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Genetic characteristics of Scots pine in Poland and reference populations based on nuclear and chloroplast microsatellite markers

Highlights

- Similar genetic variation was found between Polish Scots pine populations from a wide variety of habitats based on nSSR and cpSSR markers.
- Homogeneity was observed in the genetic structures of Polish and Finnish populations from the continuous pine range.
- Genetic differentiation in microsatellite markers was identified only when populations from the central pine distribution were compared to the marginal stands.

Abstract

Polymorphisms at a set of eighteen nuclear (nSSR) and chloroplast (cpSSR) microsatellite loci were investigated in sixteen populations of Scots pine (*Pinus sylvestris* L.) derived from the provenance trial experiment and representative of the species distribution range and climatic zones in Poland. The patterns of genetic variation were compared to the reference samples from the species distribution in Europe and Asia. A similar level of genetic variation and no evidence of population structure was found among the Polish stands. They showed genetic similarity and homogenous patterns of allelic frequency spectra compared to the Northern European populations. Those populations were genetically divergent compared to the marginal populations from Turkey, Spain and Scotland. The population structure patterns reflect the phylogeography of the species and the divergence of populations that most likely do not share recent history. As the analysed provenance trial populations from Poland are diverged in phenotypic traits but are genetically similar, they could be used to test for selection at genomic regions that influence variation in quantitative traits.

1   Introduction

Scots pine (*Pinus sylvestris* L.) is a species of great ecological, social and economic importance that covers large parts of Europe and Asia. It is found in a range of environments from the mountain areas of Spain, Turkey and the Scottish Highlands to the subarctic forests of northern Scandinavia and Siberia (Jaworski 2011). Across its wide distribution range, Scots pine exhibits high phenotypic variation (Eriksson 2008; Giertych 1993) that was established in response to selection and adaptation to local environmental conditions. Those mechanisms affect the species ability to succeed in changing environmental conditions (Savolainen et al. 2007). Adaptive variation has a strong genetic component, as shown in the Scots pine common garden and provenance trial experiments (Alía et al. 2001; Oleksyn et al. 1998; Savolainen et al. 2004). However, less is known about the underlying patterns of genetic variation in Scots pine determined by evolutionary and population history processes.

The present distribution of Scots pine in Europe is the result of survival, range shifts and postglacial recolonization events. There are several known areas including the Iberian Peninsula, Italy and the Balkans, where Scots pine survived the Last Glacial Maximum (LGM) (Cheddadi et al. 2006; Naydenov et al. 2007; Pyhäjärvi et al. 2008; Sinclair et al. 1999). According to the other genetic and microfossil data, the refugial areas are located in the Alps (Cheddadi et al. 2006) and the region of Moscow, potentially also on the southern slopes of the Carpathians (Müller et al. 2003; Willis and Van Andel 2004). However, the spatial distribution of maternally inherited, haploid, mitochondrial DNA markers indicates that isolated populations from the Iberian Peninsula, the Apennine Peninsula and Anatolia did not participate in recolonization of the areas of Scots pine continuous distribution in North and Central Europe (Cheddadi et al. 2006; Pyhäjärvi et al. 2008).

Climate conditions in refugial areas enabled the survival of the populations which became a source of genetic variability during and after LGM (Petit et al. 2003). Populations from a broad pine distribution showed a high level of genetic variation and a low level of among-population genetic differentiation (Belletti et al. 2012; Prus-Glowacki et al. 2003; Pyhäjärvi et al. 2007; Wachowiak et al. 2014; Wang et al. 1991). This low genetic structure in a wide distribution of Scots pine is typical for geographically core populations and among others, it results from high outcrossing and random mating in pines (Petit and Hampe 2006). According to the "centre-periphery hypothesis" the marginal populations are expected to be less genetically diverse and more prone to extinction (Hampe and Petit 2005). However, the recolonization routes and admixture patterns of Scots pine populations of different origins, which impact the genetic diversity, are not well characterized. This is particularly true for populations from Central Europe, including Poland, where Scots pine is continuously distributed in the lowlands and forms some dispersed populations in the Carpathian and Sudeten Mountains.

Many years of provenance experiments showed that the pine populations inhabiting northern Poland and the neighbouring Baltic countries are characterized by very good growth and adaptive ability (Barzdajn et al. 2016; Giertych 1979; Szeligowski et al. 2016). The lowland populations in Poland show a smaller percentage of deadwood, better growth parameters and a greater volume and growing stock compared to highland pines (Sabor 1993). The influence of terrain is visible in morphological and physiological differentiation (Staszkiewicz 1961) and variability in wood quality traits (Niedzielska et al. 2001), particularly in populations from the Carpathians and the Sudetes. However, the genetic relationships between the phenotypically divergent populations used in the progeny trial experiments are not well resolved.

In this research, the genetic variation of Scots pine populations in the Polish distribution range of the species was assessed based on the polymorphism at a set of nuclear (nSSRs) (Simple Sequences Repeats) and chloroplast (cpSSRs) microsatellite loci. We checked whether *P. sylvestris*

populations from different climatic regions in Poland are significantly different in genetic structure, as they are diverged at phenotypic traits. Furthermore, we looked at the genetic relationships at the same set of loci between Polish populations and the reference populations of the species from the continuous distribution represented by populations from Finland and the marginal distributions in Europe and Asia represented by populations from Scotland, Spain and Turkey.

2 Material and methods

2.1 Sampling and DNA extraction

Sixteen populations of Scots pine (384 individuals) derived from a provenance trial in the Carpathian Mountains were used in the study. The experimental plot was established in 1966 with provenances from the Polish distribution range of Scots pine, including climatic and seed zones (Table 1, Fig. 1). The populations were characterized based on phenotypic variability of qualitative and quantitative traits in a previous experiment (Hebda et al. 2017). The sampled populations include the races of lowland pines (e.g., from Supraśl, Bolewice and Spała) that show very good growth and productivity (Barzdajn et al. 2016; Oleksyn and Rachwał 1994; Szeligowski et al. 2016). Additionally, we examined populations from the foothills and mountain regions determined as local populations

Table 1. Geographical locations of *Pinus sylvestris* populations studied using nuclear and chloroplast micro-satellites markers. Native populations are from Poland (PL) divided for three climatic regions: I– Central Poland, II– North Poland, III– South Poland while reference populations are from Finland (F), Turkey (T), Spain (Sp), Scotland (S). Populations from Turkey, Spain and Scotland represent marginal populations, while other samples are from the central pine distribution area.

| Code	Provenance name	Latitude, Longitude	Altitude	Region
PL1	Karsko	52°54′N, 15°15′E	75	I
PL2	Tabórz	53°52′N, 19°55′E	110	II
PL3	Dłużek	53°31′N, 20°38′E	145	II
PL4	Ruciane	53°37′N, 21°35′E	145	II
PL5	Rozpuda	53°55′N, 22°55′E	205	II
PL6	Supraśl	53°15′N, 23°20′E	165	II
PL7	Starzyna	52°38′N, 23°37′E	170	II
PL8	Gubin	51°55′N, 14°50′E	70	I
PL9	Rychtal	51°12′N, 17°50′E	190	I
PL10	Bolewice	52°23′N, 16°10′E	90	I
PL11	Lipowa	53°44′N, 18°15′E	130	II
PL12	Jegiel	52°40′N, 21°40′E	95	II
PL13	Spała	51°35′N, 20°15′E	150	I
PL14	Janów Lubelski	50°40′N, 22°25′E	250	I
PL15	Nowy Targ	49°20′N, 20°20′E	590	III
PL16	Piwniczna	49°20′N, 20°17′E	500	III
F17	Finland_Joutsa	61°74′N, 26°14′E	125	IV
F18	Finland_Kielajoki	69°65′N, 29°07′E	100	
T19	Turkey_Çatacık	39°96′N, 31°11′E	1619	V
T20	Turkey_Bayabat-Sinop	41°64′N, 34°83′E	1228	
Sp21	Spain_Sierra de Neila	42°05′N, 03°01′W	1400	VI
Sp22	Spain_Puerto de Navafría	40°98′N, 03°81′W	1800	
S23	Scotland	57°30′N, 05°38′W	81	VII
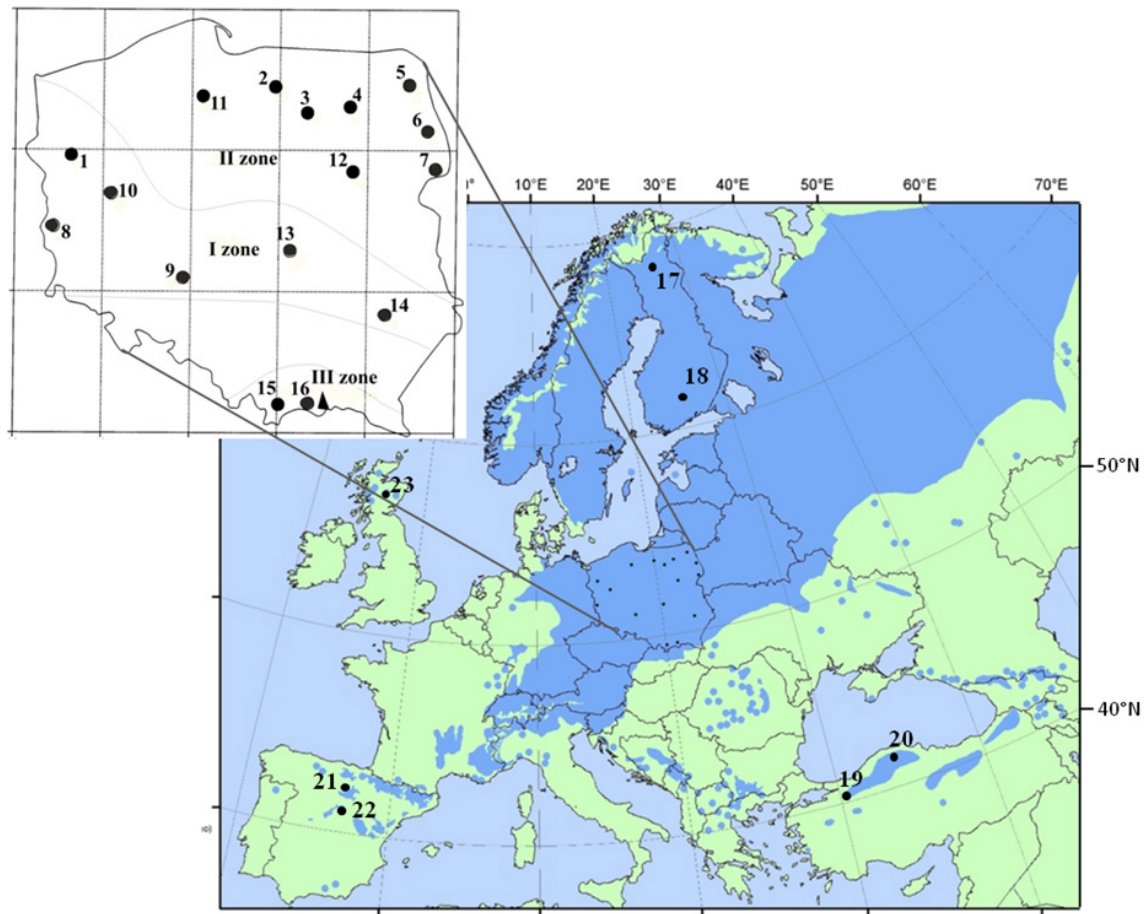
Fig. 1. Location of 16 Polish provenances of Scots pine derived from three climatic zones and reference populations of the species distribution range in Europe and Asia. Population acronyms are as in Table 1.

that displayed a weak breeding value (Barzdajn 2006; Hebda et al. 2017). Samples were collected from 24 randomly selected individuals from each population. Furthermore, reference populations of Scots pine from Finland, Scotland, Spain and Turkey (Table 1, Fig. 1) represented by about 30–50 samples each (Table 4 and in Table 5) were used in the study. Genomic DNA of each individual was extracted from needles following standard CTAB procedures.

2.2 Microsatellite genotyping and analysis

All trees were genotyped using eighteen microsatellite loci, including the following nuclear SSRs: psyl42, psyl25, psyl18, psyl44, psyl57, psyl36 (Sebastiani et al. 2012), PtTX3025 (Elsik et al. 2000), PtTX4011, PtTX4001 (Zhou et al. 2002), Spac11.4 (Soranzo et al. 1998), and the following chloroplast SSR loci: PCP36567, PCP30277, PCP102652, PCP26106, PCP87314, PCP45071, PCP1289, and PCP41131 (Provan et al. 1998). PCR (Polymerase Chain Reaction) amplifications were performed with a final volume of 10 µl containing 10 mM of each dNTP, 5 µM each of forward and reverse primers, 0.15 U Taq DNA polymerase, 1x BSA, 1.5 µM $MgCl_2$ and 1x PCR buffer. Amplification was performed using the following protocol: 95 °C for 5 min, followed by 28 cycles of 30 sec at 94 °C, 1.30 min at 60 °C, 1.30 min at 72 °C, with a final extension step of 10 min at 72 °C. The fluorescently labeled PCR products, along with a size standard (GeneSan 600 LIZ), were separated on the Applied Biosystems 3500 (Life Technologies, USA) automatic sequencer. The allele were identified using GeneMapper software ver. 5.0 (Life Technologies, USA).

2.3 Data analysis

The genetic diversity of Polish stands was analysed for individual populations and populations grouped into three climatic regions, including locations in the central (I), northern (II), and southern (III) parts of the country (Table 1, Fig. 1). The differentiation in Polish populations was compared to reference pines from the continuous (Finland) and marginal (Turkey, Spain, Scotland) species distributions. Basic genetic parameters and departure from Hardy-Weinberg equilibrium (HWE) in each population were calculated using GenAlEx 6.5 (Peakall and Smouse 2012) and Haplotype Analysis 1.05 software (Eliades and Eliades 2009). In addition, the latent genetic potential (LGP), which is defined as the difference between the effective and mean number of alleles across all loci (Bergmann et al. 1990), and the allelic richness were estimated using FSTAT 2.9.3 (Goudet 2001). Null alleles may significantly overestimate population differentiation due to the presence of false homozygotes (Chapuis and Estoup 2007). Therefore, the presence of null alleles in our dataset was assessed using Micro-Checker 2.2.3 (Van Oosterhout et al. 2004). The outlier patterns of differentiation at microsatellite loci were analysed using LOSITAN software (Tiago et al. 2008). This program allows the assessment of the expected distribution of Wright's inbreeding coefficient (F_{ST}) versus expected heterozygosity (H_e) in a model of migration with neutral markers. Departures from neutrality was evaluated for both nuclear and chloroplast microsatellites using the outlier detection approach. There were 5×10^5 simulations conducted to identify the upper and lower 95% confidence limits around the F_{ST} values.

The population structure based on allelic frequency spectra was examined using Weir and Cockerham's method as implemented in GenePop 4.4 (Rousset 2008). A significance test with 1000 permutations was performed using FSTAT 2.9.3 to determine if the F_{ST} of the tested populations from Poland and reference regions differed significantly (Goudet 2001). A hierarchical analysis of molecular variance (AMOVA) was used to estimate the partitioning of genetic variation among the populations and regions using GenAlEx 6.5 software (Peakall and Smouse 2012). The significance of the AMOVA was tested with 999 permutations. The genetic differentiation between populations and regions was also assessed using Nei's genetic distances (Nei 1972). Individual samples and populations were assigned to different genetic groups using clustering analysis in STRUCTURE 2.3 (Pritchard et al. 2000). In the Bayesian population structure analysis, the admixture model was used with the following assumptions: all markers were unlinked, all trees were admixed and all frequencies were correlated between loci. The number of tested genetic clusters (K) varied from 1 to 6, and ten independent runs were conducted for each K. The burning was set to 100 000, and the run length was set to 1 000 000. The number of clusters for each level of population comparison was inferred using Harvester software (Earl and Holdt 2012). The genetic relationships between populations were also investigated using Principal Coordinates Analysis (PCoA) based on F_{ST} values for genotype frequency at nSSRs and based on Nei's (1972) genetic distances for haplotype frequency at cpSSR loci. Differentiation among Polish populations and between Polish and reference populations due to spatial isolation was tested using the Mantel test (1967) in GenAlEx 6.5 software (Peakall and Smouse 2012). The matrixes of pairwise geographic (logarithmic scale) and pairwise genetic distances (F_{ST} values for genotype frequency at nSSR or Nei's (1972) values for haplotype frequency at cpSSR) were compared and tested for significance using 9999 permutations.

Table 2. Genetic parameters for the nuclear microsatellite loci tested in all Polish pine populations. N – number of alleles per locus, R – range of allele size, A – mean number of alleles per locus, A_e – mean effective number of alleles per locus, H_o – mean observed heterozygosity, H_e – mean expected heterozygosity, F – mean fixation index, null – null allele frequency, ns – non significant, * $P \leq 0.05$.

Locus	N	R	A	A_e	H_o	H_e	F	null	Deviation from HW equlibrium
psyl42	6	165–177	4.19	3.31	0.673	0.698	0.035	0.000	ns
psyl25$^\Delta$	2	213–216	1.56	1.07	0.040	0.062	0.343	0.014	*
psyl18$^{\Delta\Delta}$	6	287–305	2.50	1.14	0.119	0.124	0.041	0.000	ns
psyl44	5	166–178	3.06	1.27	0.209	0.215	0.030	0.000	ns
psyl57	7	184–202	5.38	2.35	0.561	0.575	0.024	0.006	*
psyl36	5	245–257	3.56	1.37	0.275	0.270	–0.016	0.000	ns
PtTX4011	10	229–279	5.19	2.94	0.459	0.660	0.304	0.079	*
Spac11.4	21	122–170	10.94	7.84	0.803	0.872	0.079	0.006	*
PtTX3025	10	206–298	5.94	2.61	0.548	0.617	0.112	0.018	*
PtTX4001	16	199–243	7.69	3.37	0.536	0.703	0.237	0.054	*

Δ – locus monomorphic for populations: PL2, PL3, PL5, PL11, PL12, PL14, F17, F18, Sp21, S23
$\Delta\Delta$ – locus monomorphic for PL11

3 Results

3.1 Genetic variation

The nuclear microsatellites were polymorphic in most populations, with a total of 88 alleles detected (Table 2). The number of alleles per locus ranged from 2 to 21. The mean observed heterozygosity ($H_o = 0.422$) was slightly lower than the mean expected heterozygosity ($H_e = 0.480$). The allele frequency spectra were skewed at some loci, as evidenced by deviations from Hardy-Weinberg equilibrium (Table 2). Some evidence of the presence of null alleles was found at 6 loci, but their frequency was low (0.006–0.079). Deviation from neutrality, which indicates divergent selection, was found only at the nuclear psyl25 locus (Supplementary file: Fig. S1, available at https://doi.org/10.14214/sf.1721). This locus showed the lowest number of alleles and the lowest observed heterozygosity of all the nuclear SSR loci studied (Table 2).

Table 3. Characteristics of the chloroplast microsatellite loci used to study Scots pine from the Polish species distribution. N – number of alleles per locus, R – range of allele size, A – mean number of alleles per locus, A_e – mean effective number of alleles per locus, uh – unbiased allelic diversity.

Locus	N	R	A	A_e	uh
PCP36567	4	108–111	2.25	1.40	0.291
PCP30277	8	130–137	5.69	3.68	0.754
PCP102652$^\Delta$	4	109–115	1.81	1.10	0.081
PCP26106	4	142–145	2.94	1.71	0.422
PCP87314	4	111–114	3.32	1.51	0.336
PCP45071	6	146–152	3.63	1.75	0.427
PCP1289	4	107–110	3.19	1.75	0.430
PCP41131$^{\Delta\Delta}$	5	135–139	2.00	1.23	0.182

Δ – locus monomorphic for populations: PL7, PL8, PL9, PL10, PL12, PL14, PL16, F18, F19, Sp21, Sp22, S23
$\Delta\Delta$ – locus monomorphic for populations: PL10, PL12, PL16, F18

The chloroplast microsatellites were polymorphic at six loci in the analysed populations, with a total of 39 alleles detected (Table 3). The number of alleles ranged from 4 to 8 per locus. The most polymorphic was the PCP30277 locus, which showed the highest effective number of alleles ($A_e = 3.68$) and mean heterozygosity (uh = 0.754) (Table 3).

For 16 Polish provenances the mean number of alleles ranged from 4.40 to 5.50 (Table 4). The effective number of alleles varies from 2.24 to 2.84. Variable levels of genetic diversity were found across Polish populations from different climatic regions, in Central and North Poland (I and II climatic zones) (Table 4). The local population (PL15) from South Poland (III climatic zone) was characterized by the lowest observed heterozygosity. Pines from North Poland had the highest number of private alleles at nuclear loci. The inbreeding coefficient ranged from 0 to 0.180, with a mean of 0.055. Pines from North and South Poland had the highest values of latent genetic potential (Table 4).

Table 4. Genetic diversity of Scots pine populations in Poland based on nuclear microsatellite loci. A – mean number of alleles per locus, A_e – mean effective number of alleles per locus, A_p – number of private alleles, A_R – allelic richness, H_o – mean observed heterozygosity, H_e – mean expected heterozygosity, F – mean fixation index, LGP – latent genetic potential, * statistically significant differentiation of populations ($P \leq 0.05$).

Population	Sample size	nSSR diversity							
		A	A_e	A_P	A_R	H_o	H_e	F	LGP
PL1 Karsko	19	4.80	2.66	0	4.59	0.496	0.506	0.005	21.42
PL8 Gubin	19	5.30	2.84	0	5.16	0.487	0.492	−0.001	24.61
PL9 Rychtal	24	4.50	2.63	0	4.81	0.388	0.491	0.180	18.70
PL10 Bolewice	21	5.00	2.48	0	4.99	0.407	0.436	0.053	25.20
PL13 Spała	20	5.10	2.72	1	4.41	0.457	0.473	0.010	23.82
PL14 Janów Lubelski	23	4.90	2.41	0	4.83	0.376	0.445	0.108	24.91
PL2 Tabórz	23	4.40	2.45	0	4.76	0.390	0.425	0.051	19.53
PL3 Dłużek	21	4.90	2.39	1	4.20	0.456	0.482	0.037	25.12
PL4 Ruciane	22	5.30	2.84	2	4.86	0.452	0.501	0.062	24.59
PL5 Rozpuda	23	5.00	2.74	0	5.13	0.424	0.464	0.077	22.60
PL6 Supraśl	21	5.10	2.47	0	4.82	0.438	0.465	0.010	26.35
PL7 Starzyna	22	5.50	2.70	1	4.85	0.411	0.461	0.075	27.99
PL11 Lipowa	21	5.10	2.27	2	4.74	0.415	0.445	0.037	28.29
PL12 Jegiel	21	5.10	2.55	0	4.91	0.411	0.471	0.070	25.51
PL15 Nowy Targ	23	5.10	2.24	1	4.64	0.361	0.417	0.085	28.58
PL16 Piwniczna	23	4.90	2.35	0	4.69	0.404	0.417	0.024	25.53
Population means (regions)									
I	126	4.9	2.62	1.0	4.80	0.435	0.474	0.059	23.11
II	174	5.1	2.55	6.0	4.78	0.425	0.464	0.052	25.00
III	46	5.0	2.29	1.0	4.67	0.383	0.417	0.054	27.06
Population means (central populations)									
Finland	49	5.2	2.49	0.5	4.74	0.484	0.448	−0.049	28.34
Poland	346	5.0	2.55	0.5	4.77	0.423	0.462	0.055	24.55
Populations means (marginal populations)									
Turkey	50	4.65	2.35	3	4.42	0.461	0.478	0.069	23.40
Spain	60	4.75	2.46	1	4.37	0.445	0.462	0.079	22.88
Scotland	39	5.70	2.66	1	4.82	0.476	0.482	0.000	30.38
Central vs. marginal populations									
Central	395	5.01	2.53	1.3	4.76*	0.435	0.462	0.037	25.03
Marginal	149	5.03	2.49	1.7	4.54*	0.461	0.474	0.049	25.55

In the central pine distribution area, the Polish and Finnish populations showed similar values for effective number of alleles (2.55 and 2.49, respectively), allelic richness (4.77 and 4.74, respectively) and expected heterozygosity (0.462 and 0.448, respectively) (Table 4). The populations from Finland exhibited higher observed heterozygosity than expected relative to the Polish populations and to the other geographical regions represented by marginal populations. The Scottish population showed the highest values for almost all genetic parameters based on genotypic frequency at the nSSR loci. The marginal populations from Spain and Turkey had the lowest allelic diversity, richness and latent genetic potential of the tested regions. Similar levels of genetic variation were found in populations from the continuous distribution (Poland and Finland) and marginal populations (Turkey, Spain, Scotland), except for a significant difference in allelic richness (4.76 and 4.54, respectively, P≤0.05) (Table 4).

Table 5. Genetic diversity of Scots pine populations in Poland based on chloroplast microsatellite loci. A_h – mean number of haplotypes, A_e – mean effective number of haplotypes, A_P – number of private haplotypes, A_R – haplotype richness, H_d – haplotype diversity, D^2sh – mean genetic distance of individuals within populations.

Population	Sample size	cpSSR diversity A_h	A_e	A_P	A_R	H_d	D^2sh
PL1 Karsko	23	20	18.24	7	17.51	0.988	2.518
PL8 Gubin	24	14	10.67	5	11.97	0.946	1.719
PL9 Rychtal	23	21	19.59	3	18.34	0.992	2.015
PL10 Bolewice	24	19	16.94	3	16.20	0.982	1.759
PL13 Spała	22	18	16.13	7	16.36	0.983	2.797
PL14 Janów Lubelski	23	19	16.03	8	16.60	0.980	2.536
PL2 Tabórz	23	20	18.24	9	17.51	0.988	2.875
PL3 Dłużek	21	19	17.64	8	18.00	0.990	2.818
PL4 Ruciane	24	20	18.00	8	16.96	0.986	3.706
PL5 Rozpuda	23	18	14.30	6	15.69	0.972	1.989
PL6 Supraśl	21	21	21.00	11	20.00	1.000	4.089
PL7 Starzyna	21	17	12.60	7	16.00	0.967	1.883
PL11 Lipowa	24	19	16.00	6	16.09	0.978	1.969
PL12 Jegiel	24	19	16.00	2	16.09	0.978	1.879
PL15 Nowy Targ	24	22	20.57	6	18.48	0.993	2.014
PL16 Piwniczna	24	19	14.40	5	15.98	0.971	1.757
Population means (regions)							
I	139	18.9	16.53	5.5	16.67	0.981	2.468
II	181	19.2	16.96	7.1	17.36	0.984	2.894
III	48	17.5	15.13	5.5	15.15	0.969	1.867
Population means (central populations)							
Finland	49	20.0	17.96	7.3	16.36	0.979	2.041
Poland	368	19.1	16.65	6.3	16.74	0.981	2.395
Populations means (marginal populations)							
Turkey	50	18.0	14.66	6.5	14.86	0.970	4.794
Spain	60	21.5	16.80	10.5	15.38	0.972	3.091
Scotland	38	27.0	19.00	11.0	15.74	0.973	2.208
Central vs. marginal populations							
Central	417	19.5	16.74	5.5	16.73	0.981	2.346
Marginal	148	21.2	16.38	9.0	15.24	0.971	3.596

The distribution of genetic diversity at the chloroplast loci between Polish populations was similar to that found for the nuclear markers (Table 5). The mean number of haplotypes varied from 14 to 22. The effective number of haplotypes ranged from 10.67 to 21.00. The highest number of genetic polymorphisms was found in population PL6 from North Poland, and several other populations from that region showed genetic parameter values above the means for the Polish populations. Haplotype diversity was high in all populations, ranging from 0.946 to 1.000.

The haplotypic richness, diversity and mean pairwise distance in the Polish populations were similar to those in Finish pines (Table 5). The populations from the central pine distribution showed a higher mean effective number of haplotypes, haplotype richness and diversity than the marginal populations. The highest mean number of haplotypes and private haplotypes was found in Scottish population.

3.2 Genetic differentiation and population structure

AMOVA analysis showed that less than 3% of the genetic differentiation is found among the Polish populations (Table 6). Significant differentiation was found only in some pairwise comparisons between individual populations on the basis of F_{ST} values for genotype frequency at nuclear loci (Suppl. file: Table S1). However, there is no evidence of genetic divergence in the geographical regions defined (Table 6, Suppl. file: Table S1). Overall low genetic distances between the Polish populations were evident in the PCoA analysis (Fig. 2). Similarly, in the STRUCTURE analysis, pines from Poland were assigned to one genetic cluster (Fig. 2). No evidence of isolation by distance was found among the Polish populations in the Mantel test (Suppl. file: Fig. S2).

There was no significant differentiation in the allelic frequency spectra among the Polish and Finnish populations growing in the central pine distribution area. Significant differentiation was found between the central and marginal populations of Scots pine (Table 6). A variation of

Table 6. Hierarchical analysis of molecular variance (AMOVA) at nuclear loci (nSSR diversity) and at chloroplast loci (cpSSR diversity) based on the allelic distance matrix, df – degrees of freedom, SS – sum of squares, MS – mean squares, p – probability.

| Variance component	df	nSSR diversity					cpSSR diversity	
		SS	MS	Variance	% Total	p	% Total	p
Polish populations								
Among Regs	2	20.185	10.093	0.016	0	0.095	0	0.165
Among Pops	13	107.865	8.297	0.139	3	0.001	0	0.956
Within Pops	330	1750.996	5.306	5.306	97	0.001	100	0.893
Total	345	1879.046						
Population means (central populations)								
Among Regs	3	28.730	9.577	0.013	0	0.120	0	0.507
Among Pops	14	115.596	8.257	0.141	3	0.001	0	0.585
Within Pops	377	1953.509	5.182	5.182	97	0.001	100	0.657
Total	394	2097.835						
Central vs. marginal populations								
Among Regs	1	122.613	122.613	0.503	8	0.001	1	0.003
Among Pops	21	258.106	12.291	0.299	5	0.001	0	0.256
Within Pops	521	2761.401	5.300	5.300	87	0.001	99	0.007
Total	543	3142.119						
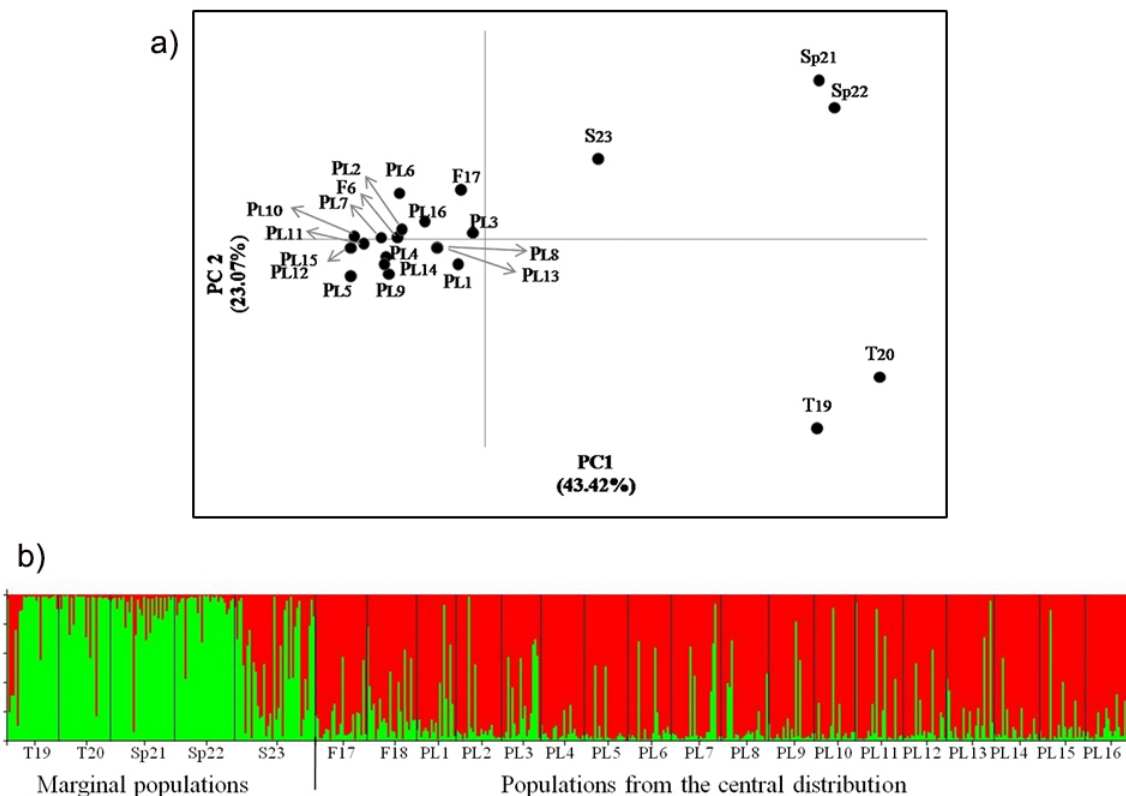
Fig. 2. a) Principal coordinates plot showing the genetic relationship of the Scots pine populations from the wide distribution of this species. The proportion of variance accounted for by the first two axes was 66.49%, **b)** Assignment of investigated populations to the two genetic clusters (K=2) found in the STRUCTURE analysis based on nuclear microsatellite markers. Population acronyms are as in Table 1.

about 8% of among populations occurred within regions. The differentiation among central populations was lower than that between marginal populations (F_{ST}=0.016 and F_{ST}=0.074, respectively, P≤0.05). The Polish and Finnish populations were also grouped in one cluster, while the marginal populations were separated from them in the PCoA analysis (Fig. 2). Genetic divergence between populations was also evident in the Bayesian STRUCTURE analysis, in which one genetic cluster covered the Polish and Finnish populations and the other cluster included Turkish and Spanish pines. The Scottish population showed an intermediate genetic structure between the two other major groups (Fig. 2). A significant positive correlation between genetic and geographic distances was found for all the studied Scots pine populations from the central and marginal distributions in the Mantel test (r = 0.602, P≤0.01) (Suppl. file: Fig. S3).

In contrast to the nuclear markers, the chloroplast microsatellites showed much less evidence of population structure in the Polish pine distribution and reference populations (Table 6). Almost all the differentiation was found within individual populations (Table 6). AMOVA analysis showed significant differentiation only between central pines and marginal populations. Less than 1% of the haplotypic variation was due to differences among these regions. A principal coordinates plot of the cpSSR loci showed no particular grouping of central versus marginal populations except for the outlier populations from Spain (Suppl. file: Fig. S4). No evidence of strong population structure was found in the STRUCTURE analysis (Suppl. file: Fig. S5). However, a significant correlation between the genetic diversity of the chloroplast loci and geographic distance was found for populations of Scots pine from the entire analysed species distribution (r=0.437, P≤0.05) (Suppl. file: Fig S6) and for populations from the Polish pine distribution (r=0.284, P≤0.05) (Suppl. file: Fig. S7).

4   Discussion

Our data show high genetic similarity amongst Polish populations at nuclear and chloroplast microsatellite markers. The genetic diversity of the investigated populations was lower relative to populations of native stands from North-eastern Poland which were determined by nuclear microsatellites with mean heterozygosity equalled 0.778 (Nowakowska 2016). However, in this study only four loci were taken into account so direct comparisons of the results are not fully informative (Kalinowski 2005). An equally high value of the genetic diversity for chloroplast microsatellites markers, paternally inherited in conifers, was observed in other Polish populations with $H_e = 0.98$ (Wójkiewicz et al. 2016b). We found a similar degree of polymorphism and no statistically significant differentiation between the three climatic regions at both markers. Some differences were found in the nuclear loci in pairwise population comparisons, but no clear geographical trend in variation could be observed. No evidence of isolation by distance at the nuclear microsatellite loci was found among the Polish pine populations in the Mantel test. A positive relationship between pairwise Nei's genetic and geographic distance was found for the chloroplast loci. However, this variation disappeared when local populations from the Carpathian Mountains were excluded from the analysis. Interestingly, the lowest genetic variation and highest inbreeding rate at the nuclear loci was found in the local population PL15 from the Carpathian Mountains, which also showed the worst growth rate and weak productivity in quantitative trait studies of the provenance trial experiments (Barzdajn 2006; Hebda et al. 2017). In this case, the poor growth and development of local pines correlate with the low levels of genetic diversity. It is a known fact that a small number of pine trees produce seeds in that area (Skrzyszewski 2004). The topographical features of the landscape, including dispersed Scots pine habitat, has likely limited gene flow and narrowed the genetic variation in the populations (Oleksyn et al. 1994). The low level of seed production by the pines growing in Carpathians likely decreased the genetic diversity of the progeny used to set up the provenance experiment plots, as suggested by our study.

The genetic structure of a population at neutral markers is influenced by population history and several processes, including mutation, migration and random genetic drift. The distribution of genetic variation is strictly related to gene flow through pollen and seeds. Free gene flow in large geographical areas may have a homogenizing effect on an allele's frequency spectra and the gene pool of individuals (Burczyk et al. 2004; Robledo-Arnuncio et al. 2004). It is seen in the genetic diversity of *P. sylvestris* at nuclear and chloroplast markers, which is widely distributed across the range. The high genetic diversity has been reported in isolated populations from Italy ($H_e = 0.847$ and 0.81; Belletti et al. 2012; Scalfi et al. 2009, respectively) and in populations from the broad pine distribution from Romania and Sweden (H_e ranged from 0.50 to 0.80; Floran et al. 2010), determined by the nuclear microsatellites. On the other hand low values of heterozygosity ($H_e = 0.338$, $H_e = 0.376$, $H_e = 0.325$, respectively) for some Scots pine populations from continuous species distribution, from Finland and two populations from Russia, have been showed (Sebastiani et al. 2012). Additionally, the high level of the genetic diversity for chloroplast microsatellites loci was observed in populations of Scots pine from Scotland, Spain, Western and Central Europe (H ranged from 0.950 to 0.987, Provan et al. 1998), in Italian (H = 0.92, Scalfi et al. 2009) and Spanish *P. sylvestris* (H = 0.978, Robledo-Arnucio et al. 2004). Although the recolonization history of Scots pine in Europe is still not fully resolved (Pyhäjärvi et al. 2008; Naydenov et al. 2007), the low divergence amongst Polish populations may indicate their common phylogeographic history. Alternatively, it may have resulted from range shifts and the effective admixture of populations of different origin that colonized Central Europe after last glacial period. Therefore, the development of high-resolution mitochondrial markers transmitted in pines across short geographical distances by seeds is essential for fine scale population structure and admixture analysis (Donnelly et al. 2017).

Compared to the reference distribution of Scots pine in Europe, Polish populations showed a very similar level of genetic variation to Finnish populations. Both geographical regions had similar allelic frequency spectra distributions, and no significant correlation between genetic differentiation and geographical distance between populations was found for the nuclear and chloroplast loci in the Mantel test. Obtained results are in accordance with other studies on *P. sylvestris* in the continuous species distribution in Europe using isoenzymes and DNA markers (Dvornyk et al. 2002; Garcia-Gil et al. 2003; Goncharenko et al. 1994; Pyhäjärvi et al. 2007; Wachowiak et al. 2014; Wang et al. 1991). Considering the large geographical areas covered by investigated populations, it seems that their genetic similarity must result from shared phylogeographic history. In contrast, those pine populations from Central and Northern Europe differed from marginal pines in characteristics such as allelic richness, the number of private haplotypes and the mean genetic distance. Allelic richness is a measure of genetic diversity that indicates the population's potential for adaptability and persistence (Greenbaum et al. 2014). The significant lower level of allelic richness in the marginal populations might reflect differences in the effective population size, the influence of genetic drift and population history related to population size fluctuations.

In our study, the analysis of population structure at nuclear markers showed that the marginal populations are genetically divergent and separated from the central populations. Our data support a distinct character of isolated populations which was shown using mitochondrial DNA and morphological markers (Naydenov et al. 2007; Pyhäjärvi et al. 2007; Sinclair et al. 1999; Soranzo et al. 2000). Differentiation between those geographical regions indicates limited gene flow and possibly different evolutionary history. Previous genetic studies showed that the populations from Turkey, the Iberian Peninsula and Scotland were characterized by a higher level of heterozygosity or some unique molecular markers relative to the populations in central and northern Europe (Prus-Głowacki et al. 2012; Floran et al. 2010; Pyhäjärvi et al. 2007; Wachowiak et al. 2011). The intermediate genetic structure of the Scottish pine population relative to the central and marginal locations may result from the admixture of populations with different postglacial histories (Wachowiak et al. 2011). The genetic variability of populations across the wide species distribution range was supported by the significant isolation by distance observed in our study.

Molecular markers might be used to assess the role of selection on the distribution of adaptive genetic variability. Selection at a specific locus may be reflected by deviation from Hardy-Weinberg equilibrium. One nuclear locus in our study (psyl25) exhibited patterns of genetic variation that deviated from neutral expectations. This locus showed the lowest number of alleles (just 2, 216 and 213 bp) of all the analysed loci. The allele distribution showed some geographical trend, with a rare allele (213) being present more frequently in populations from Central and Southern Poland. This locus was monomorphic in most populations from North Poland. If selection was involved, that would mean that this nuclear microsatellite could lie in a very close proximity to genomic regions under selection, as the microsatellite markers themselves are generally assumed to be selectively neutral and a level of recombination in pines (at least for coding regions) seems high (Wachowiak et al. 2009). Deviation from the expected allele frequency spectra might also be caused by the presence of null alleles at the loci. Null alleles might occur due to mutations in the primer binding sites and lead to the over estimation of homozygosity due to deficits in specific alleles at a single locus (Callen et al. 1993). However, our study showed an overall low frequency of null alleles that did not significantly skew the allelic frequency spectra across populations.

The microsatellites tested in our study provide information on genetic diversity, population structure and demographics, which are essential for distinguishing the confounding effects of demographic processes and shared population history from that of selection (Chhatre and Rajora 2014). The provenance trial was previously subjected to quantitative genetic studies (Hebda et al. 2017). Those studies showed that the pines from the Polish distribution are significantly differen-

tiated in growth or productivity parameters, as measured during 50 years of performance in the experimental plot in the Carpathian Mountains (Hebda et al. 2017). The examined populations from the provenance trial show a low level of genetic polymorphism at neutral loci and high heritable phenotypic diversity. Therefore, some of the excellent and poorly growing populations from the provenance trial experiment could be especially useful to test for selection patterns at the genomic regions that are potentially involved in phenotypic variation at quantitative traits.

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Total of 66 references.

Supplementary files

Suppl.pdf, available at https://doi.org/10.14214/sf.1721