and genetic diversity (*He*) in comparison with the other studied stands (Table 2). The results obtained for the KOC site confirmed the probable higher plasticity compared to the other studied stands. The high values of the genetic variability of the KOC stand may allow adaptation under dynamically changing ecological growth conditions. The results obtained were probably due to the extraction of seeds (in France) from many unrelated stands. Consequently, a mixture of unrelated seeds was used for reforestation in Poland. Similar results were obtained in other studies on Polish pine populations analysing the maternal and progeny generations [36]. The use of a seed mixture obtained from unrelated populations to establish a KOC stand further confirms the very high mean genetic distance between individuals (Table 2). An unnatural level of intrinsic genetic variability was achieved in the KOC population by human activities. This result should be considered a cumulative effect. First, pines naturally have higher genetic variability within populations than between populations [8]. Second, in the case of the KOC stand, there was artificially generated high internal genetic variability. A similar situation was described by Przybylski et al. [9] in Kampinos National Park, where the maternal and progeny generations of pine stands were subjected to genetic studies. In the study by Przybylski et al. [9], the gene pool resulting from human activities in one of the studied populations contained a number of alleles that were subjected to strong selection pressure in the progeny generation. We should probably expect similar natural regulatory mechanisms acting on the KOC stand. The results of the analyses carried out in the KOC stand additionally showed a high value of private haplotypes (*Ph*) compared to the other populations (Table 2). It is widely believed that the occurrence of private alleles and rare alleles may influence species adaptation to climate change or to changing selection pressures in the future [37]. Müller-Starck [38] showed that certain rare alleles found in stands of beech (*Fagus sylvatica* L.) growing in a highly stressful environment increased the adaptive potential of the populations studied. Cheng et al. [39] demonstrated the role of rare alleles in the expression of elm disease resistance. Rajora et al. [40] argued that the loss of private alleles observed in heavily thinned stands may compromise the integrity of locally adapted gene pools. Thus, the presence of private alleles in a population is an expected benefit of the assisted migration of genotypes. However, in the context of the previously cited study by Przybylski et al. [9], the stability of private alleles in natural ecosystems is questionable. The results of the enrichment of the gene pool by assisted migration are achieved artificially and are unlikely to be stable, and they are subject to natural selection pressures. A parameter to be analysed in the context of the assisted migration of genotypes is genetic differentiation (*Fst*) between

native and non-native populations. The genetic diversity of cpSSR between European pine populations is approximately 2.1% [13–15]. The present study showed *Fst* values for the KOC stand from 4% to 12% (Table 4). The high and statistically significant (p-value = 0.000) *Fst* value that distinguished the KOC stand from the other populations raised the question of the maximum safe genetic and geographic distances for seed transfer. This relationship should be analysed in detail for species to be included in assisted genotype migration. This question becomes particularly important when a significantly different genetic pool implies different physiological responses of a tree to external environmental factors, such as temperature, humidity, growing season, or other biotic and abiotic stress factors.

The analysed stands were characterised by an average defoliation level of 26%–60% loss of the assimilation apparatus. This result was better than the average for mature stands obtained by the State Forest Monitoring in Poland in 2021 (Monitoring Lasów w Polsce (gios.gov.pl (accessed on 24 September 2022) [41])). It should also be mentioned that there are frequent insect infestations in the study area (e.g., *Panolis flammea*), and, as a result of this, the tree crowns lose most of their needles. However, the genetic diversity of all the stands allows them to persist stably in satisfactory health through dynamic regeneration processes. The stands did not differ statistically from one another in terms of the loss of the assimilative apparatus (Figure 4), and the number of needles and the biosocial status of the trees were the most important factors influencing defoliation. In the context of stand vigour, the promoted migration of genotypes in the KOC stand did not induce better health than the other populations studied. The differences for the KOC stand compared to the other populations studied were found in the number of cones in the crowns and the number of seedlings in the stand. The KOC stand had the lowest number of estimated cones in the canopy, and this was almost 100% lower than the maximum number of cones estimated in the MIE stand. The number of cones influenced the number of pine seedlings in all the stands. In the GAC and MIE stands, 67 to 71 seedlings were estimated in the assessed sample plots (Table 5). In the KOC stand, which was in the same forest area as GAC and MIE, only two pine seedlings were found. According to the recommendations for the natural regeneration of pines in the climatic conditions of Poland, after habitat preparation, the optimal number of seedlings is 50,000 ha^{-1} [42]. The achieved value of the seedlings in the KOC stand was too low to ensure the natural regeneration of the population and the transfer of genetic information to the progeny generation. Unresolved in the present study was the question of the ecological reasons for the selection of foreign genotypes observed in the KOC stand. One hypothesis is the effect of the lack of nitrogen compounds on seeds. Seeds obtained from such plants quickly lose their ability to germinate. Deficiencies in phosphorus, potassium, calcium, and other micronutrients also negatively affect seed quality [43]. Local climate is also an important component of natural selection. However, the question of the causes of selection for alien genotypes needs further research.

5. Conclusions

Seed transfer, defined as the assisted migration of genotypes in times of rapidly changing climate, is an important component of forest science research. This paper presented an assessment of cpDNA differences between stands formed from seeds of different provenances and the reproductive potential of the studied stands. Stands formed from seeds of foreign origin were rich in alleles and, therefore, had high genetic variability. The artificial increase in genetic variability is desirable in the assisted migration of genotypes. The presence of different alleles should make a stand more adaptable, which is important in changing growing conditions. However, in the time perspective analysed, higher genetic variability did not benefit the KOC stand in the sense of its lower defoliation which had no impact on the possible greater plasticity of the population in the future. The data obtained showed that assisted genotype migration is an anthropogenic creation, and its natural persistence in an ecosystem is impossible. Selection forces act on stocks derived from seeds of foreign origin, limiting their potential for the natural transmission of genotypes to the offspring generation. Based on the obtained results, it was concluded that the research topic

of the assisted migration of genotypes should be continued in terms of the safe distance (geographically and genetically) of seed transfer and economic viability.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f13111829/s1: (1) Figure S1. Protection Plan of the Tuchola Forests National Park, which contains unpublished documents of the National Park Directorate approved by the State Administration. The document is available in Polish from the library of Tuchola Forests National Park. (2) Figures S2. Location of sample trees in the GAC stand. (3) Figure S3. Location of sample trees in the PLE stand. (4) Figure S4. Location of sample trees in the MIE stand. (5) Figure S5. Location of sample trees in the KOC stand.

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Data Availability Statement: The data are publicly available in annual reports held in the library of the Forest Research Institute in Poland.

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