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Relationships between the Allozyme and Phenotypic Diversities of *Picea ajanensis* Populations

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Abstract—The structures of *Picea ajanensis* populations were compared based on the allozyme analysis of vegetative buds and the morphometric analysis of generative organs. Six cenopopulations of *P. ajanensis* were investigated in areas with various levels of volcanic impact in the Kamchatka Peninsula. The genetic structures of spruce populations and phenotypes were determined by the analysis of ten enzyme systems (*PGM*, *GOT*, *HK*, *LAP*, *MDH*, *SKDH*, *IDH*, *GDH*, *PGI*, and *SOD*). The phenotypic variability of spruce populations was estimated based on the composition of morphotypes that were identified by using the geometric morphometrics of cone-scale shapes. Pairwise comparison of samples of cones from 170 trees from six populations revealed 12 morphotypes differing in the shape of the cone scales. Comparative assessment of the variability and similarity of the populations was carried out based on the frequency of the occurrence of the phenotypes and the frequency of the alleles of the polymorphic loci. Correlations of the genetic and phenotypic distance matrices between different phenotypes were revealed. This observation was consistent with the genetic determination of the shape of the cone scales in spruce. Genetic differences between the morphotypes with regard to nine polymorphic loci (*Got-2*, *Skdh-1*, *Idh-2*, *Pgm-2*, *Mdh-1*, *Mdh-3*, *Pgm-1*, *Pgi-2*, and *Hk*) were not significant. Statistically significant differences between the spruce morphotypes were revealed for two loci: *Pgm-2* and *Mdh-1*. Differences in the genetic diversity of spruce populations generally corresponded to differences in their phenotypic diversity. The high levels of genetic and phenotypic diversity characterized a stable population structure of spruce in the area of weak volcanic influence. Changes in the genetic structure and low levels of the phenotypic diversity of spruce were observed under catastrophic volcanic impact.

Keywords: allozyme polymorphism, phenotypic diversity, cone morphology, *Picea ajanensis*

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INTRODUCTION

The problem of studying and evaluating the phenogenetic variability and the need for the synthesis of genetic and phenotypic analysis of the populations is one of the most pressing issues in population biology (Altukhov; 2003; Sannikov and Petrova, 2003, 2007). A system of methods for studying the taxonomic structure of woody plants on the population-genetic basis, which combines the principles and methods of the phenotypic and genetic analysis of the populations, was developed and tested on the example of Scots pine by S.N. Sannikov and I.V. Petrova (2003, 2007). In estimating the intra- and interspecies differentiation of different representatives of the Pinaceae family, phenotypic markers usually consist of the polygenic traits of the generative organs, including the characteristics of the size and indices of the shape of the cones, seeds, and cone scales (Mamaev, 1972; Frolov, 1993; Popov, 2005; Putenikhin et al., 2005).

A key direction in the studies of plant phenogenetics is the development of quantitative methods of the conjugate phenotypic and genetic analysis of populations (Sannikov and Petrova, 2007). Analysis of the variability of the generative organs of plants using geometric morphometrics is promising in estimating the phenotypic variability of coniferous plants. The effectiveness of this approach for the analysis of the variability and differentiation of the populations was shown on the example of the Japanese stone pine (*Pinus pumila* (Pall) Regel.) (Vetrova, 2013; Vetrova and Sinelnikova, 2014).

The purpose of this work is to analyze the relationship of the allozyme and phenotypic diversity of populations of the Jezo spruce (*Picea ajanensis* (Lindl. ex Gord.) Fisch. ex Carr. syn. *Picea jezoensis* (Siebold et Zucc.). We studied the natural populations of the Jezo spruce in the Kamchatka Peninsula. Previously, using allozyme and phenotypic markers, we obtained the data indicating the genetic and phenotypic heterogeneity of

Table 1. Characteristics of sample sites used for material collection

Sample code	Geographical coordinates	Location in relief
E1	56°03′09″ N 160°03′02″ E	Northern part of CKD, Ushkovsky volcano foot, old bed of dry river, 175 m above sea level
E2	56°03′07″ N 160°00′47″ E	Northern part of CKD, vicinity of Kozirevsk village, plains habitat, 170 m above sea level
E3	55°04′37″ N 158°53′13″ E	Middle part of CKD, plains habitat, 176 m above sea level
E4	55°36′16″ N 159°27′40″ E	Middle part of CKD, plains habitat, 90 m above sea level
E5	55°19′39″ N 159°15′11″ E	Middle part of CKD, over-floodplain terrace of Kamchatka River, 145 m above sea level
E6	56 °32′11″ N 161°02′15″ E	Northern part of CKD, Shiveluch volcano foot, south-western slope, 150 m above sea level

CKD, Central Kamchatka depression.

populations of the Jezo spruce in this area and the relationship between the variability and the degree of volcanic effects (Vetrova et al., 2014). The objectives of this work included the allocation of Jezo spruce morphotypes by the shape of the cone scales using geometric morphometrics, a comparison of the genetic characteristics of morphologically distinct groups of trees using isozyme analysis, and estimation of the diversity and similarity of the populations by the frequency of the occurrence of morphotypes, as well as the frequency of the alleles of the polymorphic loci.

MATERIALS AND METHODS

This study included six cenopopulations of the Jezo spruce from the northern and southern areas of the coniferous island of Central Kamchatka. The three northern cenopopulations were located in areas of active volcanism: the southwestern foot of the Shiveluch volcano (E6) and at the western foot of the Ushkovsky volcano (E1 and E2), the territories that starting from different times were no longer affected by dry rivers, and three samples (E3, E4, and E5) were located in an area of weak ash falls in the southern region of the Central Kamchatka Depression (CKD), in the plain and valley spruce forests (Table 1).

The material for the study consisted of vegetative buds and cones collected from 28–30 trees in each of the six cenopopulations in September and October 2012. All of the studied spruce stands were uneven-aged. Sampling was carried out on trees whose height was 5–7 m and their age, according to dendrochronological analysis, was 60–80 years.

The variability of the shape of the cone scales was analyzed using the guidelines for the geometric morphometrics as set out in the review article of I.Ya. Pavlinov and N.G. Mikeshina (2002) and the monograph of M. Zelditch et al. (2004). The geometric morphometrics cone scales was carried out for 170 Jezo spruce trees from six cenopopulations. We selected three cones from each tree, five scales from the middle of each cone, and then the five most representative scales for each tree from these 15 samples. Tagging and allocating the coordinates were carried out on the scanned images of the scales using a screen digitizer (Rolf, 2010).

Cone scales are bilaterally symmetrical structures; thus, in order to characterize their shape, we selected 11 landmarks on one of the sides of the scales. Figure 1 shows the alignment of the landmarks by the contour of the scales in accordance with the angular algorithm. Landmarks 1 and 2 were put on the axis of symmetry at the base and apex of the scales (Fig. 1a); Landmark 3 corresponds to the point of bending of the drawn scale apex and Landmark 4 corresponds to the intersection of the lateral line of the scales with the line perpendicular to the axis of symmetry from its center. The remaining landmarks (5, 6, 7, 8, 9, 10, and 11) were arranged in accordance with the angular algorithm: at 20, 35, 50, 70, 115, 135, and 155 degrees to the symmetry axis, respectively (Fig. 1a).

The starting positions of the landmarks were normalized using the Procrustes superimposition of the sample of the scales with an average configuration in the program CoordGen6 (Sheets, 2001). Data processing and analysis was carried out using the IMP-software package (integrated morphometrics programs). To isolate the morphotypes, individual samples of cone scales from all 170 trees were compared pairwise in the Twogroup program for Goodall's test for special Procrustes distances using the bootstrap resampling (Sheets, 2001). Trees whose cone-scale samples were significantly different according to Goodall's test at a significance level of $p \leq 0.05$ were attributed to different morphotypes by the shape of the cone scales. Trees with similar morphotypes were united in groups that

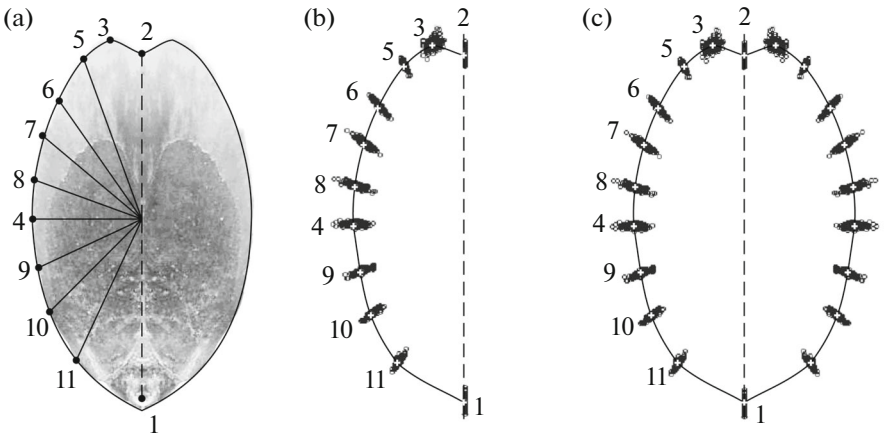

Fig. 1. Geometric morphometrics of cone scales of Jezo spruce: a, alignment of landmarks on the contour of scales; b, superimposition of sampling of scales (N = 170) with an average configuration by method of Sliding Baseline Registration (SBR); c, duplication and reflection of coordinates of landmarks from the symmetry axis for a complete image of scales.

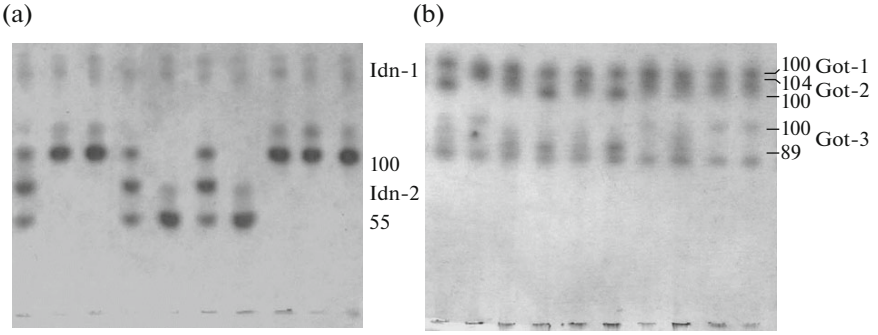

Fig. 2. Electrophoregrams of isocitrate dehydrogenase (a) and glutamate oxaloacetate transaminase (b) of Jezo spruce.

were compared for genetic diversity. The composition and frequency of the morphotypes were used to calculate the indicators of the phenotypic diversity (μ) and similarity of populations (r) and the phenotypic distances (D) between the samples (Zhivotovsky, 1991) according to the formulas below:

$$\mu = \left(\sum_{i=1}^{m} \sqrt{p_i} \right)^2; \quad r = \sum_{i=1}^{m} \sqrt{p_i q_i}; \quad D = \frac{2\sqrt{2}}{\pi} \sqrt{1 - r},$$

where m is the total number of morphotypes detected in all the samples; and p_i and q_i are the frequencies of the morphotypes in the compared populations.

The genetic variation of the spruce was determined using the allozyme analysis of the tissues of the vegetative buds. Homogenization of the vegetative buds for isoenzyme analysis was carried out in 1–2 drops of the extraction buffer 0.05 M Tris-HCl pH 7.7 containing dithiothreitol (0.06%), Trilon B (0.02%), and β-mercaptoethanol (0.05%). The extracts were separated by horizontal electrophoresis in 13% starch gel. Each extract was tested in three buffer systems: morpholin-citrate, pH 7.0 (Clayton and Tretiak, 1972) and Tris-citrate, pH 8.5; lithium borate hydroxide, pH 8.1 (Ridgway et al., 1970); and tris-EDTA-borate, pH 8.6 (Markert and Faulhaber, 1965). The histochemical staining of enzymes was carried out by the standard methods adapted to the object of study. The identified areas of activity of the enzymes and their encoding loci were numbered in descending order of their electrophoretic mobility. Alleles were designated in accordance with the mobility of the encoded allozymes with respect to the most common allozyme whose mobility was taken as 100 (Ayala, 1984). Figure 2 shows examples of the electrophoregrams of isocitrate dehydrogenase and glutamate oxaloacetate transaminase of Jezo spruce.

Preliminary analysis of the bud samples from the Kamchatka populations of Jezo spruce identified 15 isozyme loci (Vetrova et al., 2014) which were previously used as markers in the genetic studies of this species (Potenko, 2007). Electrophoretic analysis was performed for ten enzyme systems: phosphoglucomutase (*PGM*, EC 2.7.5.1), glutamate oxaloacetate trans-

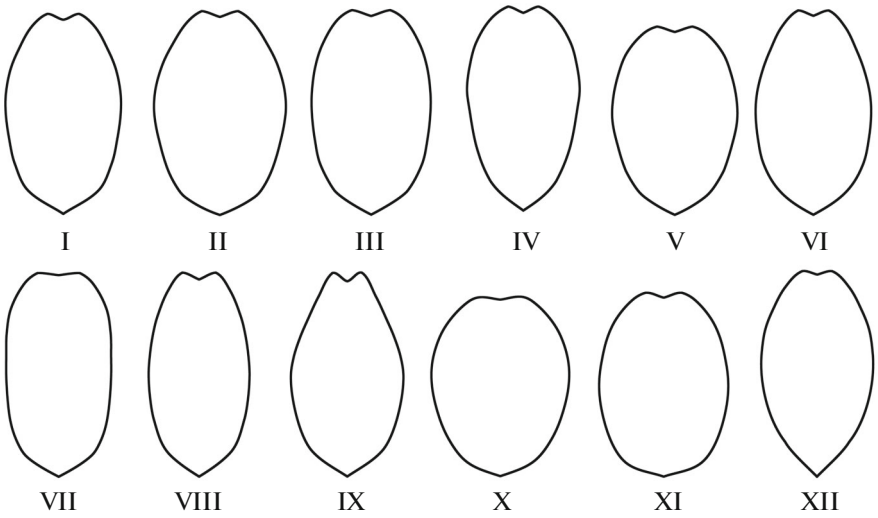

Fig. 3. Diversity of shapes of cone scales in the studied populations of *P. ajanensis*. Digits indicate numbers of morphotypes.

aminase (*GOT*, EC 2.6.1.1), hexokinase (*HK*, 2.7.1.1), leucine aminopeptidase (*LAP*, EC 3.4.11.1), malate dehydrogenase (*MDH*, EC 1.1.1.37, shikimate dehydrogenase (*SKDH*, EC 1.1.1.25), isocitrate dehydrogenase (*IDH*, EC 1.1.1.42), glutamate dehydrogenase (*GDH*, EC 1.4.2.3), phosphoglucose isomerase (*PGI*, EC 5.3.1.9), and superoxide dismutase (*SOD*, 1.15.1.1).

To determine the genetic diversity of the groups of trees selected by the similarity of the shape of the cone scales, just as for the cenopopulations of spruce, certain generally accepted variability indicators were calculated for 15 loci: the percentage of polymorphic loci (*P*), the average number of alleles per locus (N_a), the effective number of alleles (N_e), and the average observed (H_o) and expected (H_e) heterozygosity. The genetic structure of the morphotypes and populations was determined by the indicators of Wright's F-statistics (Guries and Ledig, 1982). The extent of the genetic differences between the morphotypes was estimated according to the method proposed by M. Nei (Nei, 1972). The indicators were calculated in the computer program GenAlex 6 (Peakall and Smouse, 2006). The reliability of the genetic differences between the morphotypes for allele frequencies of the 15 loci was estimated using the χ^2 test.

RESULTS

Pairwise comparison of individual samples of cones from 170 trees made it possible to allocate 12 morphological forms of spruce by the configuration of the cone scales. The average configurations of all the morphotypes of the cone scales obtained in the CoordGen6f program (Sheets, 2001) are shown in Fig. 3.

The frequency of the occurrence of different morphotypes in the total sample of spruce trees ranges from 1.2% to 24%. The highest frequency of occurrence is marked for two morphotypes: II (23.5%) and III (24.1%). Morphotypes X, XI, and XII are rare with a frequency of occurrence of 1.2%, as are morphotypes IV and VIII (1.8%).

The frequencies of the alleles of the studied isozyme loci in the selected morphotypes of Jezo spruce are shown in Table 2. Six of the fifteen analyzed isozyme loci of Jezo spruce are monomorphic (*Got-1*, *Lap-2*, *Mdh-2*, Sod-*1*, Sod-*2*, and *Gdh*). Four loci (*Got-2*, *Skdh-1*, *Idh-2*, and *Pgm-2*) were attributed as highly polymorphic, three loci (*Mdh-1*, *Mdh-3*, and *Pgm-1*) as moderately polymorphic, and two loci (*Pgi-2* and *Hk*) as weakly polymorphic. The alleles alternative to the main one detected in these loci are not found in every morphotype (Table 2). Two of them, $Pgi\text{-}2^{66}$ and $Pgi\text{-}2^{90}$, are rare: $Pgi\text{-}2^{66}$ was marked only in morphotype II (frequency 1.3%), $Pgi\text{-}2^{90}$ only in morphotype V (frequency 3.1%). Another rare allele was detected in locus *Pgm-1* ($Pgm\text{-}1^{96}$), and only in morphotype I (2.1%). A total of 27 alleles were revealed in 15 loci.

Comparison of spruce morphotypes for the significance of differences between them for the allele frequencies of nine polymorphic loci showed that for a combination of these loci the differences were insignificant; however, for two loci (*Pgm-2* and *Mdh-1*), we revealed differences for eight pairs of compared morphotypes at the significance level $p \leq 0.05$ (Table 3). Significant differences in the allele frequencies in locus *Pgm-2* were detected by comparing morphotype VII with all the other morphotypes, and the allele frequencies of locus *Mdh-1* were found to be differ for morphotypes V, II, and III (Table 3).

The parameters of genetic diversity for the trees of each of the selected morphotypes of Jezo spruce are shown in Table 4. The presented data show that the

Table 2. Allele frequencies of 15 isozyme loci in groups of trees of seven major morphotypes of Jezo spruce

Locus	Allele	Morphotypes (number of samples).						
		I (24)	II (40)	III (41)	V (16)	VI (21)	VII (10)	IX (6)
Got-1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Got-2	100	0.542	0.488	0.598	0.500	0.476	0.500	0.417
	104	0.458	0.513	0.402	0.500	0.524	0.500	0.583
Lap-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Skdh-1	80	0.354	0.438	0.341	0.438	0.357	0.400	0.417
	100	0.646	0.563	0.659	0.563	0.643	0.600	0.583
Mdh-1	91	0.083	0.063	0.061	0.188	0.119	0.050	0.000
	100	0.917	0.938	0.939	0.813	0.881	0.950	1.000
Mdh-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-3	68	0.104	0.100	0.061	0.031	0.048	0.050	0.167
	100	0.896	0.900	0.939	0.969	0.952	0.950	0.833
Idh-2	55	0.271	0.238	0.256	0.156	0.238	0.150	0.167
	100	0.729	0.763	0.744	0.844	0.762	0.850	0.833
Sod-1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Sod-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gdh	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-1	96	0.021	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.833	0.850	0.725	0.719	0.810	0.850	0.917
	108	0.146	0.150	0.275	0.281	0.190	0.150	0.083
Pgm-2	100	0.354	0.438	0.402	0.344	0.452	0.100	0.583
	105	0.646	0.563	0.598	0.656	0.548	0.900	0.417
Pgi-2	66	0.000	0.013	0.000	0.000	0.000	0.000	0.000
	90	0.000	0.000	0.000	0.031	0.000	0.000	0.000
	100	1.000	0.988	1.000	0.969	1.000	1.000	1.000
Hk	90	0.000	0.000	0.012	0.063	0.000	0.000	0.000
	100	0.958	0.975	0.963	0.906	0.952	1.000	1.000
	110	0.042	0.025	0.024	0.031	0.048	0.000	0.000

percentage of loci that exhibit variability varies in the groups of trees selected by the shape of cone scales from 40 to 60%. The average number of alleles per locus varies from 1.400 to 1.667 and the effective number of alleles varies from 1.202 to 1.307. The average values of the observed and expected heterozygosity vary in the range of 0.120 to 0.183 and from 0.124 to 0.181, respectively. The highest values of almost all the indicators of genetic diversity are observed in the group of trees of morphotype V; and the lowest, in trees of morphotype VII (Table 4.). The sample of trees with morphotype IX with a low polymorphism and the average number of alleles per locus is characterized by a relatively high observed heterozygosity.

The extent of genetic differentiation of the seven major morphotypes of Jezo spruce studied was estimated based on genetic distances D (Nei, 1972) calculated for the frequencies of the alleles of all the analyzed isozyme loci. The data presented in Table 5 show that the genetic distance D between groups of trees of different morphotypes varies in a fairly wide range of values: from 0.001 to 0.020. The closest in terms of the genetic structure (D = 0.001–0.002) were morphotypes I, II, III, and VI. The greatest difference in the genetic structure (D = 0.020) was observed in morphotypes VII and IX. Differences between morphotypes in terms of the genetic structure are consistent with their differences in terms of the shape of the cone

Table 3. Results of comparison of morphotypes of Yezo spruce for allele frequencies of loci *Pgm*-2 and *Mdh*-1

Morphotypes	Loci	Number of alleles	χ^2	df	*p*	Morphotypes	Loci	Number of alleles	χ^2	df	*p*
I–II	*Mdh-1*	2	0.199	1	0.65	I–V	*Mdh-1*	2	1.905	1	0.177
	Pgm-2	2	0.864	1	0.35		*Pgm-2*	2	0.009	1	0.924
I–III	*Mdh-1*	2	0.235	1	0.63	I–VII	*Mdh-1*	2	0.230	1	0.631
	Pgm-2	2	0.298	1	0.585		***Pgm-2***	**2**	**4.530**	**1**	**0.033**
I–IX	*Mdh-1*	2	1.071	1	0.301	II–III	*Mdh-1*	2	0.002	1	0.968
	Pgm-2	2	2.101	1	0.147		*Pgm-2*	2	0.204	1	0.651
II–IX	*Mdh-1*	2	0.793	1	0.373	II–V	***Mdh-1***	**2**	**4.032**	**1**	**0.045**
	Pgm-2	2	0.894	1	0.344		*Pgm-2*	2	0.830	1	0.362
III–V	***Mdh-1***	**2**	**4.227**	**1**	**0.040**	II–VI	*Mdh-1*	2	1.170	1	0.279
	Pgm-2	2	0.335	1	0.563		*Pgm-2*	2	0.025	1	0.875
III–VI	*Mdh-1*	2	1.263	1	0.261	II–VII	*Mdh-1*	2	0.044	1	0.833
	Pgm-2	2	0.285	1	0.594		***Pgm-2***	**2**	**7.819**	**1**	**0.005**
III–VII	*Mdh-1*	2	0.035	1	0.852	V–VI	*Mdh-1*	2	0.672	1	0.412
	Pgm-2	**2**	**6.525**	**1**	**0.011**		*Pgm-2*	2	0.889	1	0.346
III–IX	*Mdh-1*	2	0.773	1	0.380	V–VII	*Mdh-1*	2	1.997	1	0.158
	Pgm-2	2	1.401	1	0.236		***Pgm-2***	**2**	**3.900**	**1**	**0.048**
VI–VII	*Mdh-1*	2	0.739	1	0.390	V–IX	*Mdh-1*	2	2.605	1	0.106
	Pgm-2	**2**	**7.511**	**1**	**0.006**		*Pgm-2*	2	2.072	1	0.150
VI–IX	*Mdh-1*	2	1.574	1	0.210	VII–IX	*Mdh-1*	2	0.619	1	0.431
	Pgm-2	2	0.641	1	0.423		***Pgm-2***	**2**	**8.667**	**1**	**0.003**

df, number of degrees of freedom, *p*, significance level.

Table 4. Parameters of genetic variability of major morphotypes of Jezo spruce isolated by the shape of cone scales

Morphotypes (number of samples)	*P*, %	N_a	N_e	H_o	H_e	*F*
I (24)	53.3	1.600 ± 0.163	1.281 ± 0.095	0.156 ± 0.046	0.167 ± 0.051	0.022 ± 0.041
II (40)	60.0	1.600 ± 0.131	1.285 ± 0.102	0.177 ± 0.057	0.165 ± 0.052	–0.057 ± 0.019
III (41)	53.3	1.600 ± 0.163	1.286 ± 0.098	0.170 ± 0.054	0.166 ± 0.053	–0.031 ± 0.025
V (16)	60.0	1.667 ± 0.159	1.307 ± 0.098	0.183 ± 0.060	0.181 ± 0.052	0.022 ± 0.080
VI (21)	53.3	1.533 ± 0.133	1.287 ± 0.099	0.162 ± 0.052	0.168 ± 0.052	0.017 ± 0.055
VII (10)	46.7	1.467 ± 0.133	1.202 ± 0.086	0.120 ± 0.047	0.124 ± 0.045	0.024 ± 0.076
IX (6)	40.0	1.400 ± 0.131	1.252 ± 0.099	0.178 ± 0.079	0.144 ± 0.052	–0.125 ± 0.162
Mean ± SE	52.4 ± 2.7	1.552 ± 0.054	1.272 ± 0.036	0.164 ± 0.021	0.159 ± 0.019	–0.015 ± 0.027

P, percentage of polymorphic loci; N_a, average number of alleles per locus; N_e, effective number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; *F*, Wright's fixation index.

scales, which are quantitatively expressed by the Procrustes distances (Table 5).

The correlation between the matrices of the phenotypic and genetic distances, which was calculated by Spearman's rank correlation coefficient, is 0.40 (p = 0.01). This implies that the greater the differences of the spruce trees in terms of the shape of the cone scales the more likely it is that are genetic differences between them.

The populations differed in terms of the composition and frequencies of morphotypes. Each cenopopulation was represented by 5–7 morphotypes in terms of the shape of the cone scales, and usually 1–2 morphotypes were dominant. It should be noted that four of the 12 morphotypes, including three rare ones, are

Table 5. Genetic distances *D* (Nei, 1972) and phenotypic (Procrustes) distances between the studied morphotypes of Jezo spruce

Morphotypes	II	II	III	V	VI	VII	IX
I	0	0.044	0.024	0.065	0.024	0.040	0.057
II	0.002	0	0.040	0.032	0.059	0.079	0.086
III	0.002	0.003	0	0.052	0.045	0.046	0.078
V	0.005	0.005	0.004	0	0.085	0.097	0.114
VI	0.002	0.001	0.002	0.003	0	0.041	0.035
VII	0.007	0.010	0.010	0.008	0.011	0	0.063
IX	0.008	0.004	0.010	0.013	0.006	0.020	0

White part indicates phenotypic distances; gray part indicates Nei's genetic distances *D*.

Table 6. Parameters of genetic variation of the studied cenopopulations of Jezo spruce and their diversity by the shape of cone scales

Sample	*P*, %	N_a	N_e	H_o	H_e	*F*	$\mu \pm S\mu$
E1	46.67	1.467 ± 0.133	1.256 ± 0.094	0.147 ± 0.047	0.152 ± 0.050	−0.007 ± 0.034	3.621 ± 0.221
E2	53.33	1.600 ± 0.163	1.287 ± 0.100	0.147 ± 0.049	0.167 ± 0.052	0.093 ± 0.068	4.749 ± 0.199
E3	60.00	1.667 ± 0.159	1.298 ± 0.096	0.211 ± 0.068	0.176 ± 0.052	−0.132 ± 0.049	5.825 ± 0.202
E4	60.00	1.600 ± 0.131	1.248 ± 0.099	0.147 ± 0.052	0.143 ± 0.050	−0.044 ± 0.035	4.505 ± 0.273
E5	53.33	1.600 ± 0.163	1.278 ± 0.098	0.189 ± 0.064	0.163 ± 0.051	−0.101 ± 0.062	4.961 ± 0.080
E6	53.33	1.533 ± 0.133	1.283 ± 0.102	0.175 ± 0.060	0.162 ± 0.053	−0.048 ± 0.043	3.657 ± 0.395
Average	54.44	1.578 ± 0.059	1.275 ± 0.039	0.169 ± 0.023	0.161 ± 0.020	−0.043 ± 0.021	4.553 ± 0.836

P, percentage of polymorphic loci; N_a, average number of alleles per locus; N_e, effective number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; *F*, Wright's fixation index; μ, index of phenotypic diversity for frequencies of morphotypes; *S*μ, standard error of μ.

found only in the northern group of samples. Thus, morphotype VII, which is genetically different from all the morphotypes in terms of the allele frequencies of locus *Pgm-2*, is common under the Shiveluch volcano (sample E6) and was observed under the Ushkovsky volcano (sample E1), morphotype IV was observed in all three northern samples (E1, E2, and E6), morphotype X was observed only in E1, and morphotype XII was observed only in E2. Two morphotypes are found only in the southern group of populations: the rare morphotype XI is observed only in E4, while morphotype IX is common only in E5 and is not found in other populations. These phenotypic differences between the southern and northern population groups are likely to be the result of genetic differentiation in the past.

The indicators of the phenotypic and genetic diversity of the populations are listed in Table 6. Differences in the genetic diversity of the samples are generally consistent with the differences in their phenotypic diversity by the shape of the cone scales. The highest level of genetic variation and phenotypic diversity is observed in the southernmost spruce cenopopulation among all those included in the analysis (E3) and located in the subzone of weak ash falls (Table 6). The high level of observed heterozygosity and phenotypic diversity was also observed in another sample (E5) from that area (Table 6).

The lowest level of both the genetic and phenotypic diversity was observed in the spruce cenopopulation from the foot of the Ushkovsky volcano (E1), which characterizes the initial stage of spruce formation on the dry river sediments. At the final stage of the formation of a spruce forest in these conditions (sample E2), the level of phenotypic diversity is above average at an average level of genetic polymorphism and a deficit of heterozygous genotypes (Table 6). The low level of phenotypic diversity and the average level of genetic variability were observed in the sample from a spruce forest at the foot of the Shiveluch volcano (E6), which is experiencing catastrophic volcanic influence (Table 6).

The genetic distances *D* (Nei, 1972) and the phenotypic distances between the studied cenopopulations of Jezo spruce calculated based on the criterion of the similarity of the populations in terms of the composition and the frequency of morphotypes are listed in Table 7. The highest phenotypic differences from other populations were observed for the two samples from the northern group from the foot of the Ushkovsky and Shiveluch volcanoes (E1 and E6). The sample from the Shiveluch volcano (E6) is very different from most of the other populations in terms of the

Table 7. Genetic distances *D* (Nei, 1972) and phenotypic distances between the studied cenopopulations of Jezo spruce

Cenopopulations	E1	E2	E3	E4	E5	E6
E1	0	0.608	0.581	0.701	0.631	0.709
E2	0.009	0	0.348	0.527	0.524	0.576
E3	0.005	0.013	0	0.421	0.455	0.623
E4	0.009	0.017	0.016	0	0.596	0.626
E5	0.005	0.012	0.008	0.005	0	0.538
E6	0.015	0.024	0.008	0.019	0.010	0

White part indicates phenotypic distances calculated by the composition and frequency of morphotypes; gray part indicates Nei's genetic distances D.

genetic structure, while the sample from the Ushkovsky volcano (E1) is poorly differentiated from the other investigated cenopopulations (Table 7). The correlation between the genetic and phenotypic distances of the populations is not identified.

DISCUSSION

The differences between the genetic and phenotypic structure of the Jezo spruce population at the foot of the Shiveluch volcano are due to the strong and prolonged volcanic impact. It was found that this volcano had at least 60 large eruptions just in the Holocene, and in recent years, its activity and the intensity of ash falls have been increasing. Currently, spruce forests in this area are at the stage of withering away. The upper canopy of trees has been observed to shrink and the number of trees of the second layer, which has still preserved vitality, is decreasing. Perhaps, in these circumstances, the reproductive ability is retained in the spruce genotypes that are most resistant to ash falls, which explains the differences in the genetic structure of this population and a reduction of the phenotypic diversity of the trees by the shape of the cone scales. The decrease in the genetic and phenotypic diversity, which was marked at the initial stage of the formation of the spruce forest on deposits of the dry river at the foot of the Ushkovsky volcano is due to the small initial population size, and probably indicates that this population originates from a small number of parental specimens.

We have previously found (Vetrova et al., 2014) that the most significant contribution to the interpopulation differentiation of the Jezo spruce populations in Kamchatka belongs to loci *Pgm-1* (F_{st} = 0.085) and *Idh-2* (F_{st} = 0.083). The weak differences between the morphotypes for these loci explain the lack of association between the genetic and phenotypic differentiation of the spruce populations in the current study.

The correlation of the genetic and phenotypic distances between the groups of trees of different morphotypes is consistent with the genetic determination of a trait such as the shape of the cone scales of spruce trees. Significant differences between the spruce morphotypes were identified for two loci: the highly polymorphic locus *Pgm-2* and moderately polymorphic *Mdh-1.*

The data on the population variation of Jezo spruce, which were obtained based on molecular genetic analysis, are generally consistent with the data on the phenotypic diversity of the populations in terms of the shape of the cone scales. A high level of genetic and phenotypic diversity characterizes the stable population structure of Jezo spruce outside the influence of volcanoes. Changes in the genetic structure and a low level of phenotypic diversity are observed in pessimal growing conditions under the catastrophic volcanic effects.

The geometric morphometrics of cones made it possible to differentiate spruce morphotypes and to quantify the diversity and similarity of the populations. This method enhances the phenotypic approach in the analysis of the population structure and can be recommended for other conifers. Further studies of the relationship between the phenotypes and genotypes of spruce need to extend the set of DNA and phenotypic markers.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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