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Genetic Diversity and Differentiation of Siberian Stone Pine Populations at the Southern Range Margin in the West Siberia Lowland Area

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Abstract—Based on the analysis of the variability of 25 allozyme loci, genetic variation within and differentiation between populations of Siberian Stone Pine growing at the *trailing*-edge range margin in the southern taiga up to the forest-steppe transition zone in Western Siberia have been estimated. At the current stage, climate change and anthropogenic impact have not caused a substantial reduction in genetic diversity within and increase in differentiation between isolated small stands at the southern edge of their distribution in the lowland areas of the Siberian Stone Pine range.

Keywods: Siberian stone pine, West Siberia, trailing-edge range margin, allozyme variability, genetic differentiation

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INTRODUCTION

Most forest woody plants are characterized by high intraspecific genetic variability, over 90% of which are concentrated within populations, while the level of their genetic differentiation depends upon many factors, including the location of a population relative to the border of its range (Hamrick et al., 1992; Hamrick and Godt, 1996). According to the center-periphery theory, marginal populations are more exposed to extinction (degradation) and more genetically depauperized than populations from the central part of a range, since they grow in less favorable environments and have a low effective size (Aitken and Libby, 1994; Ledig, 2000). This theory is corroborated by case studies on conifers, particularly *Pinus rigida* Mill (Gurles and Ledig, 1982), *Pinus contorta* Douglas ex Loudon (Aitken and Libby, 1994), and *Pseudotsuga menziesii* (Mirb.) Franco (Li and Adams, 1989). In some cases, however, peripheral populations can maintain a degree of variability typical for a population from the central part of a range, as demonstrated for *Picea abies* (L.) Karst (Muona et al., 1990), *Alnus rubra* Bong (Hamann et al., 1998), *Pinus strobus* L. (Beaulieu and Simon, 1994), and *Picea mariana* Mill. (Gamache et al., 2003). This paradox may be explained by the sufficient inflow of genetic material into isolates, their relatively young age, the peculiarities of the reproductive system of species, etc. (Kramer et al., 2008), as well as by the different origins of marginal stands. At present, a relatively recent developing scientific trend is phylogeography, focusing on the postulated prevalence of the impact of climate change on the formation of the structure of genetic diversity over stochastic demographic processes. The results of phylogeographic inferences indicate that the contemporary spatial structure of gene pools of most species in North America and Eurasia has been formed by the dynamics of habitats due to climate oscillations in the Quaternary (Hewitt, 2000, 2004). It is assumed that boreal species of a leading edge population located at the front edge for colonization of new areas suitable for vegetation and reproduction may be more adaptive compared to the species of populations located at the opposite trailing edge of a habitat (Hampe and Petit, 2005). The latter are in less suitable environmental conditions for growth and renewal; in addition, the genetic material inflow from the central populations is extremely limited (Davis and Show, 2001). The Quaternary climate oscillations occurred in Western Siberia, producing an interchange of colder to warmer periods, followed by a marked shift of botanical geographical zones, the boundaries of plant habitats, and the permafrost boundary (Kats, 1969). At the current stage, global climate warming may also lead to changes in vegetation cover, particularly in Siberia, as well as the redistribution of vegetation zones and populations of forest-forming woody species (Chebakova et al., 2003). A change in the margins of a forest-forming

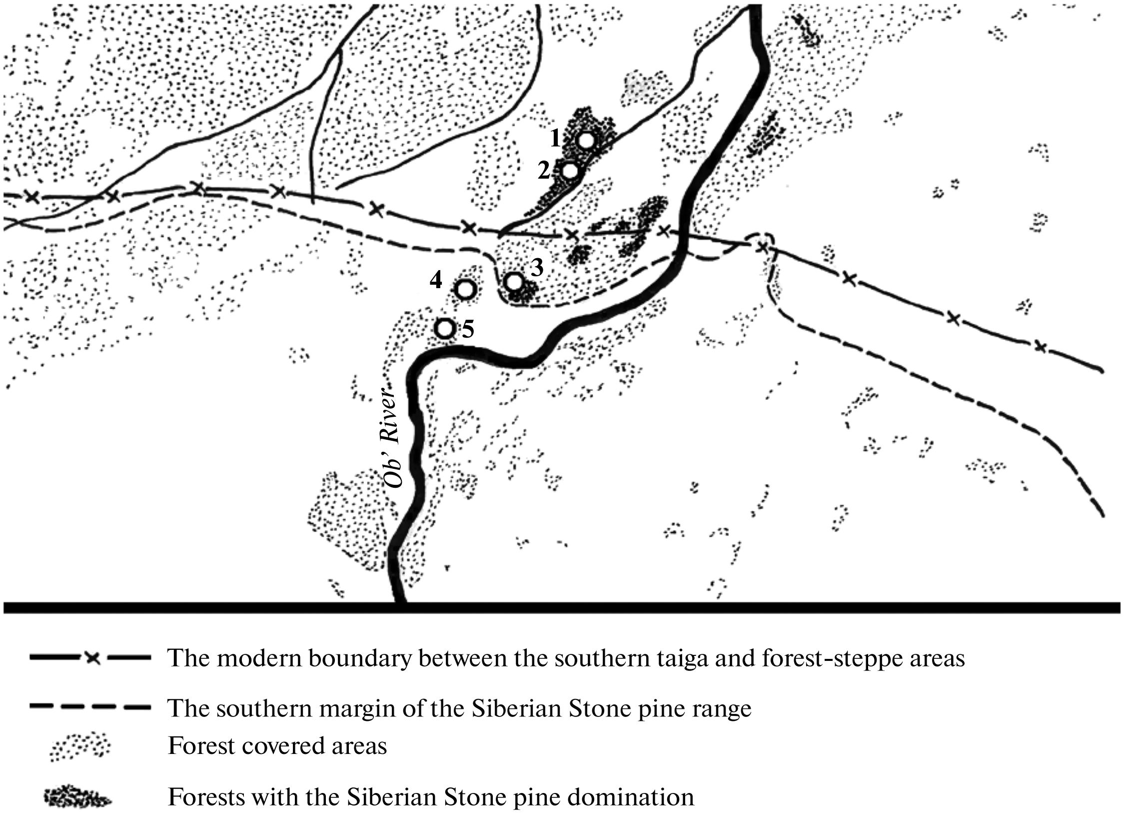

Fig. 1. A scheme of the sample plots location and the southern limit of the Siberian Stone Pine range relative to the boundary between the southern taiga and forest-steppe subzones.
1—Bazoi, 2— Kinda, 3—Krasnyi Yar, 4—Vyuny, 5—Yurt-Ora.

species range in the Western Siberia Plain in Holocene was determined by the data on the spore and pollen analysis of wetland and lake deposits (Blyacharchuk, 2010). One of the edificators of the contemporary vegetation cover in Western Siberia, which underwent changes after the last glaciation, is the Siberian Stone Pine (*Pinus sibirica* Du Tour), a pine species of the Quinquefolia section of the Strobus subgenus (Gernandt et al., 2005). At present, the Siberian Stone pine have a vast geographic distribution from the Polar circle in the north to the mountain systems in Southern Siberia and from the eastern permafrost boundary to the northeastern European part of Russia, where the range of the Siberian Stone pine is limited by the competition with the European spruce and the anthropogenic factor (Semechkin et al., 1985). In the Western Siberian Plain, Siberian Stone pine forms high productive stands. Here, the southern margin of its range generally coincides with the southern boundary of the taiga zone. The southern edge region of the range is fragmented and represents small population *islands* among the pine and birch-aspen forests (Bekh, 1974). In terms of phylogeography, the transition zone between the southern taiga and forest-steppe areas represents a *trailing* range margin (Hampe and Petit, 2005) shifting northwards at present (Chebakova et al., 2003). Previously, the studies of stem radial growth (Bekh, 1972), seed productivity (Nekrasova and Mishukov, 1974), and the generative sphere and structure of the Siberian Stone pine yields (Velisevich and Petrova, 2009; Velisevich et al., 2013) were carried out in this transition zone. The objectives of this study are both the analysis of genetic variation and differentiation of the Siberian Stone pine populations at the southern margin of its distribution in lowlands of Western Siberia, and the analysis of the causes and the genetic consequences of the species range fragmentation.

MATERIALS AND METHODS

The region of surveying is referred to as the southern Ob' river area, where the boundary runs between the southern taiga and forest-steppe subzones and generally coincides with the Siberian Stone pine range margin. Here, five sample plots were established along to a vector perpendicular to this boundary (Fig. 1). The data on morphological descriptions of soils and laboratory analyses of soil samples, using standard methods (Arinushkina, 1970; Vadyunina and Korchagina, 1986) could show that the sample plots differed from each other in soil moisture reserves in the upper 20 cm layer.

1. *Bazoi.* The most southern large Siberian pine woodland in the south of the southern taiga subzone is a Bazoi landform, Kozhevnikovo forestry, Tomsk oblast', woodland block 119, subsections 6 and 17. The forest type is a Siberian Stone pine forest with sedges and mixed herbs (8 units of *Pinus sibirica* 2 units of *Picea obovata*); bonitet, estimated productivity is of class 2. Forest soils are sod-podzolic; the soil moisture reserve is 43 cm (satisfactory); the reserve of humus in the soil is in the order of 44 tons per hectare (average). *Carex macroura* and mixed herbs dominate in the surface cover (total project cover is 60%). The average height of a Siberian Stone pine trees is 26 m; the average stem diameter is 65 cm, the age is 160–210 years (the average age is 189 years). The area of this stand is about 25 square km.

2. *Kinda* is a plot in the Bazoi landform, Kozhevnikovo forestry, Tomsk oblast', a valley of the Kinda river, woodland block 126, subsections 13 and 16. The sample plot is located 2.5 km southwest of *Bazoi*; it is the edge of the southern area of the south taiga subzone. This is a typical dwarf shrub-sedge-moss sogra, a lowland Siberian Stone pine forest swamp (6 units of *P. sibirica*, 2 units of *Picea obovata* and 2 units of *Betula* spp.); bonitet, estimated productivity is of class 4. Forest soils are humic-gley; the soil moisture reserve is 89 mm (good); the reserve of humus in the soil is in the order of 17 tons per hectare (high). Tufted sedge, *Carex caspitosa* dominates in the grass cover (55%); hydrophilous species of green mosses dominate in the moss cover. The average height of a Siberian Stone pine trees is 23 m; the average stem diameter is 41 cm, and the age is 160–330 years (the average age is 225 years). The Siberian Stone pine stand area is about 1.5 square km.

3. *Krasnyi Yar* is the Orsk-Simanskii landform, Kolyvan' forestry, Novosibirsk oblast', woodland block 36, subsections 15. This small island stand is located 15 km southwest of *Kinda*, at the northern edge of the forest-steppe zone. It is a dwarf-shrub-sledge pine forest with 9 units of Scots pine and 1 unit of Siberian Stone pine and birch); bonitet, estimated productivity (of Siberian Stone pine) is of class 3. Forest soils are iluvial-ferroferous podzolic; the soil moisture reserve is 20 mm (unsatisfactory); the reserve of humus in the soil is in the order of 32 tons per hectare (average). The grass layer is sparse (total project cover is 60%); with domination of shrubs and *Carex macroura*. The height of a Siberian Stone pine trees is 22 m; the stem diameter is 45 cm, and the age is 120–140 years (the average age is 136 years). The Siberian Stone pine stand subsection is situated among pure Scots pine forest; its area is about 0.21 square km.

4. *Vyuny* is an isolated stand in the northern area of the forest-steppe zone, located in the Vyuny landform, Kolyvan' forestry, Novosibirsk oblast', woodland blocks 12, 16, and 17. The forest type is a fern-mixed herb pine forest (10 units of *P. sylvestris* with some *Betula* spp. and *Pinus sibirica*); bonitet, estimated productivity (of Siberian Stone pine) is of class 3. Forest soils are illuvial-ferriferous podzolic; the soil moisture reserve is 16 mm (unsatisfactory); the reserve of humus in the soil is in the order of 44 tons per hectare (high). Grass cover is very dense (90%); bracken, glague and smooth-cone small reed (*Calamagrostis obtusata*) dominate in it. The average height of a Siberian Stone pine tree is 21 m; the average stem diameter is 44 cm, and the age is 120–140 years (the average age is 131 years). Siberian Stone pine trees are scattered in the pine forest as three small stand groups at a significant distance from each other (1–1.5 km). The distance to the *Krasnyi Yar* population is 8.5 km. The total area of the subsection where these groups are located is about 0.8 square km.

5. *Yurt-Ora* is the most southern Siberian Stone pine isolate at a maximum distance from the boundary between taiga and forest-steppe areas; it is located in the Orskii Borok landform, Kolyvan' forestry, Novosibirsk oblast', woodland block 40. The forest type is a dwarf-shrub-green moss pine forest (10 units of *Pinus sylvestris* with some *P. sibirica*); bonitet, estimated productivity (of Siberian Stone pine) is of class 4. Forest soils are illuvial-ferriferous podzolic; the soil moisture reserve is 11 mm (unsatisfactory); the reserve of humus in the soil is in the order of 27 tons per hectare (average). The grass layer is very sparse (5–7%); dwarf shrubs such as bilberry and cowberry dominate in the surface cover. The moss cover is composed of forest green mosses. The average height of a Siberian Stone pine tree is 18 m; the average stem diameter is 38 cm, and the age is 110–320 years (the average age is 154 years). A few trees grow separately a kilometer away among the Scots pine stand. The distances to the *Krasnyi Yar* and *Vyuny* isolates are 14 km and 7 km, respectively. The area of the subsection composing Siberian Stone pine is about 0.11 square km.

The material (shoots) for genetic analysis was sampled from 25 trees in each of the *Bazoi* and *Kinda* stands; in the other three isolates, the material was sampled from each Siberian Stone pine tree that vegetated in the given area.

Diploid tissues of vegetative buds were used for the allozyme analysis. The tissues were homogenized in an extraction buffer (0.05 M Tris-HCl, pH 7.7, with addintion of soluble polyvinilpyrrolidone (3%), β-mercaptoethanol (0.05%), and insoluble polyvinilpyrrolidone with an equal volume of each sample). Electrophoresis was performed in 13% starch gel. Electrophoretic resolution of each sample was performed in two buffer systems, as follows: A—morpholine-citrate buffer at pH 7.8 (Clayton and Tretiak, 1972); and B—Tris-EDTA-borate buffer system at pH 8.6 (Markert and Faulhaber, 1965) (Table 1). Multiple zones of activity and corresponding gene loci were marked by the abbreviated names of enzymes and numbered according to their electrophoretic activity in decreasing order, from anode to cathode. Genetic control of the isoenzyme systems did not generally differ from the previously described one for the Siberian Stone Pine

Table 1. Studied enzyme systems, number of analyzed loci, and buffer systems used for electrophoresis

Enzyme	Abbrevation	Number by Enzyme Classification	Number of analyzed loci	Buffer system[1]
Alcohol dehydrogenase	ADH	1.1.1.1	1	B
Formate dehydrogenase	FDH	1.2.1.2	1	A
Fluorescent esterase	FEST	3.1.1.2	1	B
Glutamate dehydrogenase	GDH	1.4.1.2	1	B
Glutamate-oxaloacetate transaminase	GOT	2.6.1.1	3	B
Isocitrate dehydrogenase	IDH	1.1.1.42	1	A
Leucine aminopeptidase	LAP	3.4.11.1	2	B
Malate dehydrogenase	MDH	1.1.1.37	2	A
Menadione reductase	MNR	1.6.99.2	1	A
6-phosphogluconate dehydrogenase	6-PGD	1.1.1.44	3	A
Phosphoglucose isomeras	PGI	5.3.1.9	2	B
Phosphoglucomutase	PGM	2.7.5.1	2	B
Phosphoenolpyruvate carboxylas	PEPCA	4.1.1.31	1	A
Shikimate dehydrogenase	SKDH	1.1.1.25	2	A
Superoxide dismutase	SOD	1.15.1.1	2	B

[1]—See text for buffer system symbols.

and closely related pine species of the subgenus *Strobus* (Krutovskii et al., 1987; Politov, 1989; Politov et al., 1999; Belokon' et al., 2005). Histochemical staining procedured were performed according to the standard methods (Manchenko, 1994) with insignificant modifications.

Based on the obtained genotypes, the allele frequencies of the analyzed isoenzyme loci have been calculated, a comparison of the observed distribution of genotypes with the expected distribution under the Hardy-Weinberg equilibrium using a χ^2 test was carried out, and the heterogeneity of the allele frequencies among samples was estimated. The basic parameters of genetic variability have also been calculated; they are as follow: the percentages of the polymorphic loci (P) by the 99% criterion, the mean number of alleles per locus (A) and the mean number of alleles of more than 5% frequency per locus ($A > 5\%$), the mean effective number of alleles per locus (A_E), the expected heterozygosity (H_E), the observed heterozygosity (H_O), and the Shannon information index (I). Population subdivision and differentiation were assessed using F-statistics (Wright, 1972) and Nei's standard genetic distances D_N (Nei, 1972). The genetic data have been processed using the PopGene v32 (Yeh and Boyle, 1997) and GenAlEx v6.5 programs (Peakall and Smouse, 2006, 2012).

RESULTS

Genotypes of 104 Siberian Stone pine trees were identified at 25 loci encoding 15 enzymes. Loci *Gdh*, *Got-1*, *Got-3*, *Mdh-1*, *6-Pgd-2*, *6-Pgd-3*, *Pgi-1*, *Pgm-2*, *Idh*, *Lap-2*, *Mnr-1*, and *Sod-2* appeared monomorphic in all of the analyzed samples. Genetic variability at 13 loci was indicated (Table 2).

Genetic Structure

In all of the polymorphic loci, the most frequent alleles appeared to be common for the analyzed stands. At the same time, some characteristics of the analyzed stand's allelic composition of certain loci should be noted. Thus, the allele $Adh\text{-}1^{84}$ rare in Siberian Stone Pine can only be found in the *Bazoi* sample. In the *Kinda* stand, located in the river valley of the same name, the frequency of the rare Fdh^{20} allele is more than double that of other samples, although it remains at a level of less than 10%. Two trees heterozygous at the *Got-2* locus are found in the *Krasnyi Yar* isolated sample; in other samples, no polymorphism at this locus is revealed. Three alleles at the *Mdh-2* locus have been detected only in the *Kinda* sample; two of these alleles are detected in each of the *Vyuny* and *Yurt-Ora* isolates; in the other samples, this locus was monomorphic. The rare $Skdh\text{-}2^{110}$ allele occurred in the samples of the southern taiga subzone (*Bazoi* and *Kinda*) and was absent in all of the three southern isolates. The polymorphism at the *Pepca* locus was detected in the *Kinda* population confirming once again its most genetically diverse status. The χ^2-test for heterogeneity of the allele frequencies did not reveal any significant differences among the stands. The distribution of the genotypes in the analyzed stands generally corresponds to Hardy–Weinberg equilibrium; some cases of significant deviations ($p \le 0.05$) can be

Table 2. Frequencies of alleles at polymorphic loci in Siberian Stone Pine stands at the Southern Range Edge in West Siberia Lowland Area

Locus	Allele	Bazoi	Kinda	Krasnyi Yar	Vyuny	Yurt-Ora
Adh-1	100	0.720	0.700	0.796	0.786	0.731
	93	0.260	0.300	0.204	0.214	0.269
	84	0.020	0.000	0.000	0.000	0.000
Fdh	100	0.960*	0.920	0.981	0.964	0.962
	20	0.040	0.080	0.019	0.036	0.038
Fest-2	127	0.440	0.440	0.444	0.429	0.346
	100	0.560	0.560	0.556	0.571	0.654
Got-2	100	1.000	1.000	0.963	1.000	1.000
	75	0.000	0.000	0.037	0.000	0.000
Lap-3	100	0.880	0.900	0.907*	0.857	0.962
	87	0.120	0.100	0.093	0.143	0.038
Mdh-2	100	1.000	0.920	1.000	0.964	0.962
	95	0.000	0.020	0.000	0.000	0.038
	88	0.000	0.060	0.000	0.036	0.000
Mdh-3	106	0.240	0.160	0.204	0.357	0.385
	100	0.760*	0.840*	0.796*	0.643	0.615
Pepca	100	1.000	0.980	1.000	1.000	1.000
	75	0.000	0.020	0.000	0.000	0.000
Pgi-2	115	0.140	0.100	0.037	0.115	0.192
	100	0.860	0.900	0.963	0.885	0.808
Pgm-1	100	0.760	0.860	0.870*	0.750	0.923
	96	0.240	0.140	0.130	0.250	0.077
Skdh-1	114	0.040	0.040	0.000	0.000	0.038
	104	0.360	0.300	0.333	0.308	0.231
	100	0.440	0.560	0.556*	0.577	0.538*
	95	0.160	0.100	0.111	0.115	0.192
Skdh-2	110	0.080	0.060	0.000	0.000	0.000
	100	0.920	0.940*	1.000	1.000	1.000
Sod-3	100	0.920	0.940	0.981	0.893	0.962
	60	0.080	0.060	0.019	0.107	0.038

* Frequent alleles at loci, where deviations in the genotype composition from the Hardy—Weinberg equilibrium have been observed.

related to deficiencies in the heterozygous genotypes. This tendency is most pronounced in the *Krasnyi Yar* stand, where deviations from the Hardy-Weinberg equilibrium were revealed in four loci. In each of the samples from the southern taiga subzones, deviations were revealed in two loci; in the *Yurt-Ora* stand, a deviation was revealed only in the *Skdh-1* locus; whereas in the *Vyuny* stand, the numbers of genotypes at all loci corresponded to the Hardy-Weinberg equilibrium (Table 2).

Genetic Diversity

According to our data, the percentage of polymorphic loci in the *Kinda* sample was higher than that in any other compared sample (Table 3). The mean number of alleles per locus and the mean number of alleles having more than 5% frequency per locus in the samples from the taiga southern subzone (*Bazoi* and *Kinda*) appeared higher than the analogous values obtained for the forest-steppe isolates. Trees in the

Table 3. Genetic diversity of Siberian Stone Pine Populations at the Southern Range Edge in the West Siberia Lowland Area

Population	N	P, %	A	$A > 5\%$	A_E	H_O	H_E	I
Bazoi	25	40	1.520 (±0.154)	1.400 (±0.115)	1.228 (±0.086)	0.107 (±0.035)	0.124 (±0.038)	0.204 (±0.062)
Kinda	25	48	1.600 (±0.153)	1.480 (±0.117)	1.195 (±0.070)	0.102 (±0.030)	0.117 (±0.034)	0.200 (±0.055)
Krasnyi Yar	27	40	1.440 (±0.117)	1.280 (±0.108)	1.159 (±0.066)	0.062 (±0.022)	0.093 (±0.033)	0.153 (±0.051)
Vyuny	14	40	1.440 (±0.117)	1.360 (±0.114)	1.203 (±0.070)	0.117 (±0.038)	0.120 (±0.037)	0.190 (±0.056)
Yurt-Ora	13	40	1.480 (±0.143)	1.280 (±0.108)	1.197 (±0.080)	0.102 (±0.035)	0.108 (±0.037)	0.177 (±0.059)
Mean		41.6 (±1.6)	1.496 (±0.029)	1.360 (±0.038)	1.196 (±0.011)	0.099 (±0.010)	0.112 (±0.006)	0.185 (±0.009)

N—Sample size, P—percentage of polymorphic loci by the 99% criterion, A—mean number of alleles per locus, $A > 5\%$—mean number of alleles of more than 5% frequency per locus, A_E—mean effective number of alleles per locus, H_E—average expected heterozygosity, H_O—average observed heterozygosity, I—shannon information index, standard deviations of the averages are in brackets.

Vyuny isolate proved to be the most heterozygous ($H_O = 0.117$). The expected heterozygosity and Shannon information index were higher in the southern taiga samples than in the compared southern isolates; however, the differences in the mean values for the parameters of genetic diversity were statistically insignificant.

Genetic Subdivision and Differentiation

Values for inbreeding coefficient of individual relative to population F_{IS}, individual relative to species F_{IT}, and population relative to species F_{ST}, which have been calculated for the polymorphic loci of the Siberian Stone Pine and as the average value for the species in total, are presented in Table 4.

Table 4. Measures of genetic subdivisions (F-statistics) of Siberian Stone Pine stands at the Southern Range Edge

Locus	F_{IS}	F_{IT}	F_{ST}
Adh-1	–0.019	–0.011	0.007
Fdh	0.143	0.152	0.010
Fest-2	–0.071	–0.065	0.006
Got-2	–0.038	–0.007	0.030
Lap-3	0.304	0.314	0.014
Mdh-2	–0.054	–0.024	0.029
Mdh-3	0.377	0.401	0.039
Pepca	–0.020	–0.004	0.016
Pgi-2	–0.009	0.017	0.025
Pgm-1	0.212	0.238	0.032
Skdh-1	0.229	0.237	0.010
Skdh-2	0.231	0.265	0.045
Sod-3	–0.083	–0.065	0.017
Mean	0.092 ± 0.032	0.111 ± 0.032	0.021 ± 0.003

Value of F_{ST} coefficient, reflecting the degree of population subdivision, indicates that approximately 98% of the revealed genetic variability in Siberian Stone Pine can be found within populations, while only 2% ($F_{ST} = 0.021$) is conditioned by differences among samples. The obtained mean value F_{ST} indicates a low genetic subdivision in the analyzed samples. The contributions to the interpopulation component of variability from the *Skdh-2* (F_{ST} 0.045), *Mdh-3* (0.039), and *Pgm-1* (0.032) loci are the greatest, whereas the contributions from the *Fest-2* (0.006) and *Adh-1* (0.007) loci are the least important.

According to our data, the genetic differentiation of the analyzed Siberian Stone pine stands was extremely low; the genetic distance D_N varied from 0.001 to 0.004. The UPGMA-dendrogram of the studied samples plotted based on cluster analysis using D_N values can offer an opportunity to assess the genetic similarity between patricular Siberian Stone pine stands (Fig. 2).

The genetic differentiation between the samples *Kinda* and *Krasnyi Yar* ($D_N = 0.001$), as well as with respect to the *Bazoi* and *Vyuny* samples ($D_N = 0.001$) was the lowest. These pairs of samples form certain clusters in the diagram. The southernmost Siberian Stone pine outpost, *Yurt-Ora*, is the most differentiated from the other samples and allocated in a separate branch of the dendrogram.

DISCUSSION

The analysis of the distribution of genetic variability among the studied Siberian Stone pine stands indicated that the stands in the southern taiga subzone (*Bazoi* and *Kinda*) are characterized by both the pre-

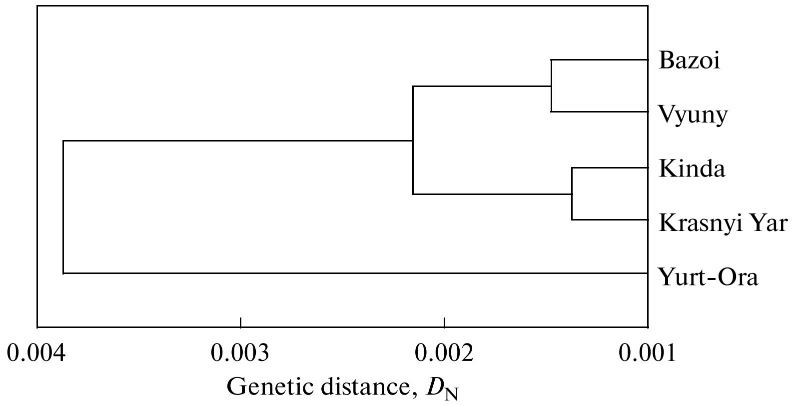

Fig. 2. Clustering of the Siberian Stone Pine stands based on genetic distances D_N.

sense of rare alleles at some loci and higher values for the most number of parameters of genetic variability compared to the southern isolates. The diversity of Siberian Stone Pine tree genotypes in the *Kinda* stand should be especially noted; it is characterized by a high percentage of polymorphic loci, the maximum number of alleles at the *Mdh-3* locus, and the occurrence of the *Skdh-2*$_{110}$ allele, which is rare for the species. An interesting feature of the genotypic composition in the *Kinda* stand located in the river valley of the same name can be the doubling in the frequency of a rare allele at the *Fdh*^{20} locus compared to its frequency in the other samples. A similar increase in the frequency of the given allele in the Siberian Stone pine populations vegetating in the Western Siberia river valleys compared to neighboring stands located on the river terraces (Velisevich and Petrova, 2009) has been previously mentioned. Specifically the *Kinda* from the studied stands can represent an all-aged natural Siberian Stone pine forest distinct from the others without passing through the stage of reforestation following a fire. It can be assumed that natural reforestation in various periods of the stand's formation was carried out with seeds from various Siberian Stone pine forests, distant from each other, which resulted in an increase in the allelic and genotypic diversity of the *Kinda* stand.

The expected heterozygosity and the Shannon information index are characterized by higher mean values for the samples from the southern taiga subzone when compared with the forest-steppe isolates, since these differences are statistically insignificant.

The sufficiently high values for H_O, H_E, and I in the *Vyuny* isolate can be explained by the fact that it consists of three spatially separated groups, which, (1) appears to be the descendants of trees from different isolates, and (2) grows under different microecological conditions, which cannot exclude the possibility of action of multidirectional natural selection in these groups. The *Krasnyi Yar* stand, which demonstrates the traits of a classical *isolate*, can be distinguished among all of the studied stands by the minimal values for parameters of genetic variability. Within this location, trees of the same age class vegetate in small areas with a high density; in addition, they may be the descendants of unsaved trees previously isolated from the main habitat and grown among a small number of Siberian Stone pine trees. The observed effects under the conditions of both some degree of relationship between hypothetic parent trees and an increase in the rate of self-pollination in their breeding patterns can be expected. Therefore, different genetic effects of fragmentation of Siberian Stone pine stands at the margin of the *trailing* edge range, which are related with the history of formation and specificity of ecological conditions suitable for germination and the growth of isolates, are detected.

High intrapopulation and low interpopulation allozyme diversity is typical for woody, particularly coniferous plants, and represents the consequences of highly effective size of their populations, anemochory, and predominant cross-pollination (Hamrick et al., 1992). Approximately 2% of the total genetic variability of the continental populations and isolates at the southern Siberian Stone pine range margin is conditioned by differences between these populations. The previously derived subdivision estimates by different sets of loci for various Siberian Stone pine populations can vary from 1.6% to 8% (Krutovskii et al., 1989; Politov et al., 1992; Krutovskii et al., 1995; Goncharenko and Silin, 1997; Politov, 2007; Petrova et al., 2014). The proportion of genetic variation among samples along the wetland – forest transect of the Siberian Stone pine (total length is approximately 2 km) in the Western Siberia southern taiga subzone comprised 2.1% (Petrova et al., 2008); i.e., it coincides with the result obtained in the given research study. Consequently, the genetic subdivision of the Siberian Stone pine stands at the boundary between the southern taiga subzone and the forest-steppe area is low; in addition, it can be comparable with a subdivision in populations of trees growing under different microecological conditions in the southern subzone of the taiga.

The genetic differentiation of the Siberian Stone Pine stands located in the transition zone between the southern taiga and forest-steppe areas in Western Siberia is insignificant ($D_N \leq 0.004$). According to the classification proposed by K.V. Krutovskii with coauthors, sample pairs, for which the genetic distance can be expressed as $D_N < 0.007$, representing samples from the same population (Krutovskii et al., 1989). A clustering of pairs, such as: *Kinda* – *Krasnyi Yar* and *Bazoi* – *Vyuny* ($D_N = 0.001$) at the dendrogram may be explained by the specificity of a zoochoric transfer of seeds (when analyzing the objects, the possibility of the origin of Siberian Stone pine trees in the *Krasnyi Yar* stand from the seeds carried there from the *Kinda* stand was noted), as well as by the differences in the properties of the surface soil cover. The *Bazoi* and *Vyuny* stands can be referred to the mixed-herb group of forest types characterized by matted turf and a thick grass layer. The *Kinda* and *Krasnyi Yar* stands are characterized by a well-developed moss cover and an insignificant grass layer. In the mossy forest types, competition between Siberian Stone Pine seedlings and species of vegetation cover is hardly expressed. Within motley-grass forest types, competition between Siberian Stone Pine seedlings and plant species forming soil cover was more intense, since after the fire in these habitats, the recovery of burnt out places began with not only Siberian Stone pine but also birch and high grass. In addition, aggravation of intraspecific competition within the clumps of seedlings appeared due to the zoochorous dispersal of Siberian Stone pine seeds is quite probable under the given conditions. Conversely, a soil cover is an indicator of forest-vegetation conditions, including soil and light characteristics. Consequently, the differences in the genotypic structure of fragments in a population consisting of different types of forest can be most probably due to the effect of the whole complex of factors of natural selection at all of the stages of dynamics during age-dependent regeneration. It has been previously determined that against a background adjusted for ecological factors under the conditions of forest cultivation, the height and the age of Siberian Stone pine trees at the beginning of seed-bearing and, consequently, the success in the intraspecies competition are related with the allozyme heterozygosity of a genotype (Petrova et al., 2004). Therefore, the genotypic structures of Siberian Stone pine stands with similar soil covers can acquire common properties, which favor the minimization of their genetic differentiation under the influence of the natural selection that combines the soil's specificity and intra- and interspecific competition in this case. In general, the weak genetic subdivision and differentiation of the studied Siberian Stone pine populations testify to the fact that they are parts of a single population, in spite of the range fragmentation. It conforms to the conception of a shifting *trailing* edge range margin.

At the same time, it should be noted that the climatogenic range dynamics concept and the classification of the range margins applied by us are by convention attributed to the species, whose distribution is confined to a certain climatic zone, i.e., to the so-called zonal species. The causes of formation of population fragments in a plant species having zonal and intra-zonal ranges are principally different, as follows: in the first case, the fragments can occur under gradually deteriorating conditions when approaching to the range margin; in the second case, isolates are mosaically located, and in addition, their existence is generally conditioned by edaphic factors.

Among the forest-forming trees in the Euro-Asian continent, Scots pine (*Pinus sylvestris*) is a striking representative of intrazonal types. This species is distributed in habitats with poor (sandy and stony) soils under a wide range of climatic conditions, from 37° to 70° N and from the Atlantic ocean to 120° E (Pravdin, 1964). As a result of the comparative analysis of allozyme variability in 26 marginal island populations and 20 populations in the central part of the range of *Pinus sylvestris* L., the S. Wright—N. Dubinin *island model* of automated genetic processes and the E. Mayr hypothesis of homozygotization of small isolated populations at the range margins are corroborated at the species level (Sannikov et al., 2011). It has been ascertained that a significant decrease in the polymorphism of isolates of this species can be observed when a reduction of their area to a size less than the critical value (15–20 km^2) was observed. In other cases, the genetic diversity level of marginal populations can vary compared to the populations in the central part of the range; reliable positive correlation between the mean number of alleles per locus, as well as the observed mean heterozygosity and the area of marginal isolated populations, have been detected (Sannikov et al., 2011). Therefore, patterns of genetic variation in the natural isolates coincide with the views of classical population genetics, according to which, a decrease in or cessation of the genetic material exchange with the population of a basic part of a range is typical for small isolated groups of species individuals, which results in a decrease of their effective number, fixation of alleles, and a loss of genetic diversity.

In the range of phenomena accompanied by the fragmentation in a marginal area of the range into small populations, the heterozygote deficiency recorded in a number of our samples may be indicated as well. In many publications, it was demonstrated that a similar deficiency can be a normal component of population genetic structure in conifers, and this is being caused by inbreeding due to both partial self-pollination and cross-pollination among closely related individuals under occurrence a spatial family structure of a stand. As a rule, the consequences of inbreeding are primarily expressed at the early ontogenetic stages (seed embryos and seedlings); therefore, selection against inbred progeny incompletely eliminates the heterozygote deficiency in the mature trees of a stand (Guries and Ledig, 1978; Hamrick et al., 1992; Politov, 1989). In our case, similar deviations have not expressed any certain systematic character, which can testify to the elimination of most of the inbred progeny by selection.

In our view, the formation of natural isolates in zonal species may take place only within the *stable edge* margin (Hampe and Petit, 2005), predominantly under mountainous conditions, when relict populations have no relations with the mainland part of the species range for a long time period. Such a Siberian Stone pine isolate may be exemplified by a population inhabiting the Mount Alkhanai in the Southern Transbaikalia, one of the most southeastern patches of the species' range. In this population, the analysis of genetic structure has revealed a low proportion of polymorphic loci compared to other populations in the eastern part of the range, which may be related to a long-term isolation and a low a number of trees involved in a reproductive process (Petrova et al., 2012).

An anthropogenic impact, such as lumbering, transfer of forest lands into other categories of land use, and climate change are often referred to as factors responsible for forest stand fragmentation. These processes can result in either a change of margins or a reduction of areas suitable for the natural processes of species reproduction and recovery (Davis and Show, 2001). Considering the southern range boundaries of the Siberian Stone pine distribution in the Western Siberian Plain, it may be affirmed that both the abovementioned factors have had an impact on the stand structure. Exploration and land-reclamation of Western Siberia was carried out under severe natural conditions of the Little Ice Age (LIA), whose timeframe in the region was limited to 1550–1850 (Zhilina, 2010). During the LIA, sea glaciation increased, the ice regime in rivers changed, and permafrost development progressed. The LIA may be divided into three phases differing in heat and moisture supplies, as well as in the peculiarities of seasons: the first phase is a descending branch (from warmth to coldness) between 1550 and 1600; the second phase is the nucleus between 1600 and 1800; and the third phase is an ascending branch (from coldness to warmness) between 1800 and 1850 (Maloletko, 2003). The Siberian Stone pine is well adapted to the severe conditions of a boreal zone; it can endure through a frozen winter quite well maintaing its regular seed-bearing; this is why, the second LIA phase was optimal for the species. The northward shift in the boundary of the Siberian Stone pine range is connected with the climatic changes in the LIA's third phase, which is the period when the analyzed stands occur. After the LIA, the forests in the analyzed area developed for a century and a half under the conditions of the subsequent warming, initially at a slow rate and for the past 50 years, at a quick rate. It could not avoid contributing to the further fragmentation of the area occupied by the Siberian Stone Pine.

The modern boundary between the southern taiga and forest-steppe areas has long been commonly accessible for humans and is a well developed territory. According to historical reports (Matveyeva and Gusachenko, 1996), the nearest settlement, Kolyvan', was established in 1713. Agriculture was a traditional way of life for the local population; in addition, the *shifting* cultivation system based on regularly involving the new lands coming into use was extended in the 18th century. The impact of anthropogenic pressures arose from each construction of the Siberian Route at the end of the 18th century, followed by the Trans-Siberian main railway line from 1891 to 1916 and the bridge over the river Ob' in the early 20th century. All of these have apparently led to the continued expansion of the development of forest resources and to an increased impact on the nearest forests. Therefore, both climate change and the anthropogenic transformation have contributed to the decrease in the area and fragmentation of forests, including Siberian Stone pine stands, in the studied territory.

The prospects of the existence and reforestation, as well as conservation of the gene pool of the islands Siberian Stone pine stands at the *trailing* edge range margin are directly related to the state of the reproductive system. It has been previously determined that in the transition zone between the southern taiga and forest-steppe areas, the Siberian Stone pine is characterized by rather high seed productivity; however, the yields are unstable in this region (Nekrasova and Mishukov, 1974). The study on the seed yield structure in the Siberian Stone pine stands at the southern range margin in Western Siberia whose genetic structures are described in this paper, has determined (Velisevich, 2013) that a decrease in the values for cone formation and ripening, with an increase of the percentage of underdeveloped seeds, took place towards the boundary with the forest-steppe zone. Within the isolated island populations, the high mortality rates of female cones in the period of the winter crop's spring-growth after dormancy in winter, an increased number of sterile scales in the medial and distal zones of a cone, as well as a high percentage of seed buds perishing at the earliest development stages, are indicated. The latter is especially expressed in the two southernmost isolates; in addition, it may be related to both the low probability of the cone pollination of these trees, with the pollen transferred from the large northern woodlands inhabited by Siberian Stone pine, and the low flying qualities of the pollen from their own trees (Velisevich et al., 2011). In spite of the limitation in the female generative morphogenesis in populations at the southern range margins, in general, the cone-bearing trees should therefore be considered as actual participants in the reproductive process as seed donors, since their reproduction is ensured by their own seeds (Velisevich et al., 2013).

Over the past 100 years, the *trailing* edge range margin has shifted 50 km northwards (Bekh, 1974). In the long term perspective, a considerable change in the genetic structure, a reduction in genetic diversity, and an increase in fragmentation, as well as the subsequent vanishing of the Siberian Stone pine isolates at the boundary between the southern taiga and forest-steppe areas should be expected in the event that the boundary between the forest-steppe and taiga areas shifts further northward and distances itself from the isolates, and if the temperature regime becomes more unfavorable for developing male and female generative spheres.

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