ISSN 1022-7954, Russian Journal of Genetics, 2010, Vol. 46, No. 12, pp. 1417–1425. © Pleiades Publishing, Inc., 2010.
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PLANT GENETICS

Characterization of Gene Pools of Three *Pinus pumila* (Pall.) Regel Populations at the Range Margins

O. V. Nakonechnaya[a], A. B. Kholina[a], O. G. Koren, V. Janeček[b], A. Kohutka[b], R. Gebauer[b], and Y. N. Zhuravlev[a]

[a] *Institute of Biology and Soil Science, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, 690022 Russia; e-mail: kholina@biosoil.ru.*

[b] *Department of Dendrology and Forest Tree Breeding, Czech University of Life Sciences in Prague, Prague, 12916521 Czech Republic*

Received May 19, 2010; in final form, June 17, 2010

Abstract—Genetic variation of Siberian dwarf pine *Pinus pumila* (Pall.) Regel was characterized in three marginal populations in southwestern, southern and eastern parts of the natural species range (Baikal Area, Primorye, Kamchatka) using isozyme analysis. Analysis involving 16 isozyme loci encoding ten enzyme systems was conducted. Our results confirm that *P. pumila* is one of the most polymorphic species in the genus *Pinus*. Three marginal populations exhibited high genetic variation ($P_{95} = 68.8\%$, $H_o = 0.247$, $H_e = 0.291$). Populations heterogeneity and significantly high level of divergence in coniferous ($F_{ST} = 0.050$, $D_N = 0.044$) reflect their genetic originality. In summary, it was shown that the level of genetic variation characteristic for *P. pumila* in other parts of the not only is reproduced in the populations examined but even is close to maximum there.

DOI: 10.1134/S1022795410120033

INTRODUCTION

Members of the genus *Pinus* belong to the most valuable wood-forming trees in Eurasia, being of high commercial value and substantial water-controlling, antierosive, sanitary, and esthetic significance. Investigation of genetic diversity in these species is essential for conservation of their gene pools and for reproduction of the genetic variation characteristic for them upon breeding and reproduction.

Siberian dwarf pine *Pinus pumila* (Pall.) Regel has several features discriminating it from other pines. This species possesses high genetic variation [1–5] and ecological plasticity. It is undemanding to edaphic conditions, growing mainly as branchy bushes, reproducing in nature both by seeds and vegetatively by rooting creeping shoots [6]. Brushwoods of Siberian dwarf pine were shown to play the unique role building the constructors of biogeocenosis cover at a certain stage of colonization of volcano slopes by plants after eruptions [7]. Considering the issues of the preservation of the wood genetic resources of Northeast Asia, it should be kept in mind that populations of many valuable arboreal species have been formed under severe and diverse conditions of Siberia and Russian Far East and frequently possess unique gene pools, which particularly concerns marginal, hybrid, high-productive populations. Accordingly, the main preservation objects in this region should not involve the species as a whole, but individual populations of these species [8]. Allozyme variation of individual *P. pumila* populations in the Russian and Japanese parts of the species range was studied previously [1, 3–5, 19]. The species was shown to possess heterozygosity maximum among cedar pines and close to the maximum values known for conifers [1, 3]. For preservation of the *P. pumila* genetic resources, any information on the gene pool conditions of previously unstudied populations that could possess additional variation reserves and interest for breeding is valuable. It is also urgent to carry out comparative analysis of genetic variation in marginal populations from various parts of the range significantly distant from each other. The aim of the present study was to characterize diversity of *P. pumila* gene pool in three marginal populations in southwestern, southern, and eastern parts of the range.

MATERIALS AND METHODS

The seeds for analysis were collected from individual trees in three natural populations of Siberian dwarf pine (Fig. 1) in 2008: population 1, abbreviations BL - Baikal Lake, Svyatoi Nos Peninsula, northwest to the town of Ust-Barguzin (28 trees); population 2, OM - Primorye, Chuguevskii district, Oblachnaya mountain (22 trees); population 3, EV - Kamchatka, Bystrinskii district, outskirts of the Esso village (25 trees). Six endosperms were analyzed from each tree in order to identify the individual genotype. The hard shell and nucellus were removed from each seed; then, a thin layer of megagametophyte tissue was cut and homogenized in 100–120 μl of extraction buffer (1% PVP-40,

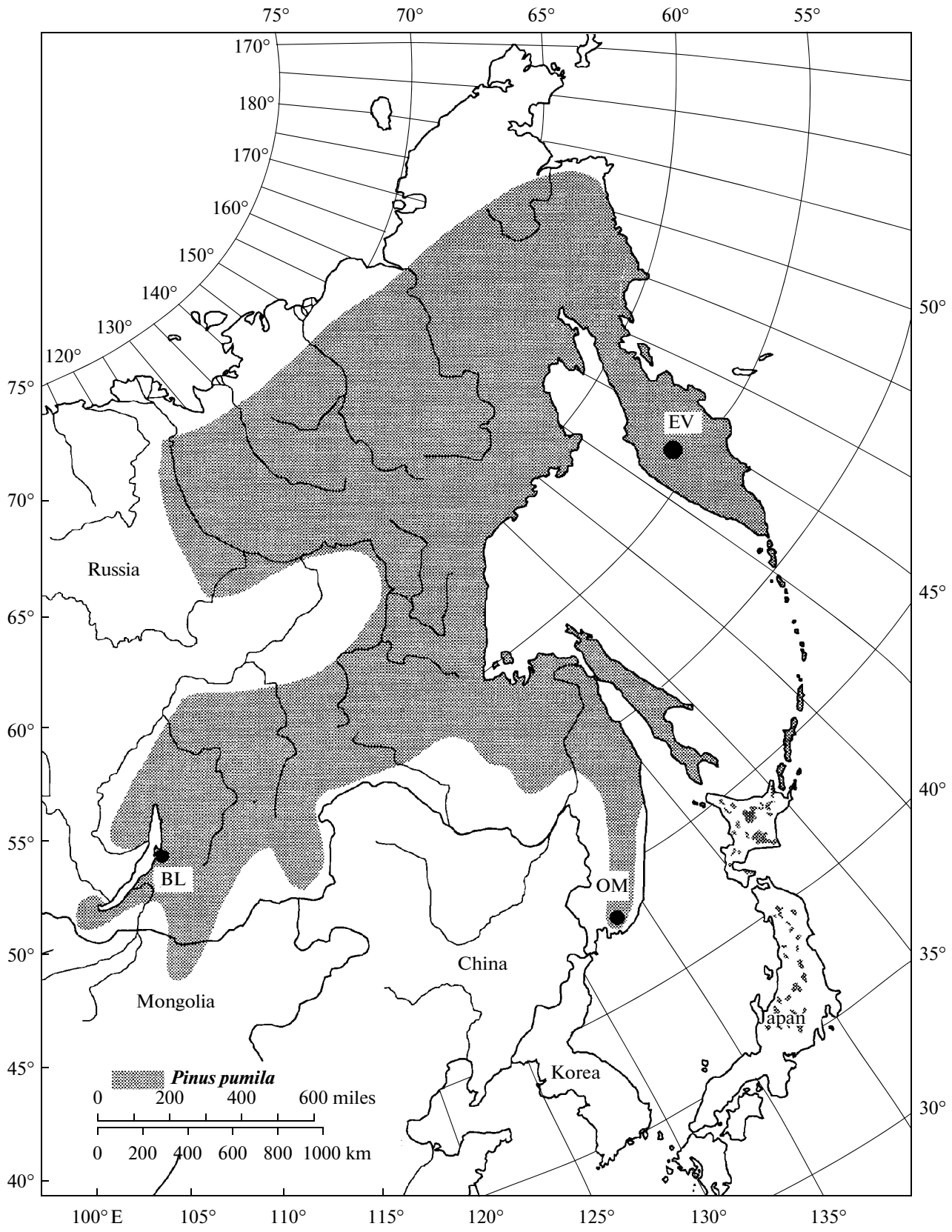

Fig. 1. Geographic distribution of *Pinus pumila* (marked in grey) and sampling localities(●).

1% Triton X-100, 0.2% of ascorbic acid in Tris-HCl buffer) [9].

Electrophoresis was performed in 13% starch gel in two buffer systems, Tris-citrate (pH 6.2) and Tris-EDTA-borate (pH 8.6) [10]. Histochemical staining of enzyme activity zones was conducted according to standard techniques [10, 11]. Ten enzyme systems were used as molecular genetic markers, while 16 loci (*Adh-1, Aat-1, Aat-2, Aat-3, Gdh, Dia-1, Dia-2, Idh, Lap-1, Mdh-2, Fe-2, Fe-3, Pgm-1, Pgm-2, Skdh-1, Skdh-2*) were examined for genetic variation. Nomenclature of Prakash was used for enzymes specification [12]. Alleles were designated according to the relative electrophoretic mobility; the mobility of the most common variant was taken to be 1.00.

The mode of inheritance of the enzyme systems was determined on the basis of significance of the deviation of the observed allelle frequency distribution from the expected ratio 1 : 1 in haploid endosperms of heterozygous trees calculated using χ^2 test. Polymorphism indices (*P*), the average number of alleles per locus (*A*), the average observed (H_o) and expected (H_e) heterozygosities were calculated using standard methods [10, 13]. Allele and genotype heterogeneities were assessed using standard χ^2 test [13]. Wright's F-statistics were calculated for subdivision analysis in each population [14]. Genetic distances D_N between the populations were calculated according to Nei [15]. Method of unweighted pair group method with arithmetic mean (UPGMA) was used for clustering. Statistical analysis was conducted using the TFPGA software [16].

RESULTS AND DISCUSSION

According to the results of electrophoretic analysis of ten enzyme systems, we detected 44 allelic variants at 16 structural loci in three investigated *P. pumila* populations. Fifteen loci were polymorphic (Table 1), while the *Aat-1* locus was shown to be monomorphic in all populations. Schematic representation of aspartate aminotransferase, isocitrate dehydrogenase, fluorescent esterase, phosphoglucomutase, and shikimate dehydrogenase electrophoregrams is shown in Fig. 2. The detected electrophoretic variants of ten enzymes and their genetic interpretation were similar to that previously demonstrated for cedar pines [9, 10, 17] and in particular for *P. pumila* [1, 5].

Allelic character of all 44 detected electrophoretic variants was established based on analysis of their distribution in haploid endosperms of heterozygous trees (Table 1). Segregation ratios with respect to single loci with some exceptions in general corresponded to Mendelian ones (1 : 1). Deviations from the expected segregation were observed in 2 from 35 studied heterozygotes, both for *Fe-2* locus. In both cases, the heterogeneity of the segregation ratios was caused by a segregation bias towards the most common allele. Moreover, disturbances in segregation were observed only in the case of particular allele combinations. The segregation corresponded to 1 : 1 (Table 1) in other combinations involving the same alleles. Disturbances in alleles segregation have been repeatedly observed in studies of enzyme inheritance in the genus *Pinus* [1, 5, 9, 18], while disturbances in segregation at this locus were detected in *P. pumila* [1, 5]. In all, our results

Table 1. Segregation of allele variants in *P. pumila* heterozygous trees

Locus	Allele combination	Number of trees	Segregation	χ^2
Adh-1	1.00 : 1.10	1	3 : 3	0.000
Aat-2	1.00 : 1.22	16	44 : 49	0.269
Aat-3	1.00 : 2.00	26	77 : 79	0.026
	1.00 : 2.25	10	34 : 26	1.067
	1.50 : 2.00	2	4 : 6	0.040
	1.50 : 2.25	1	1 : 5	2.667
	2.00 : 2.20	11	31 : 35	0.242
	2.00 : 2.75	4	9 : 15	1.500
	2.25 : 2.75	1	1 : 3	1.000
Gdh	1.00 : 1.20	22	62 : 65	0.071
Dia-1	0.80 : 1.00	46	119 : 149	3.358
Dia-2	1.00 : 1.08	2	6 : 6	0.000
Idh	0.95 : 1.00	3	8 : 10	0.222
Lap-1	0.00 : 1.00	17	51 : 48	0.091
	0.00 : 1.10	1	1 : 5	2.667
	1.00 : 0.90	6	15 : 19	0.471
	1.00 : 1.10	11	34 : 32	0.061
Mdh-2	0.86 : 1.00	44	130 : 132	0.015
Fe-2	0.95 : 1.00	4	5 : 19	8.167*
	0.95 : 1.07	1	2 : 2	0.000
	1.00 : 1.07	17	59 : 35	6.128*
	1.00 : 1.00/1.07	5	13 : 15	0.143
Fe-3	0.78 : 1.00	2	8 : 4	1.333
	0.78 : 1.06	1	5 : 1	2.667
	1.00 : 1.06	31	86 : 90	0.091
Pgm-1	0.90 : 0.95	2	6 : 6	0.000
	0.90 : 1.00	21	65 : 58	0.398
	0.90 : 1.05	2	6 : 6	0.000
	0.95 : 1.00	19	65 : 49	2.246
	0.95 : 1.05	1	1 : 5	2.667
	1.00 : 1.05	7	25 : 17	1.524
Pgm-2	0.95 : 1.00	3	9 : 9	0.000
	1.00 : 1.40	12	36 : 36	0.000
Skdh-1	1.00 : 1.10	7	23 : 19	0.381
Skdh-2	1.00 : 1.10	6	20 : 16	0.444

* Significant deviation at the 0.01 significance level.

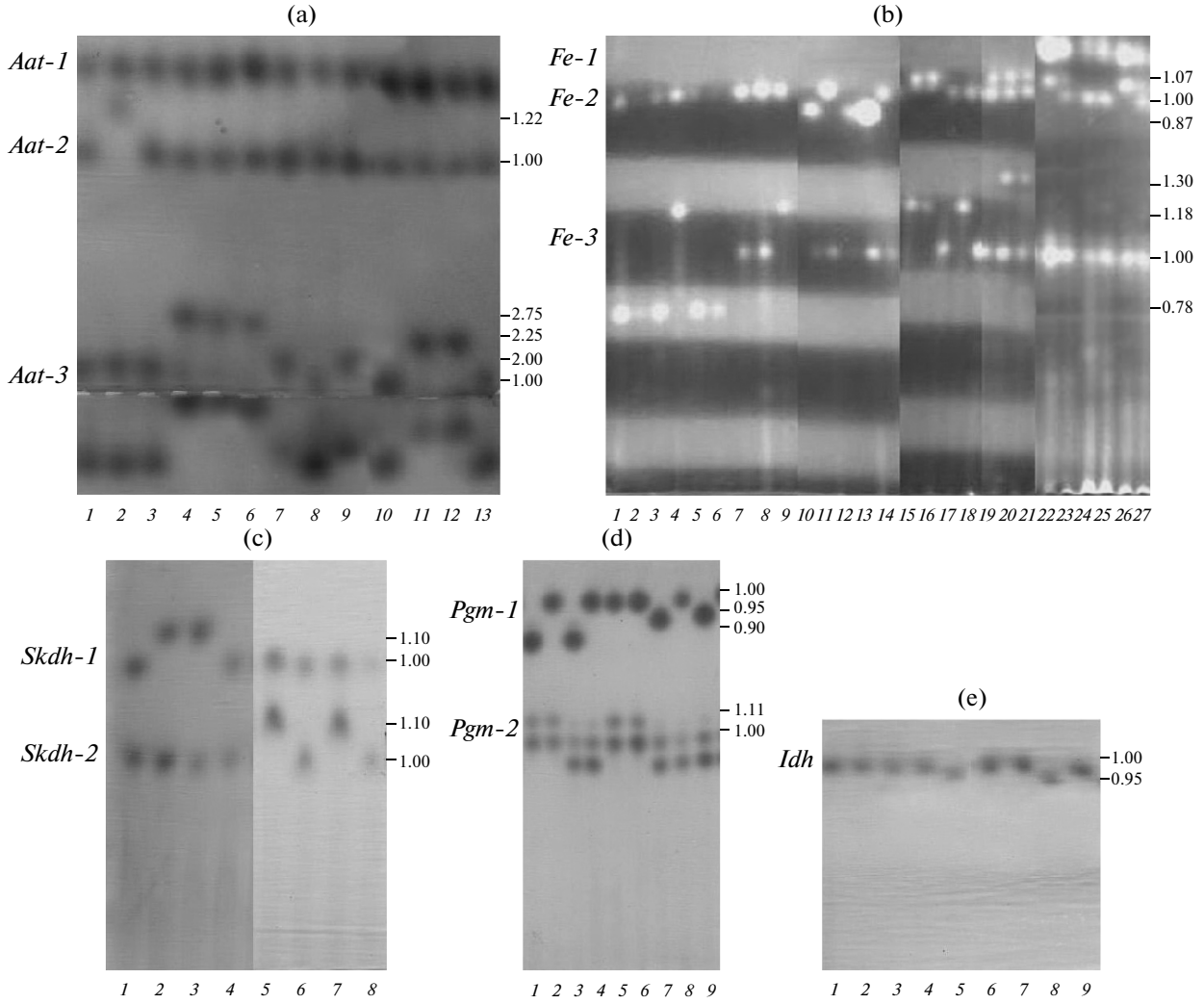

Fig. 2. Electrophoregrams of some polymorphic loci in *Pinus pumila*. (a) *Aat*: *1*, *3*, *7*, *9 Aat-2*$^{1.00}$/*Aat-3*$^{2.00}$; *2 Aat-2*$^{1.22}$/*Aat-3*$^{2.00}$; *4*, *5*, *6 Aat-2*$^{1.00}$/*Aat-3*$^{2.75}$; *8*, *10*, *13 Aat-2*$^{1.00}$/*Aat-3*$^{1.00}$; *11*, *12 Aat-2*$^{1.00}$/*Aat-3*$^{2.25}$. (b) *FE*: *1*, *2*, *3*, *5*, *6 Fe-2*$^{1.00}$/*Fe-3*$^{0.78}$; *4*, *9*, *18 Fe-2*$^{1.00}$/*Fe-3*$^{1.18}$; *7*, *8*, *11*, *14*, *17*, *23–25 Fe-2*$^{1.00}$/*Fe-3*$^{1.00}$; *10*, *12*, *13 Fe-2*$^{0.87}$/*Fe-3*$^{1.00}$; *15*, *16 Fe-2*$^{1.07}$/*Fe-3*$^{1.18}$; *19*, *27 Fe-2*$^{1.07/1.00}$/*Fe-3*$^{1.00}$; *20*, *21 Fe-2*$^{1.07/1.00}$/*Fe-3*$^{1.00/1.30}$; *22*, *26 Fe-2*$^{1.07}$/*Fe-3*$^{1.00}$. (c) *SKDH*: *1*, *4*, *6*, *8 Skdh-1*$^{1.00}$/*Skdh-2*$^{1.00}$; *2*, *3 Skdh-1*$^{1.10}$/*Skdh-2*$^{1.00}$; *5*, *7 Skdh-1*$^{1.00}$/*Skdh-2*$^{1.10}$. (d) *PGM*: *1 Pgm-1*$^{0.90}$/*Pgm-2*$^{1.11}$; *2*, *5*, *6 Pgm-1*$^{1.00}$/*Pgm-2*$^{1.11}$; *3 Pgm-1*$^{0.90}$/*Pgm-2*$^{1.00}$; *4*, *8 Pgm-1*$^{1.00}$/*Pgm-2*$^{1.00}$; *7*, *9 Pgm-1*$^{0.95}$/*Pgm-2*$^{1.00}$. (e) *IDH*: *1–4*, *6*, *7*, *9 Idh*$^{1.00}$, *5*, *8 Idh*$^{0.95}$.

confirmed genetic nature of the electrophoretic variants found in the Siberian dwarf pine populations.

We detected two- to five alleles at each polymorphic locus. The test for heterogeneity demonstrated significant differences in allele frequencies among the populations (Table 2). Eight rare alleles with frequencies ≤0.05 and nine unique alleles (present only in one population) were revealed in the *P. pumila* populations examined. The *Aat-3, Dia-1, Lap-1, Mdh-2,* and *Pgm-1* loci exhibited the highest variation in the populations studied (the mean observed heterozygosity in three populations exceeded 35%), while the *Adh-1, Dia-2, Idh, Skdh-2* loci demonstrated the lowest variation (the mean observed heterozygosity lower than 5%). No marked geographic trend in the allele frequency in the populations was shown that indirectly testifies to the absence of directed gene flow amon gthe populations. Apparently, independent processes primarily associated with the adaptation of these populations to the unique environmental conditions occur in these localities; gene drift and hybridization on the range borders play here a significant role. The presence of rare (BL, four; OM, three; EV, six) and unique (BL, four; OM, two; EV, three) alleles reflects the action of microevolutionary processes in the marginal populations.

Populations testing at single loci for goodness-of-fit to the Hardy-Weinberg equilibrium demonstrated (Table 3) significant deviations from it in five cases: in the BL populations at the *Fe-2* locus, in the OM pop-

Table 2. Allele frequencies at 15 polymorphic loci in three *P. pumila* populations and test for heterogeneity

Locus	Alleles	Population			Locus	Alleles	Population		
		BL ($N = 28$)	OM ($N = 22$)	EV ($N = 25$)			BL ($N = 28$)	OM ($N = 22$)	EV ($N = 25$)
Adh-1	1.00	1.000	0.932	1.000		1.00	0.534	0.523	0.400
	1.10	0.000	0.068	0.000	Test for heterogeneity: $\chi^2 = 2.71$; $d.f. = 2$; $p > 0.5$				
Test for heterogeneity: $\chi^2 = 18.92$; $d.f. = 2$; $p < 0.01$					*Fe-2*	0.87	0.000	0.000	0.040
Aat-2	1.00	0.929	0.795	1.000		0.95	0.054	0.000	0.040
	1.22	0.071	0.205	0.000		1.00	0.536	0.773	0.800
Test for heterogeneity: $\chi^2 = 3.16$; $d.f. = 2$; $0.10 < p < 0.25$						1.07	0.339	0.227	0.060
Aat-3	1.00	0.446	0.409	0.380		1.00/1.07	0.071	0.000	0.060
	1.50	0.000	0.023	0.000	Test for heterogeneity: $\chi^2 = 10.52$; $d.f. = 2$; $p < 0.01$				
	2.00	0.304	0.318	0.480	*Fe-3*	0.78	0.018	0.000	0.060
	2.25	0.214	0.227	0.120		1.00	0.661	0.818	0.680
	2.75	0.036	0.023	0.020		1.18	0.321	0.182	0.240
Test for heterogeneity: $\chi^2 = 0.49$; $d.f. = 2$; $p > 0.9$						1.00/1.30	0.000	0.000	0.020
Gdh	1.00	0.429	0.591	0.900	Test for heterogeneity: $\chi^2 = 3.40$; $d.f. = 2$; $p > 0.25$				
	1.20	0.571	0.409	0.100	*Pgm-1*	0.90	0.179	0.114	0.160
Test for heterogeneity: $\chi^2 = 25.76$; $d.f. = 2$; $p < 0.01$						0.95	0.179	0.250	0.140
Dia-1	0.80	0.625	0.591	0.460		1.00	0.642	0.636	0.460
	1.00	0.375	0.409	0.540		1.05	0.000	0.000	0.240
Test for heterogeneity: $\chi^2 = 3.16$; $d.f. = 2$; $0.10 < p < 0.25$					Test for heterogeneity: $\chi^2 = 4.44$; $d.f. = 2$; $p > 0.1$				
Dia-2	1.00	0.982	1.000	1.000	*Pgm-2*	0.95	0.018	0.000	0.000
	1.08	0.018	0.000	0.000		1.00	0.893	0.954	0.820
Test for heterogeneity: $\chi^2 = 1.69$; $d.f. = 2$; $p > 0.5$						1.11	0.089	0.046	0.180
Idh	0.95	0.089	0.000	0.000	Test for heterogeneity: $\chi^2 = 4.25$; $d.f. = 2$; $p > 0.1$				
	1.00	0.911	1.000	1.000	*Skdh-1*	1.00	1.000	0.773	0.960
Test for heterogeneity: $\chi^2 = 8.68$; $d.f. = 2$; $0.01 < p < 0.025$						1.10	0.000	0.227	0.040
Lap-1	0.00	0.161	0.045	0.140	Test for heterogeneity: $\chi^2 = 18.92$; $d.f. = 2$; $p < 0.01$				
	0.90	0.071	0.000	0.040	*Skdh-2*	1.00	0.911	1.000	1.000
	1.00	0.625	0.864	0.640		1.10	0.089	0.000	0.000
	1.10	0.143	0.091	0.180	Test for heterogeneity: $\chi^2 = 8.68$; $d.f. = 2$; $0.01 < p < 0.025$				
Test for heterogeneity: $\chi^2 = 7.97$; $d.f. = 2$; $0.01 < p < 0.025$					Test for heterogeneity: $\chi^2 = 120.67$; $d.f. = 30$; $p < 0.01$				
Mdh-2	0.86	0.445	0.477	0.600					

Note: N, the number of plants studied.

ulation at the *Pgm-1* locus, and in the EV population at the G*dh, Lap-1,* and *Skdh-1* loci. In all cases, deviation from Hardy-Weinberg equilibrium was caused by heterozygote deficiency. Probably, some of the cases of heterozygote deficiency could be explained by the usage of six endosperms while detecting individual genotypes, which could probably result in incomplete detection of some heterozygotes. In total, no significant deviation from Hardy-Weinberg equilibrium was demonstrated in the BL and OM populations with respect to all loci examined.

From the allele frequencies at 16 genes, we calculated the main parameters of genetic polymorphism measuring variation in *P. pumila* (Table 4). About 70%

Table 3. Concordance between the observed and the expected at Hardy–Weinberg equilibrium genotype frequencies in the *P. pumila* populations

Locus	Population						Total for three populations	
	BL		OM		EV			
	d.f.	χ^2	*d.f.*	χ^2	*d.f.*	χ^2	*d.f.*	χ^2
Adh-1	1	0.00	1	0.12	1	0.00	3	0.12
Aat-2	1	1.17	1	1.47	1	0.00	3	2.64
Aat-3	4	0.29	5	6.52	4	2.23	13	9.04
Gdh	1	2.05	1	1.35	1	8.33*	3	11.73*
Dia-1	1	0.003	1	0.08	1	0.06	3	0.14
Dia-2	1	0.01	1	0.00	1	0.00	3	0.01
Idh	1	3.26	1	0.00	1	0.00	3	3.26
Lap-1	4	4.74	3	1.44	4	11.87*	11	18.05
Mdh-2	1	0.10	1	0.00	1	0.69	3	0.79
Fe-2	4	17.04*	1	1.10	5	6.59	10	24.73*
Fe-3	3	0.44	1	1.49	4	2.73	8	4.66
Pgm-1	3	1.22	3	8.52*	4	7.14	10	16.88
Pgm-2	3	0.38	1	0.05	1	1.10	5	1.53
Skdh-1	1	0.00	1	1.10	1	6.35*	3	7.45
Skdh-2	1	3.26	1	0.00	1	0.00	3	3.26
For all loci	30	33.96	23	23.24	31	47.09*	84	

Notes: * The observed genotype frequencies differ from the expected ones.
d.f., degrees of freedom.

of genes in the populations examined proved to be polymorphic, while each tree was heterozygous for 24.7% of the loci. The BL population exhibited highest polymorphism and heterozygosity, while the EV population showed highest allelic diversity. The level of expected heterozygosity and polymorphism increased from east westward, whereas no such tendency was observed for the average number of alleles per locus and the observed heterozygosity.

Our results confirmed that *P. pumila* is one of the most polymorphic species in the genus *Pinus* as had been previously reported [1, 3–5]. The values of genetic polymorphism parameters for the marginal

Table 4. The main parameters of genetic polymorphism in *Pinus pumila* populations

Population	*N*	P_{95}, %	P_{99}, %	H_o	H_e	*A*	n_e
BL	28	81.3	81.3	0.275	0.320	2.19	1.47
OM	22	68.8	75.0	0.227	0.284	2.06	1.40
EV	25	56.3	62.5	0.240	0.267	2.31	1.36
Means	75	68.8	72.9	0.247	0.291	2.19	1.41

Note: *N*, the number of trees examined; P_{95}, P_{99}, %, polymorphism by 95 and 99% criteria; H_o, observed heterozygosity; H_e, expected heterozygosity; *A*, the number of alleles per locus; n_e, effective number of alleles.

populations of Siberian dwarf pine investigated in the present study are similar to those in populations of this species from other parts of its range. For instance, in a study of allozyme variation in *P. pumila* at 20 loci in five populations from Chukotka and Sakhalin, H_o varied from 0.288 to 0.322 and H_e, from 0.230 to 0.283 [5]. In 18 Japanese populations examined at 19 loci, these parameters varied more significantly (H_o, from 0.134 to 0.285; H_e, from 0.151 to 0.286 [2]). The range of change in H_o was from 0.230 to 0.268; in H_e, from 0.239 to 0.258 [1] for three populations from Kamchatka examined at 20 loci, while for four populations from Koryakiya (1), Kamchatka (2) and Kunashir Island (1) studies at 23 loci, H_o varied from 0.202 to 0.264 and H_e, from 0.225 to 0.240 [19]. At the same time, the mean H_o was 0.219 and H_e, 0.225 for nine populations from the central and eastern parts of the range studied at 26 isoenzyme loci [4], while the mean H_e was 0.234 for 29 populations from various parts of the species range examined at about 30 loci [3]. The differences in the heterozygosity level in the populations of Koryakiya, Kamchatka, Chukotka, Sakhalin, Pribaykal'ye, and Japan are related to various set of loci used in the studies conducted by various authors. Our for three marginal populations (Table 3) are closer to the maximum values reported for various regions. The values of polymorphism parameters P_{95} and P_{99} are also close to the maximum values recorded previously [1, 2, 5]. In general, we would like to note that

the variation level in the marginal *P. pumila* populations is characteristic for populations of this species from various parts of the range. Isolated plant populations at the range boundaries in poor environments usually become depressed [20]. Moreover, populations in any parts of the range may be "marginal" from the ecological point of view [21]. Effects of several factors in ecologically marginal populations characterized by severe environmental conditions lead to elimination of some genotypes decreasing the polymorphism in the population and resulting in strict adaptation to particular conditions. Apparently, the marginal populations of Siberian dwarf pine examined are not marginal from the ecological viewpoint, which is evidenced by the high values of genetic polymorphism parameters.

Geographical isolation of the Siberian dwarf pine populations studied does not significantly affect the level of their subdivision (Table 5). The F_{IS} averaged over all loci was 0.149, which confirms the deficiency of heterozygotes in these populations. The F_{ST} value (0.050) points that each single population of Siberian dwarf pine maintains up to 95% of the species genetic variation and only 5% of it accounts for the interpopulation component, which is characteristic for many species from the genus *Pinus* [1, 3, 9, 20]. The most significant contribution to the interpopulation variation is made by loci *Aat-2* (10.7%), *Gdh* (21.7%), and *Skdh-1* (15.5%), while the minimun contribution, by *Aat-3* (0.1%), *Dia-1* (1.1%), *Mdh-2* (0.5%), and *Fe-3* (0.6%). It may well be that the geographic variation of these loci is adaptive, and the former group with the maximum F_{ST} values is affected by diversified selection, whereas the latter group with the minimum F_{ST} values is influenced by stabilizing selection as was demonstrated for *P. pumila* populations from Pribaykal'ye [22].

The low subdivision level was shown for previously studied populations of Siberian dwarf pine in Russia. For instance, F_{ST} in three populations from Kamchatka was 0.021 [1], in four populations from Pribaykal'ye, 0.042 [22], in five populations from Chukotka (3) and i. Sakhalin (2), 0.043 [5], in nine populations from the central and eastern parts of the range, 0.038 [4], and in 29 populations from various parts of the range, 0.070 [3]. Low subdivision with high interpopulation variation is mainly characteristic for conifers, widespread and cross-pollinated: the proportion of interpopulation variation varies from 0.02 to 0.08 [1]. In this connection, the important role is played also by the way of seed spreading by Eurasian nutcrackers *Nucifraga caryocatactes* L. for cedar pine and Siberian dwarf pine [1, 19]. The seed spreading in zoochoric species is a more significant component of genes flow than pollen transfer and has stronger impact on population homogenization. The low differentiation of marginal distant and isolated *P. pumila* populations reported here allows mediated (through several neighboring populations) genes exchange between them and thus suggests the common gene pool of the specvies in the Russian part of the range. At the same time, balanced selection is likely to play a certain role, reducing genetic differences among the populations.

Table 5. Parameters of Wright's F-statistics for *Pinus pumila* at 16 loci

Locus	F_{IS}	F_{IT}	F_{ST}
Adh-1	–0.053	0.004	0.054
Aat-1	0.000	0.000	0.000
Aat-2	–0.176	–0.050	0.107
Aat-3	0.137	0.137	–0.001
Gdh	0.359	0.498	0.217
Dia-1	0.025	0.036	0.011
Dia-2	–0.057	–0.004	0.050
Idh	0.359	0.397	0.059
Lap-1	0.090	0.115	0.028
Mdh-2	0.097	0.101	0.005
Fe-2	0.321	0.369	0.070
Fe-3	0.190	0.195	0.006
Pgm-1	0.049	0.082	0.036
Pgm-2	0.205	0.221	0.020
Skdh-1	0.395	0.489	0.155
Skdh-2	0.359	0.397	0.059
Total	0.149	0.192	0.050

Note: F_{IS}, inbreeding coefficient of individual relative to populations; F_{IT}, inbreeding coefficient of individual relative to the total species; F_{ST}, populations subdivision index.

Heterozygote deficiency detected in the populations studied was also observed in other species of cedar pine [9, 20] and in *P. pumila* in the Japanese part of the range [2]. According to some authors, one of the main reasons of heterozygote deficiency belongs to the crossing scheme (inbreeding due to the partial self-fertilization and (or) consanguineous crossing) together with the mode of seed dissemination and environmental microheterogeneity [1, 20]. At the same time, along with the formation of "familial" population structure and related Wahlund's effect, an important role in heterozygosity dynamics belongs to selection [1, 9, 19]. In view of an increase in heterozygosity with age, Politov et al. [19] explained the presence of expressed heterozygote deficiency in one of the investigated Siberian dwarf pine populations by lower selection intensity against homozygotes in the younger population . The adaptation maximum in population was shown to be related to the mean heterozygosity level in *P. pumila* populations from Pribaykal'ye [22]. High heterozygosity parameters recorded in the studied populations and known to be characteristic for marginal populations may be in some cases probably unfavorable for the species survival, and in that case

Table 6. Coefficients of Nei's genetic distance (D_N) between *Pinus pumila* populations

Populations	BL	OM	EV
BL	–	0.0225	0.0442
OM	–	–	0.0346

selection could be directed to the maintenance of two or more homozygotes [9] resulting in the equilibrium shift toward heterozygote deficiency. The action of balanced selection in favor of heterozygotes is known under the optimal environmental conditions usually in the central parts of wood-forming species range, whereas the role of disruptive selection could be increased under severe conditions (suboptimal for this species) [23], which could be observed also in the marginal *P. pumila* populations investigated in the present study.

The coefficient of genetic distance D_N reflecting the degree of interpopulation differentiation (Table 6) was on average 0.034, the maximum value (D_N = 0.044) was observed between populations from Pribaykal'ye and Kamchatka. The calculated values exceed those for previously studied Siberian dwarf pine populations, both closely located and isolated [1, 5], but are in the range of mean values for conspecific coniferous populations $D_N = 0.008–0.050$ [5, 10, 20].

Thus, Siberian dwarf pine in three populations on the periphery of the range was characterized by high genetic diversity despite the vegetative mode of reproduction uncharacteristic for conifers. According to the results of studying allozyme variation, vegetative reproduction does not prevail in the marginal *P. pumila* populations, which is indirectly demonstrated by the absence of allelic and genotypic homogeneity in them. It should be noted that established heterogeneity and the populations divergence rather high for conifers reflect their genetic specificity. It may be explained by the action of a set of factors including population isolation and intensification of microevolutionary processes related to the contrasting edaphic and climatic conditions. In total, it could be concluded that the level of genetic variation characteristic for *P. pumila* in other parts of the range is not only reproduced but maximum in the populations on the borders of the natural *P. pumila* range, which may be partly explained by the dynamics of the structural organization of marginal populations. The increase in the total genetic diversity could be caused by hybridization, intensive migration, and gene drift. At the same time, selection under particular conditions could result in a shift of the population genetic optimum toward various homozygous genotypes. The specific character and intensity of genetic processes on the borders of the *Pinus pumila* range should be taken into account in forestry management, since the conservation of the gene pools of marginal populations may be the key component in the maintenance of genetic diversity of this pine species.

ACKNOWLEDGMENTS

We are grateful to Dreslerova Jaromira, Fundova Irena, Klapatek Vaclav, Klapste Jaroslav, Koutecky Tomas, Leugnerova Gabriela for their help in the study.

This work was supported by the project "Monitoring *Pinus pumila* (Pall.) Regel in the Range of Its Natural Distribution", of the Program of the President of the Russian Federation (grant no. NSh 1635-2008.4); the Program of the Presidium of the Russian Academy of Sciences "Biological Diversity", the Program "Genetic Diversity of Natural Populations of Far East Plants" (project no. 09-1-P23-06), Program of the Presidium of the Russian Academy of Sciences "Molecular and Cellular Biology" (project no. 09-1-P22-03), "Biotechnology and Molecular Biology of Cell Cultures of Far Eastern Pharmaceutical Plants"; "Close-to-Nature Silviculture for Stability, Production and Non-Production Function of Forests" (Ministry of Agriculture of Czech Republic, grant no. 1G58031); "The genetic Variability of *Pinus pumila* (Pall.) Regel in the Svyatoy Nos Area" (IGA FLD no. 200943130017).

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