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Genetic Diversity, Structure, and Differentiation of *Pinus sylvestris* L. Populations in the East European Plain and the Middle Urals

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Abstract: Genetic diversity is important for the long-term survival of species and plays a critical role in their conservation. To manifest the adaptive potential, it is necessary to preserve the allelic diversity of populations, including both typical and region-specific alleles. Molecular genetic analysis of 22 populations of Scotch pine (*Pinus sylvestris* L.; *Pinaceae*) in 10 subjects of the Russian Federation in the East European Plain and the Middle Urals was carried out. Molecular genetic analysis of 22 populations of *P. sylvestris* revealed 182 polymorphic PCR fragments. The studied populations are characterized by a medium level of genetic diversity. A high subdivision coefficient (G_{ST}) of the studied populations was established; the intensity was 0.559. At the same time, the level of subdivision differed for different regions; for the populations from the Middle Urals, it was 15.5% (G_{ST} = 0.155), and for the populations from the East European Plain, it was 55.8% (G_{ST} = 0.558). The dendrogram of genetic similarity shows five clusters of the studied populations of *P. sylvestris* according to their geographical location. The populations from the East European Plain are mostly characterized by typicality, while the populations from the Middle Urals, on the contrary, are more specific in gene pools. The use of the coefficient of genetic originality to identify populations with typical and specific alleles allows for solving the problem of selecting populations for the conservation of forest genetic resources. The data obtained on genetic diversity, and the structure of populations growing in areas of active logging, are important for determining the geographical origin of plant samples, which is an integral part of the control of illegal logging.

Keywords: inter simple sequence repeats (ISSR); genetic diversity; genetic structure; *Pinus sylvestris* L.

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1. Introduction

Genetic diversity is important for the long-term survival of species and plays a critical role in their conservation [1–3]. To manifest the adaptive potential, it is necessary to preserve the allelic diversity of populations, including both typical and region-specific alleles [4]. Genetic diversity also plays an important role in the response of populations to environmental factors [5,6]. Thus, knowledge about the genetic diversity of populations and their underlying individual and subpopulation components is important for the conservation and rational use of genetic resources, especially in the context of global climate change [7].

Coniferous forests form the basis of boreal ecosystems and are of enormous economic importance. They have a huge local and global impact on ecosystems, playing an important role in the regulation of water flow and soil conservation, being the most important part of the carbon cycle and a tool for cleaning atmospheric air from pollution [8–10]. In addition,

the tissues of coniferous plants are rich in biologically active compounds (BAC), such as terpenoids, steroids, alkaloids, flavonoids, a complex of polysaccharides (holocellulose), and others, which are promising raw materials for the pharmaceutical industry [11,12].

Scotch pine (*Pinus sylvestris* L.) is the second most common coniferous species in the world and is of great economic and ecological importance [13]. Pine forests cover 37% of the total land area in the world and about 70% of the land area in the Northern Hemisphere, making Scotch pine one of the most important forest-forming species [13]. The current area of this species is the result of re-colonization events and post-glacial shrinkage of a once-larger distribution area [14,15]. *P. sylvestris* is a species tolerant of a wide range of ecological habitats and plays an important economic and ecological role in the forest ecosystems of Europe [8,9]. Scotch pine has a high genetic diversity that determines quantitative, qualitative, and adaptive traits [16].

Molecular genetic studies of *P. sylvestris* have been carried out both in Europe and Russia [7,12–19]. The results of the analysis of mitochondrial markers indicate that in the entire space from the east of the East European Plain, at least to the Yenisei River, the species is almost genetically homogeneous [18]. However, in the Mediterranean and the southern part of species distribution, there is a significantly greater differentiation of populations [19]. At the same time, analysis of chloroplast DNA revealed a significant genetic heterogeneity of the species throughout its distribution area. This difference can be associated with the use of different types of DNA markers, with different types of inheritance, and is also a consequence of different genetic processes. However, the genetic conservatism of the spacer of internal transcribed ribosomal genes and sequences of chloroplast genes for a particular species makes it unsuitable to study these sequences for determining intraspecific diversity. Molecular markers based on non-coding regions of DNA are usually highly variable and thus provide high-resolution information about genetic diversity within populations and the genetic structure of populations [20–23]. The method of studying the genetic diversity of woody plant species using ISSR (Inter Simple Sequence Repeats) is simple PCR and accessible technique [24]. Due to the large number of copies of microsatellite sequences and their large number in eukaryotic genomes, the use of SSR (Simple Sequence Repeats) sequences as an efficient PCR-based DNA fingerprinting method is convenient [25–27].

Scotch pine is widely used in economic activities, and its timber is actively harvested. To draw up programs for the rational use of forest resources, knowledge about the genetic diversity, and the structure of populations growing in areas of active logging, obtained by identifying polymorphic DNA fragments, is necessary.

In this regard, the study of the molecular genetic diversity and genetic structure of the *P. sylvestris* populations of the Middle Urals and the East European Plain using the ISSR profiling PCR method is promising for the development and optimization of protocols for assessing the state of the gene pools of boreal coniferous species. In addition, this approach is effective for the selection of objects to conserve species of coniferous plants that are productive and resistant to various environmental factors.

Thus, the present work is aimed at a detailed study of the genetic diversity, genetic structure, and differentiation of natural populations of *P. sylvestris* under the conditions of their natural growth over a large area (about 55470 thousand ha.) in two distinct regions, the East European Plain and the Middle Urals.

2. Materials and Methods

2.1. Materials

Twenty-two populations of Scotch pine were chosen as objects of research (*Pinus sylvestris* L.; *Pinaceae*). The studied populations of *P. sylvestris* in Perm Krai were taken from the locations of Kochyovo's (*PS_KOCh*) Gainy's (*PS_SOSN*), Kishert's (*PS_KISH*), Kudymkar's (*PS_LENI*), Cherdyn's (*PS_ChER*) and Berezniki's (*PS_ROMA*) forestries; in the Komi Republic from the locations Lokchim's (*PS_LOKC*) and Sysolsky's (*PS_SYSO*) forestries; in Arkhangelsk Oblast from Krasnoborsk's (*PS_KRAS*) forestry; in Vologda Oblast from Velikoustyugsky's (*PS_VELI*) forestry; in Kostroma Oblast from Pyshchugsky's

(*PS_PYSH*) forestry; in Nizhny Novgorod Oblast from Urensky's (*PS_UREN*) forestry; in Mari El Republic from Korotni's (*PS_KORO*) and Kokshaysk's (*PS_KOKSh*) forestries; in Chuvash Republic from Kirsk's (*PS_KIRS*) forestry; in Ulyanovsk Oblast from Inzensky's (*PS_INZE*) forestry; and in Kirov Oblast from the locations Shabalinsky's (*PS_SHAB*), Yezhikhinsk's (*PS_YEZH*), Darovskoy's (*PS_DARO*), Yuryansky's (*PS_YURY*), Slobodskoy's (*PS_SLOB*) and Belokholunitsky's (*PS_BELO*) forestries (Table S1, Figure 1 and Figure S1).

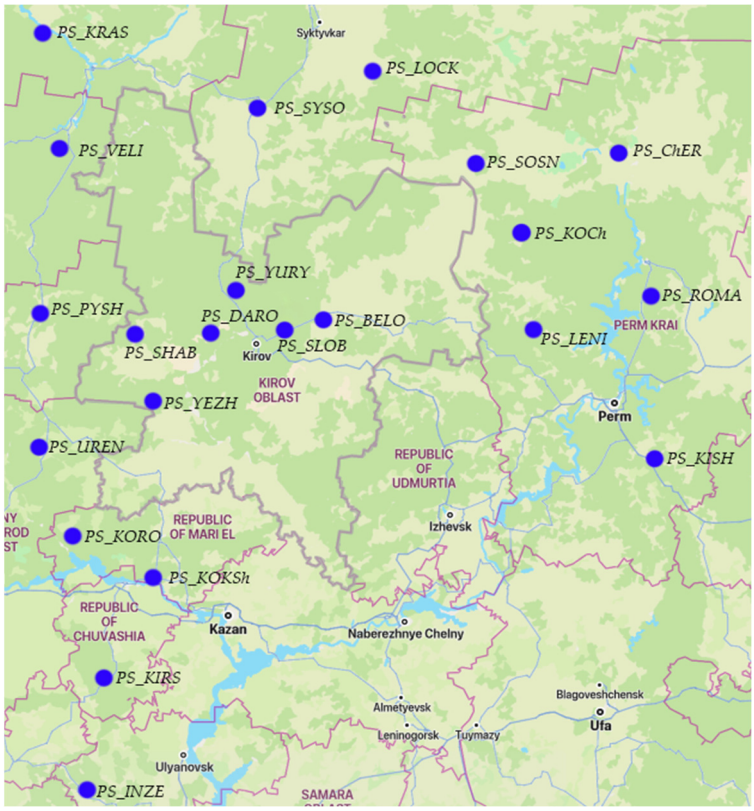

Figure 1. Schematic map of the location of the studied populations of *P. sylvestris*: *PS_KOCh*—Kochyovo; *PS_SOSN*—Gainy; *PS_KISH*—Kishert; *PS_LENI*—Kudymkar; *PS_ChER*—Cherdyn; *PS_ROMA*—Berezniki; *PS_LOKC*—Lokchim; *PS_SYSO*—Sysolsky; *PS_KRAS*—Krasnoborsk; *PS_VELI*—Velikoustyugsky; *PS_PYSH*—Pyshchugsky; *PS_UREN*—Urensky; *PS_KORO*—Korotni; *PS_KOKSh*—Kokshaysk; *PS_KIRS*—Kirsk; *PS_INZE*—Inzensky; *PS_SHAB*—Shabalinsky; *PS_YEZH*—Yezhikhinsk; *PS_DARO*—Darovskoy; *PS_YURY*—Yuryansky; *PS_SLOB*—Slobodskoy; *PS_BELO*—Belokholunitsky.

The collection of plant material was carried out from trees located at a distance of at least 50–150 m from each other [17,28,29]. The geographic distances between the populations varied from a minimum of 49 km (*PS_SHAB* and *PS_DARO* located in Shabalinsky's and Darovskoy's forestries) to a maximum of 941 km between the *PS_ChER* and *PS_INZE* populations. Pairwise geographic distances between all studied populations are presented in Table S2.

2.2. DNA Extraction and PCR

For research, samples of plant tissues were collected individually from 30 to 46 trees in each of the studied populations. DNA isolation was carried out according to the method for complex biological samples [30]. The weight of each sample of dry plant material was approximately 20 mg. To determine the concentration and quality of DNA we used NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). For the Polymerase Chain Reaction (PCR), the DNA concentration of each sample was leveled to 10 ng/μL.

The ISSR profiling analysis method was used to assess the genetic diversity and genetic structure of populations [24,31]. PCR reactions were performed in a 25 µL reaction mixture containing 25 ng of template DNA, 1 × PCR buffer with 2.5 mM $MgCl_2$, 1 µM ISSR primer, 0.25 mM each dNTP, and 2 U Taq DNA polymerase (Sileks M, Russia). PCR amplification was carried out in a SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific Inc., Waltham, MA, USA) under the following conditions: initial denaturation step at 94 °C for 2 min, followed by 32 amplifications at 94 °C for 20 s, at 52–64 °C (depending on primer sequence) [32] for 30 s, and at 72 °C for 60 s, followed by a final extension of 72 °C for 3 min (Table 1) [33].

Table 1. The information of ISSR primers is used to assess the genetic diversity of *P. sylvestris*.

Primer ID	Sequence 5′–3′	Tm (°C)	Ta (°C) *	Total Bands	PIC *
ISSR-1 ($(AC)_8T$)	ACACACACACACACACT	55.0	56	31	0.196
CR-212 ($(CT)_8TG$)	CTCTCTCTCTCTCTCTTG	55.9	56	43	0.260
CR-215 ($(CA)_6GT$)	CACACACACACAGT	52.6	56	33	0.256
M27 ($(GA)_8C$)	GAGAGAGAGAGAGAGAC	54.9	52	33	0.261
X10 ($(AGC)_6C$)	AGCAGCAGCAGCAGCAGCC	72.4	64	42	0.224

* Ta—optimal annealing temperature; Polymorphism Information Content.

All primers were tested to assess the genetic diversity of *P. sylvestris* using PCR amplification for ISSR profiling. PCR products were separated by electrophoresis at 70 V for 5 h in 1.5% agarose gel with 1xTBE buffer, stained with ethidium bromide, and photographed in transmitted ultraviolet light using Gel Doc XR+ Gel Documentation System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) gel documentation system. To determine the length of DNA fragments, a molecular weight marker (100 bp + 1.5 + 3 Kb DNA Ladder, LLC. SibEnzim-M, Moscow, Russia) and the Quantity One 1-D Analysis Software (Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used. In total, polymorphism of ISSR markers with 5 primers was analyzed within 922 individual *P. sylvestris* trees. To check the reliability of the obtained results, a PCR was performed at least three times.

2.3. Data Analysis

To quantify the genetic polymorphism and determine the genetic structure of the twenty-two populations studied, the data were presented in the form of a matrix of binary characters, in which the presence or absence of fragments of the same size in the spectra was considered, respectively, as 1 or 0 state.

Computer processing of the data was performed using the specialized macro GenAlEx for MS Excel to determine the number of alleles (n_a), effective (n_e) number of alleles [34], and expected (*He*) heterozygosity and Shannon's information index (*I*). The following parameters calculated in the POPGENE 1.31 software were used to describe the genetic structure of populations [35]: the expected proportion of heterozygous genotypes in the entire population as a measure of total genetic diversity (H_T); the expected proportion of heterozygous genotypes in a subpopulation, as a measure of intrapopulation diversity (H_S); share of interpopulation genetic diversity in total diversity or the coefficient of gene differentiation (G_{ST}); and AMOVA (Analysis of Molecular Variance) with the calculation of the *PhiPT* index (population subdivision index) using 1000 rounds of permutations [36]. Genetic distances between populations (D_N) were determined using the method of M. Nei [37]. To determine the correlation between pairwise genetic distances (D_N and *PhiPT*) and geographic distances in the general population group, the commonly used Mantel test was used. Regression analysis was carried out in MS Excel.

Based on the binary trait matrix, a genetic distance matrix was calculated, based on which dendrograms reflecting the degree of similarity between the studied populations and trees were generated by the spectrum using the MEGA X program [38].

To identify the structure of intrapopulation diversity, we used the indicator of the proportion of rare alleles (*h*). In addition, Principal Coordinates Analysis (PCA), implemented in the PAST 4.10 program, was performed to verify the obtained data. In the PAST 4.10 program [39], a detailed dendrogram was constructed for all trees using the Neighbor-joining method, and analysis and visualization were performed using the UMAP (Uniform Manifold Approximation and Projection) method [40]. The specificity of the gene pools of *P. sylvestris* populations was characterized using the genetic originality coefficient (GOC), which makes it possible to characterize populations in terms of the proportion of rare and typical alleles [4].

3. Results

3.1. Genetic Diversity of P. sylvestris

Molecular genetic analysis of twenty-two populations of *P. sylvestris* revealed 182 PCR fragments. The primers used detected between 31 and 43 PCR fragments, and the maximum number of fragments was amplified with the primer CR-212 [$(CA)_6GT$]. On average, one primer identified 36 PCR fragments. The lengths of PCR fragments varied from 150 to 1600 base pairs. The greatest genetic diversity was observed in the populations *PS_DARO* (I = 0.249; H_e = 0.164; n_e = 1.268) from Darovskoy's forestry, *PS_YURY* (I = 0.263; H_e = 0.176; n_e= 1.299) from Yuryansky's forestry and *PS_BELO* (I = 0.264; H_e = 0.177; n_e = 1.303) from Belokholunitsky's forestry. The lowest genetic diversity was observed in the populations *PS_PYSH* (I = 0.096; H_e = 0.063; n_e = 1.106) from Pyshchugsky's forestry, *PS_SHAB* (I = 0.087; H_e = 0.056; n_e= 1.092) from Shabalinsky's forestry, *PS_YEZH* (I = 0.089; H_e = 0.057; n_e= 1.092) from Yezhikhinsk's forestry, *PS_UREN* (I = 0.092; H_e = 0.059; n_e = 1.092) from Urensky's forestry (Table 2). No specific alleles were found in the populations studied (Figure S2).

Table 2. Genetic diversity of the studied populations of *P. sylvestris*.

Populations	H_e	n_e	I	Populations	H_e	n_e	I
PS_KOCh	0.131 (0.012)	1.199 (0.020)	0.213 (0.017)	*PS_SHAB*	0.056 (0.010)	1.092 (0.017)	0.087 (0.015)
PS_SOSN	0.119 (0.012)	1.182 (0.020)	0.194 (0.017)	*PS_YEZH*	0.057 (0.010)	1.092 (0.017)	0.089 (0.015)
PS_KISH	0.143 (0.013)	1.228 (0.023)	0.225 (0.019)	*PS_UREN*	0.059 (0.010)	1.092 (0.016)	0.092 (0.014)
PS_LENI	0.119 (0.011)	1.181 (0.020)	0.195 (0.017)	*PS_KORO*	0.122 (0.014)	1.207 (0.024)	0.182 (0.020)
PS_ChER	0.142 (0.012)	1.223 (0.022)	0.227 (0.018)	*PS_KOKSh*	0.117 (0.014)	1.204 (0.025)	0.172 (0.020)
PS_ROMA	0.125 (0.012)	1.194 (0.021)	0.202 (0.017)	*PS_KIRS*	0.121 (0.014)	1.206 (0.025)	0.180 (0.020)
PS_LOKC	0.112 (0.013)	1.187 (0.023)	0.169 (0.019)	*PS_INZE*	0.104 (0.013)	1.183 (0.025)	0.152 (0.019)
PS_SYSO	0.106 (0.013)	1.175 (0.023)	0.161 (0.019)	*PS_DARO*	0.164 (0.014)	1.268 (0.025)	0.249 (0.020)
PS_KRAS	0.129 (0.014)	1.216 (0.024)	0.195 (0.020)	*PS_YURY*	0.176 (0.015)	1.299 (0.027)	0.263 (0.021)
PS_VELI	0.118 (0.013)	1.193 (0.023)	0.180 (0.019)	*PS_SLOB*	0.151 (0.014)	1.249 (0.025)	0.230 (0.020)
PS_PYSH	0.063 (0.011)	1.106 (0.019)	0.096 (0.016)	*PS_BELO*	0.177 (0.015)	1.303 (0.027)	0.264 (0.021)
Total	0.119 (0.003)	1.195 (0.005)	0.183 (0.004)	Total	0.119 (0.003)	1.195 (0.005)	0.183 (0.004)

H_e—expected heterozygosity; n_e—effective number of alleles per locus; I—Shannon's information index; all of the above parameters have standard deviations in standard deviations given in brackets.

3.2. Population Genetic Structure of P. sylvestris

Analysis of the genetic structure of the studied *P. sylvestris* populations revealed that the expected proportion of heterozygous genotypes (H_T) per total sample was 0.270, while

the expected proportion of heterozygous genotypes in a subpopulation (H_S) was 0.119. The population subdivision coefficient (G_{ST}) shows that the interpopulation component accounts for 0.559 of the total genetic diversity.

The values of pairwise *PhiPT* genetic distances detected by the AMOVA (Table S3) package ranged from 0.081 (*PS_KOCh/PS_KISH*) to 0.731 (*PS_SHAB/PS_INZE*). For the total sample of *P. sylvestris*, the *PhiPT* index was 0.647, which approximates the G_{ST} = 0.559. Analysis of molecular variability (AMOVA) showed that differences between regions account for 28% of diversity, differences between populations 37%, and intrapopulation differences account for 35% (Table 3). At the same time, the level of subdivision for different regions differed; for the populations from the Middle Urals, it was 15.5% (G_{ST} = 0.155), and for the East European Plain 55.8% (G_{ST} = 0.558).

Table 3. Assessment of genetic intra- and interpopulation variability in *P. sylvestris* populations by AMOVA.

Subdivision Indicator	df	SS	MS	Dispersion	%	*p*
Among groups	1	3442.465	3442.465	10.041	28%	<0.001
Among populations	20	11379.929	568.996	13.168	37%	<0.001
Within populations	900	11373.549	12.637	12.637	35%	<0.001

df—degrees of freedom, SS—the sum of squares, MS—standard deviation, %—the percentage of total genetic diversity, p—significance level when using 1000 rounds of permutation.

The smallest genetic distance was observed between populations *PS_KOCh/PS_KISH* (D_N = 0.012), the largest (D_N = 0.322) between populations *PS_SOSN* and *PS_SHAB* (Table S4). Based on the matrix of pairwise genetic distances (D_N), a cluster analysis was performed using the Neighbor-joining method, and a dendrogram reflecting the degree of similarity in the ISSR profiles of the populations studied was constructed (Figure 2). In the dendrogram, the studied populations were divided into five clusters in accordance with their geographical location: East (I), Center (II), North (III), South (IV), and West (V).

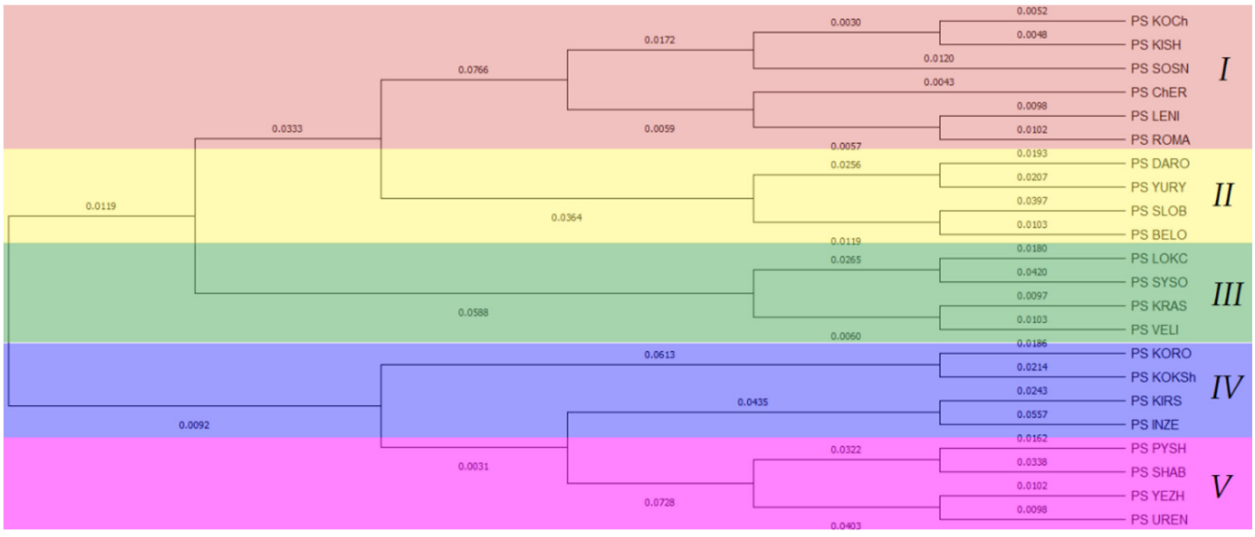

Figure 2. Dendrogram of genetic similarity of twenty-two studied populations of *P. sylvestris*, built based on polymorphism of ISSR profiles by Neighbor-joining method.

The separation of populations into five clusters is supported by the results of the Principal Coordinates Analysis (PCA), based on the *PhiPT* index calculated with the AMOVA package (Figure S3).

STRUCTURE analysis showed the presence of five groups of genotypes in the studied populations. The distribution of genotypes corresponds to the differentiation of populations according to the results of PCA analysis and the Neighbor-joining method (Figure S4).

Analysis of the population structure using UMAP, carried out for populations in the Middle Urals, indirectly confirms their low differentiation, G_{ST} = 0.155 (Figure 3). At the same time, the genotypes of individuals from the *PS_KOCh* population are distributed over all groups (Table 3).

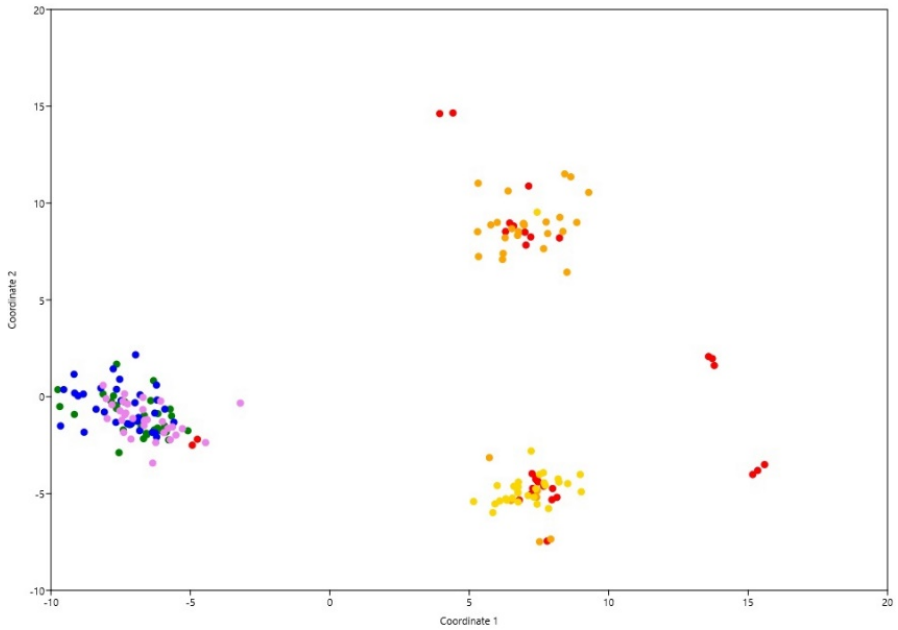

Figure 3. Distribution of individuals in the studied populations of *Pinus sylvestris* L. of the Middle Urals using UMAP.

During the study of *P. sylvesrtis* populations, their spatial and genetic structure was checked for consistency with the "isolation-by-distance" model by the Mantel test. Thus, a pairwise comparison of all twenty-two studied populations revealed the presence of a weak positive correlation (R^2 = 0.2534) between geographic and genetic distance (D_N) (Figure S5). A regression analysis was also carried out on these data, and a significant relationship was found, but the R^2 value (0.2534) showed that the correlation was weak (Table S5).

Using a specific and typical allele approach, populations in the studied regions were examined and characterized. The populations from the East European Plain are mostly characterized by the typicality of gene pools (GOC < 1.000); only the *PS_INZE* selection (GOC = 1.041) is characterized by the specificity of the gene pool. The populations from the Middle Urals, on the contrary, are more specific in gene pools (GOC > 1.200), the most specific gene pool belongs to the *PS_KISH* selection (GOC = 1.665).

The proportion of rare alleles indicator (h) assesses the structure of intrapopulation diversity, the lower the value h of the threshold (0.3) level, the more balanced the structure of diversity is characterized by populations. Of the studied populations, h was above the threshold value in *PS_SHAB* (h = 0.309) and *PS_YEZ* (h = 0.304). Two more populations were close to the threshold, *PS_PYSH* (h = 0.289) and *PS_UREN* (h = 0.290).

4. Discussion

4.1. Genetic Diversity of P. sylvestris

As a result of the study, a medium level of genetic diversity in *P. sylvestris* populations was revealed (I = 0.183; H_e = 0.119; n_e= 1.195), it is common for populations from the Southern part of the East European Plain, but less than the level of genetic diversity of the South Urals populations [41]. The greatest genetic diversity was revealed in the central populations, *PS_DARO*, *PS_YURY* and *PS_BELO*. The least genetic diversity was observed among the group of Western populations, *PS_PYSH*, *PS_SHAB*, *PS_YEZH* and *PS_UREN*; this may be due to anthropogenic pressure, in particular active logging. In addition, these

populations also differ in a less balanced genetic structure in terms of the proportion of rare alleles (*h*), which in turn may also be due to active logging in these regions. The genetic structure and diversity of populations can be greatly affected by random genetic drift, which can lead to the erosion of genetic variation due to the loss of rare alleles [42,43]. No specific alleles were found in the populations studied, which may indicate that these populations are genetically homogeneous.

4.2. Population Genetic Structure of *P. sylvestris*

The studied populations were divided into five clusters, in accordance with their geographical location. At the same time, the populations from the Middle Urals were distinguished. Differentiation between the populations from the Urals and the East European Plain amounted to about a third (28%) of the observed genetic diversity, another third is due to the interpopulation component (37%), and a third to intrapopulation differences (35%). The data obtained indicate the origin of several genetically differentiated populations and their groups in *P. sylvestris* in the study areas. A similar pattern was observed in previous studies in these regions, on a smaller number of populations [33,44]. The high differentiation may be due to the fragmentation of the area of *P. sylvestris* in the region under study. Significant differentiation between the populations from the East European Plain and the populations from the Middle Urals (28%) may be related to the history of the distribution of the species in the Pleistocene. Populations of the Middle Urals were settled mainly from the South Ural refugium, while this refugium made a smaller contribution to the gene pools of the East European populations [45,46].

In general, there is a genetic homogeneity of *P. sylvestris* populations in the Middle Urals, which is confirmed by the analysis of UMAP and AMOVA. For the *PS_KOCh* population, according to the UMAP analysis, the distribution of genotypes over all identified groups is observed. This may be due to the fact that the population was settled from several different directions. The settlement of the territory of the Urals occurred mainly from the South Ural refugium, but in addition, the settlement came from the Balkan refugium and the refugia of the second order in South Siberia [46]. Correlation analysis between genetic and geographical distances revealed the presence of a medium relationship between them ($R^2 = 0.2534$).

The weak differentiation of the populations in the Middle Urals may be due to the similarity of the habitats of the populations since all the studied populations are located at 180–200 m above sea level. Similar genetic homogeneity of Scotch pine populations was also observed in the study of populations in the territory from the east of the East European Plain at least to the Yenisei River using mitochondrial markers [18].

Within the East European Plain, *P. sylvestris* populations, despite high differentiation, are characterized by typical gene pools. Populations from the Middle Urals are characterized by specific gene pools. Populations with a specific gene pool, such as the selection from the Kishert's forestry (*PS_KISH*), can serve as a source of genetic diversity in reforestation programs. Additionally, populations with a more typical gene pool, having the most common alleles in the region, can be preserved as forest genetic reserves to preserve the genetic resources of the species. Populations from the East European Plain with the most typical gene pools can serve as an example of such populations. Data on the typicality and specificity of gene pools, as well as on the differentiation of populations, can be used in further studies of *P. sylvestris* in the study region.

The obtained data on genetic diversity and the structure of populations growing in areas of active logging are important for drawing up programs for the rational use of forest resources, identifying populations, and determining the geographical origin of plant specimens, including timber, which is an integral part of controlling illegal logging.

The use of the coefficient of genetic originality to identify populations with typical and specific alleles makes it possible to solve the problem of selecting populations for the conservation of forest genetic resources.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f13111798/s1, Supplementary Table S1. The studied populations of *P. sylvestris* were used in ISSR analysis. Supplementary Table S2. Pairwise geographic distances (km) between the studied populations of *P. sylvestris*. Supplementary Table S3. Paired PhiPT genetic distances between the studied populations of *P. sylvestris* by AMOVA. Supplementary Table S4. Pairwise genetic distances (DN) between the populations studied *Pinus sylvestris* L. Supplementary Table S5. Regression analysis of genetic and geographical distances. Supplementary Figure S1. Schematic map of the location of the studied populations of *P. sylvestris*. Supplementary Figure S2. Allele patterns of *P. sylvestris* populations. Supplementary Figure S3. Ordination of the studied populations of *P. sylvestris* using PCA, obtained on the basis of PhiPT matrix of genetic distances. Supplementary Figure S4. The structure of the distribution of genotypes in *P. sylvestris* populations. Supplementary Figure S5. Graph of dependence of genetic (DN) and geographical distances of *P. sylvestris* populations.

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