GENETIC VARIATION OF YEDDO SPRUCE POPULATIONS IN RUSSIA

Vladimir V. Potenko & Yuri D. Knysh

Department of Genetics and Breeding, Forestry Breeding and Seed Production Center, 12 Nagornaya Str., Sosnovka, 680555 Khabarovsk Territory, Russia

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ABSTRACT

Genetic diversity and genetic differentiation of eight populations of Yeddo spruce (*Picea jezoensis* (Sieb. et Zucc.) Carr.) from the Russian Far East mainland and one from the Kamchatka Peninsula were studied analyzing allozyme variation in 20 loci. The mean number of alleles per locus (*A*) was 2.63, the percent of polymorphic loci (*P*) was 88.1 %, the observed heterozygosity (H_o) was 0.181 and the mean value of expected heterozygosity (H_e) amounted to 0.189 for eight mainland populations. The values of expected heterozygosity of the northern and central mainland populations were higher than in the southern part of the natural range. Regional differences in genetic variability could be due to genetic drift in the southern populations in the middle Holocene when warmer temperatures caused an upward elevational shift of spruce populations, resulting in small, isolated populations. Only 2.4 % of the total genetic variability can be attributed to variation among mainland populations. The values of genetic variation in the Kamchatka Peninsula population were different from those in the mainland populations: $A = 1.85$, $P = 70.0$, $H_e = 0.241$. Unbiased Nei's genetic distance values (D_N) were low between the mainland populations of *P. jezoensis* and averaged 0.006. The largest values were revealed between the mainland populations and the Kamchatka Peninsula population (at average, $D_N = 0.081$) that were similar to the genetic distances observed between closely related conifer species.

Key words: *Picea jezoensis*, allozymes, genetic variation, genetic differentiation

INTRODUCTION

Yeddo spruce, *Picea jezoensis* (Sieb. et Zucc.) Carr., occurs in natural and artificial stands in Russia, China, Korea, and Japan. In the Russian Far East, *P. jezoensis* is distributed in the Primorski Territory, in the central and southern parts of the Khabarovsk Territory, in the Jewish Autonomous Region, in the Amur Territory, in the southeast of the Republic of Sakha (Yakutia), on Sakhalin Island, on the southern Kuriles, and in the central part of the Kamchatka Peninsula. Yeddo spruce is a dominant species in the mountain spruce-fir forests (mainly with Manchurian fir, *Abies nephrolepis* (Trautv.) Maxim.) of the Russian Far East that support hydrologic and climatic conditions of the territory (MAN'KO 1987). In Russia, the usual Latin name for Yeddo spruce is *P. ajanensis.*

Various studies of morphological and genetic diversity of Yeddo spruce were conducted. Four spruce species (*P. kamtchatkensis* Lacas., *P. ajanensis* Fish., *P. microsperma* (Lindl.) Carr. and *P. komarovii* V. Vassil.) were described on the basis of morphological traits (KOMAROV 1934, VASIL'EV 1950) within the Russian part of Yeddo spruce natural range. However, recent results of morphological diversity research in native Russian populations on the Sikhote-Alin mountain range do not support the subdivision of *P. jezoensis* into a number of distinct species (FROLOV 1993, POTEMKIN 1994). Higher morphological diversity of the northern Sikhote-Alin populations in comparison with the southern Sikhote-Alin populations was also revealed (FROLOV 1993). Studies of allozyme variation in one of *P. jezoensis* populations on the Kamchatka Peninsula (GÖMÖRY & PAULE 1990), three populations on Sakhalin Island and one population in the Khabarovsk Territory (GONCHARENKO & POTENKO 1992, GONCHARENKO & PADUTOV 2001), and one population on Shikotan Island (GONCHARENKO & PADUTOV 2001) have been conducted. High levels of genetic diversity in the populations were revealed. Levels of genetic differentiation between Yeddo spruce and other Eurasian spruces were also described in the studies. Almost all previously analyzed populations are geographically isolated and represented marginal parts of Yeddo spruce natural range. However, assessments of range-wide allozyme variation of Yeddo spruce from the mainland areas have not been reported. Therefore, the aim of this study was to investigate levels of genetic variation and differentiation of the nine Yeddo spruce populations representing a range-wide collection from the mainland and the Kamchatka Peninsula.

MATERIALS AND METHODS

Seeds for electrophoresis were collected in nine native populations of Yeddo spruce (Fig. 1). In eight populations seeds were collected from 282 individual trees. A seed lot collected from a native population by state forest farm for artificial reforestation provided sample from the Kamchatka Peninsula. Table 1 displays the data on the population locations and the number of trees analyzed.

Six megagametophytes per tree or 94 megagametophytes per seed lot were subjected to horizontal starch gel electrophoresis. Details of laboratory procedures were described in POTENKO & VELIKOV (1998). Seed tissues were analyzed for 14 enzyme systems (abbreviations and EC number in parentheses): aspartate aminotransferase (AAT, 2.6.1.1), alcohol dehydrogenase (ADH, 1.1.1.1), fluorescent esterase (Fl-EST, 3.1.1.1), glucose-6-phosphate isomerase (GPI, 5.3.1.9), glutamate dehydrogenase (GDH, 1.4.1.3), hexokinase (HK, 2.7.1.1), isocitrate dehydrogenase (IDH, 1.1.-1.42), leucine aminopeptidase (LAP, 3.4.11.1), malate dehydrogenase (MDH, 1.1.1.37), malic enzyme (ME, 1.1.1.40), phosphoglucomutase (PGM, 2.7.5.1), 6-phosphogluconate dehydrogenase (6-PGD, 1.1.1.44), shikimate dehydrogenase (SkDH, 1.1.1.25) and sorbitol dehydrogenase (SDH, 1.1.1.14). Enzyme staining followed standard methods (CHELIAK & PITEL 1984) with non-significant modifications. Inheritance of electrophoretic enzyme variants of Yeddo spruce was determined earlier (GONCHARENKO & PADUTOV 2001).

Some attention must be paid to inheritance of enzyme variants attributed to the *Aat-2* zone. Unlike the other Eurasian spruces (LUNDKVIST 1979, POULSEN *et al.* 1983, MUONA *et al.* 1987, GONCHARENKO & PADUTOV 2001, LEWANDOWSKI *et al.* 2002), the two-banded AAT-2 zone of Yeddo spruce has both simultaneously ($Aat\text{-}2^{0.65}$, $Aat\text{-}2^{1.00}$, $Aat\text{-}2^{1.15}$, and $Aat\text{-}2^{1.17}$)

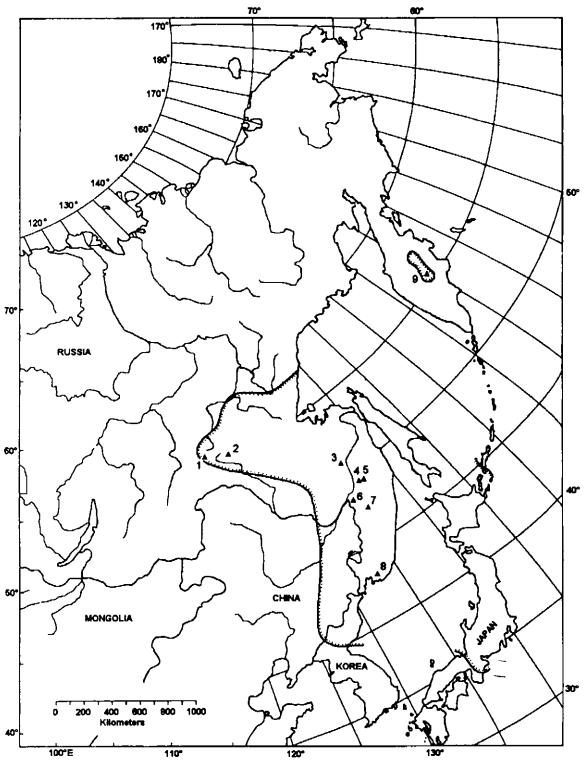

Figure 1. Range of *P. jezoensis* (MAN'KO 1987) and the locations of nine populations sampled for electrophoretic analysis

and non-simultaneously ($Aat\text{-}2^{1.20}$ and $Aat\text{-}2^{0.80}$) migrating variants (Fig. 2). No significant deviations from the expected 1:1 segregation ratio were observed in any heterozygous tree with simultaneously or non-simultaneously migrating bands at the *Aat-2* zone tested by Chi-square criterion ($P < 0.001$). Segregation patterns were received from 16 and 22 megagametophytes of two heterozygous trees with non-simultaneously migrating bands in combination $Aat\text{-}2^{1.00/1.20}$

Table 1. Location of the sampled population and number of analyzed trees.

Population / Location		Latitude (° N)	Longitude (° E)	Number of trees
12	Khorogochi (Amur Territory)	55.3	123.8	28
34	Dipkun (Amur Territory)	55.1	126.8	28
56	Snezhnyi (Khabarovsk Territory)	50.8	136.3	34
78	Burga (Khabarovsk Territory)	49.1	136.9	33
9	Tukhala (Khabarovsk Territory)	49.1	137.3	54
	Khekhtsir (Khabarovsk Territory)	48.3	135.0	37
	Dolmi (Khabarovsk Territory)	47.4	135.7	26
	Uglekamensk (Primorski Territory)	43.4	133.2	42
	Atlasovo (Kamtchatka Peninsula)[a]	55.3	159.7	94[b]

[a] Bulked seed lot analyzed. Total weight of seed lot is 50 kg.
[B] Number of analyzed seeds per seed lot.

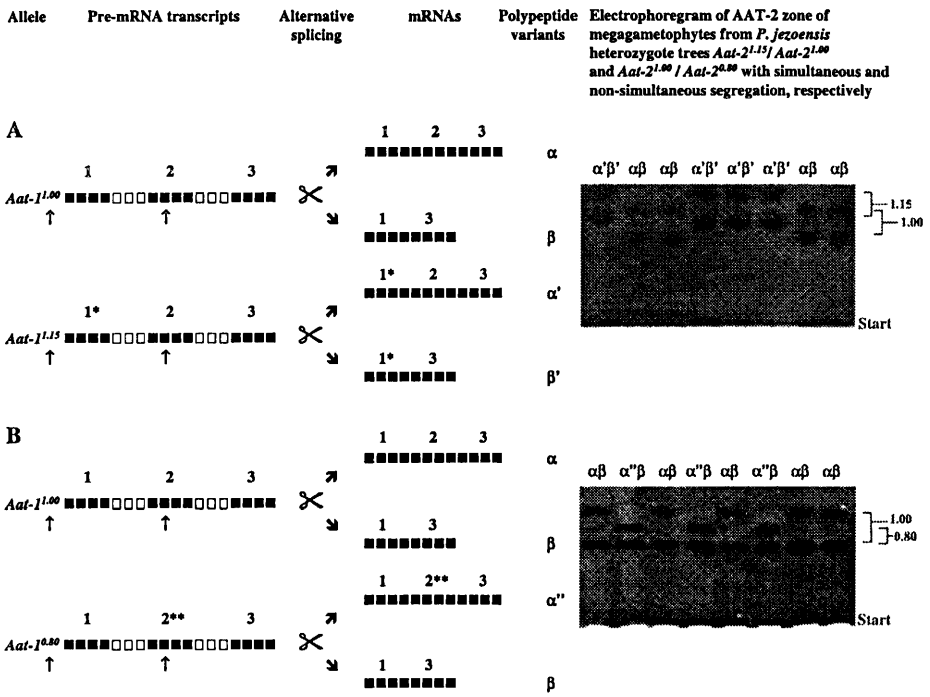

Figure 2. Generalized schemes of two types (A and B) of alternative splicing of pre-mRNAs transcribed from a single enzyme-coding gene (modified from MANCHENKO 2001) that explain the enzyme electrophoregram phenotypes of megagametophytes of *P. jezoensis* with simultaneously and non-simultaneously migrating bands of *Aat-2* zone. Introns and exons are numbered and shown by light (□ ▥□) and dark boxes (▮▮▮), respectively. The pre-mRNA transcripts are spliced (✂) in two alternative ways. Vertical arrows (⇑⇑) indicate sites of alternative splicing. Exons containing nucleotide substitutions responsible for different allozyme variations are marked by different number of asterisks (*, **). Allelic variants of polypeptides are designated α', α'' and β', respectively.

and *Aat-2*$^{1.00/0.80}$, respectively. A possible explanation is that the two-banded *Aat-2* isozymes are the result of alternative splicing. Alternative splicing is a mechanism where parts of the pre-mRNA are either excluded from or included in the mature mRNA. It allows different protein isoforms to be created from a single gene. At the single gene level, nucleotide substitutions in exon not subjected to alternative splicing is expressed in simultaneously migrating protein isoforms (Fig. 2, A), while nucleotide substitutions in exon subjected to alternative splicing is expressed in non-simultaneously migrating protein isoforms (Fig. 2, B) (SMITH *et al.* 1989, MANCHENKO 2001). In this case simultaneously and non-simultaneously migrating variants at the *Aat-2* zone can be considered as products of different alleles of single gene. It may be noted that in humans about 30% of all pre-mRNA transcripts are subject to alternative splicing (GELFAND *et al.* 1999).

Allele frequencies were analyzed using the BIOSYS-1 computer program (SWOFFORD & SELANDER 1989). For each population, the mean number of alleles per locus (A), percentage of polymorphic loci (P and P_{95}), observed (H_o) and expected heterozygosity (H_e) were computed. In addition, the mean number of alleles with frequency more than 5 % per locus ($A_{5\%}$) was calculated. Fixation indices (F_{IS}, F_{IT} and F_{ST}) were used for assaying the population genetic structure (NEI 1977). Calculations of the F-statistics, observed heterozygosity (H_o) and chi-square tests of homogeneity for allele frequency variation among populations were performed for the eight populations where individual tree seed collections were conducted (Table 1). NEI's unbiased genetic distances (NEI 1978) were calculated. The relationships among the populations were visualized by constructing of phylogenetic tree by unweighted pair-group method with arithmetic means (UPGMA), using the genetic distances and bootstrap estimates to test the reliability of the tree using the DISPAN computer program (OTA 1993).

RESULTS

Staining of the 14 enzymes revealed gene products of 20 loci. Without exception, variant alleles were found at every locus (Table 2). Most polymorphic loci were *Aat-2*, *Idh-1*, *Sdh* (6 alleles at each locus) and *Idh-2*, *Mdh-3* and *Hk* (5 alleles at each locus). The mainland

Table 2. Allele frequencies in the natural populations of *P. jezoensis*.

Locus/ allele	Population								
	Khorogochi	Dipkun	Snezhnyi	Burga	Tukhala	Khekhtsir	Dolmi	Uglekamensk	Atlasovo
Aat-1									
0.90	.0	.0	.0	.0	.009	.0	.0	.0	.0
1.00	1.000	.982	.926	.894	.963	.905	.981	.905	.564
1.05	.0	.0	.0	.061	.0	.0	.0	.012	.0
1.10	.0	.018	.074	.045	.028	.095	.019	.083	.436
Aat-2									
0.65	.0	.0	.0	.015	.0	.0	.038	.0	.0
0.80	.0	.0	.0	.0	.0	.014	.0	.0	.340
1.00	1.000	1.000	.956	.985	1.000	.973	.962	.976	.660
1.15	.0	.0	.029	.0	.0	.014	.0	.012	.0
1.17	.0	.0	.0	.0	.0	.0	.0	.012	.0
1.20	.0	.0	.015	.0	.0	.0	.0	.0	.0
Adh									
0.90	.093	.093	.118	.076	.093	.027	.058	.025	.0
1.00	.407	.741	.750	.636	.713	.878	.827	.925	.574
1.10	.500	.167	.132	.288	.194	.095	.115	.050	.426
Fl-Est									
0	.0	.0	.0	.0	.0	.027	.019	.0	.0
0.70	.0	.0	.015	.0	.009	.027	.0	.012	.0
1.00	.982	.911	.897	.970	.935	.932	.962	.917	1.000
1.30	.018	.089	.088	.030	.056	.014	.019	.071	.0
Gdh									
0.65	.0	.0	.0	.0	.0	.014	.0	.0	.0
0.80	.0	.0	.0	.0	.0	.014	.019	.0	.0
1.00	.982	.964	1.000	1.000	1.000	.973	.981	1.000	1.000
1.35	.018	.036	.0	.0	.0	.0	.0	.0	.0
Gpi									
0.80	.0	.036	.059	.030	.028	.068	.019	.012	.011
1.00	1.000	.964	.926	.894	.926	.919	.942	.976	.979
1.20	.0	.0	.015	.076	.046	.014	.038	.012	.011
Hk									
0.80	.0	.0	.0	.0	.009	.0	.0	.0	.0
0.90	.018	.125	.059	.015	.074	.054	.019	.024	.0
1.00	.696	.750	.824	.833	.741	.757	.769	.881	.979
1.10	.286	.125	.103	.152	.176	.189	.212	.095	.021
1.20	.0	.0	.015	.0	.0	.0	.0	.0	.0
Idh-1									
0	.0	.0	.044	.0	.0	.0	.0	.0	.0
0.90	.0	.0	.0	.0	.009	.0	.019	.012	.0
1.00	.875	.821	.838	.894	.880	.905	.865	.917	1.000
1.05	.0	.0	.015	.0	.0	.0	.0	.0	.0
1.10	.125	.179	.103	.106	.111	.081	.115	.071	.0
1.15	.0	.0	.0	.0	.0	.014	.0	.0	.0
Idh-2									
0.70	.0	.0	.0	.0	.009	.0	.0	.0	.128
0.90	.0	.018	.015	.0	.019	.014	.0	.0	.0
1.00	1.000	.982	.985	.970	.963	.986	1.000	1.000	.872
1.05	.0	.0	.0	.030	.0	.0	.0	.0	.0
1.20	.0	.0	.0	.0	.009	.0	.0	.0	.0

populations had quite similar allele frequencies. However, the *Aat-1*, *Aat-2*, *Adh*, *Idh-2*, *Lap-1*, *Mdh-3*, *Pgm-2* and *Skdh-1* loci showed some differences in allele frequencies between the mainland populations and the Atlasovo population from the Kamchatka Peninsula.

Table 2. (continued).

Locus /allele	Population								
	Khorogochi	Dipkun	Snezhnyi	Burga	Tukhala	Khekhtsir	Dolmi	Uglekamensk	Atlasovo
Lap-1									
0	.071	.054	.015	.0	.019	.014	.019	.024	.436
0.94	.107	.054	.0	.037	.028	.027	.0	.0	.0
1.00	.821	.875	.985	.944	.954	.946	.981	.929	.564
1.04	.0	.018	.0	.019	.0	.014	.0	.048	.0
Lap-2									
0.95	.036	.0	.059	.022	.047	.014	.038	.012	.0
1.00	.911	.964	.897	.978	.925	.932	.962	.964	1.000
1.05	.054	.036	.044	.0	.028	.054	.0	.024	.0
Mdh-1									
0.90	.0	.018	.088	.030	.028	.0	.058	.012	.0
1.00	1.000	.964	.897	.970	.963	1.000	.942	.988	1.000
1.14	.0	.018	.015	.0	.009	.0	.0	.0	.0
Mdh-2									
0.80	.054	.143	.088	.076	.046	.027	.0	.048	.0
1.00	.946	.857	.897	.909	.944	.973	1.000	.952	1.000
1.20	.0	.0	.015	.015	.009	.0	.0	.0	.0
Mdh-3									
0	.214	.161	.191	.197	.120	.149	.212	.214	.404
0.45	.018	.0	.0	.0	.0	.014	.0	.0	.0
0.75	.321	.339	.382	.394	.444	.432	.442	.345	.053
0.90	.018	.0	.015	.0	.0	.014	.0	.0	.0
1.00	.429	.500	.412	.409	.435	.392	.346	.440	.543
Me									
0.20	.107	.018	.0	.0	.0	.0	.0	.0	.0
1.00	.625	.786	.882	.750	.898	.797	.865	.929	.819
1.65	.214	.089	.044	.094	.046	.176	.096	.060	.011
2.00	.054	.107	.074	.156	.056	.027	.038	.012	.170
6-Pgd-1									
0.90	.0	.018	.118	.136	.065	.095	.019	.0	.011
1.00	1.000	.982	.882	.864	.935	.865	.981	.988	.989
1.10	.0	.0	.0	.0	.0	.041	.0	.012	.0
Pgm-1									
0.90	.214	.107	.088	.076	.111	.054	.096	.071	.0
0.95	.036	.0	.059	.045	.037	.014	.0	.024	.0
1.00	.750	.875	.853	.879	.833	.905	.904	.881	.883
1.10	.0	.018	.0	.0	.019	.027	.0	.024	.117
Pgm-2									
0.85	.054	.018	.088	.081	.056	.054	.038	.012	.0
1.00	.768	.857	.838	.903	.861	.905	.942	.988	.457
1.15	.179	.125	.074	.016	.083	.041	.019	.0	.543
Sdh									
0	.0	.036	.0	.015	.0	.0	.0	.0	.0
0.95	.0	.0	.0	.0	.009	.0	.0	.0	.0
1.00	.821	.768	.824	.758	.759	.784	.596	.583	.553
1.05	.0	.036	.029	.030	.028	.027	.0	.0	.0
1.10	.179	.161	.147	.182	.204	.189	.404	.405	.447
1.15	.0	.0	.0	.015	.0	.0	.0	.012	.0
Skdh									
0.95	.0	.0	.015	.0	.0	.014	.058	.012	.0
1.00	1.000	.982	.970	1.000	1.000	.959	.885	.964	.404
1.05	.0	.018	.015	.0	.0	.014	.058	.024	.0
1.20	.0	.0	.0	.0	.0	.014	.0	.0	.596

Table 3. Genetic variability at 20 loci in 11 populations of *P. jezoensis* (standard errors in parentheses).

Population	A	$A_{5\%}$	P_{95}	P	H_e[a]	H_o
Khorogochi	2.20	1.85	55.0	65.0	0.211 (0.051)	0.176 (0.039)
Dipkun	2.55	1.80	55.0	95.0	0.203 (0.039)	0.188 (0.025)
Snezhnyi	2.85	2.00	75.0	95.0	0.210 (0.034)	0.204 (0.034)
Burga	2.55	1.75	65.0	90.0	0.201 (0.040)	0.207 (0.037)
Tukhala	2.85	1.70	65.0	85.0	0.188 (0.037)	0.181 (0.023)
Khekhtsir	3.05	1.70	70.0	95.0	0.184 (0.034)	0.185 (0.036)
Dolmi	2.35	1.60	55.0	90.0	0.171 (0.039)	0.169 (0.039)
Uglekamensk	2.65	1.45	50.0	90.0	0.145 (0.037)	0.136 (0.034)
Mean for 8 mainland populations	2.63 (0.28)	1.73 (0.16)	61.3 (8.8)	88.1 (10.0)	0.189 (0.023)	0.181 (0.022)
Atlasovo	1.85	1.60	55.0	70.0	0.241 (0.052)	-

[a] Unbiased estimate (see NEI, 1978)

Table 4. F-statistics for 20 polymorphic loci and test of homogeneity of allele frequency.

Locus	F_{IS}	F_{IT}	F_{ST}	χ^2	df	P
Aat-1	-0.015	0.010	0.024	40.809	21	*
Aat-2	-0.017	-0.002	0.015	41.970	35	ns
Adh	0.126	0.191	0.075	69.926	14	**
Fl-Est	-0.060	-0.045	0.014	25.591	21	ns
Gdh	-0.010	0.007	0.017	27.741	21	ns
Gpi	0.050	0.067	0.018	20.614	14	ns
Hk	-0.069	-0.051	0.017	37.265	28	ns
Idh-1	-0.040	-0.024	0.015	45.272	35	ns
Idh-2	-0.008	0.005	0.012	28.024	28	ns
Lap-1	-0.008	0.019	0.028	41.132	21	*
Lap-2	-0.012	-0.000	0.012	12.724	14	ns
Mdh-1	0.007	0.029	0.022	20.313	14	ns
Mdh-2	0.037	0.056	0.019	18.198	14	ns
Mdh-3	0.019	0.028	0.009	21.388	28	ns
Me	0.062	0.111	0.052	86.958	21	**
6-Pgd-1	0.055	0.092	0.039	40.300	14	**
Pgm-1	0.001	0.016	0.015	24.154	21	ns
Pgm-2	-0.031	0.003	0.033	34.621	14	*
Sdh	0.020	0.043	0.024	57.611	35	*
Skdh	0.024	0.043	0.020	20.419	21	ns
Mean	0.007	0.03	0.024	20.419	21	ns

*$p < 0.01$; **$p < 0.001$

Parameters of genetic variation were calculated on the basis of frequencies of 82 alleles of 20 loci. For the eight mainland populations, the mean number of alleles per locus ranged from 2.20 to 3.05, and averaged 2.63. The percent of polymorphic loci (P) ranged from 65.0 to 95.0%, with an average of 88.1%. The observed heterozygosity was from 0.136 to 0.207, with an average of 0.181. The mean value of expected heterozygosity amounted to 0.189, with variation from 0.145 to 0.211. The values of expected heterozygosity of the northern and central mainland populations were higher than in the southern part of the natural range. The values of genetic variation in the Atlasovo population were different from those for the mainland populations and reached: A = 1.85, P = 70.0, H_e = 0.241 (Table 3).

F_{IS} values ranged from -0.069 for *Hk* to 0.126 for *Adh*, with an overall mean of 0.007. F_{IT} values at the

Table 5. Estimates of NEI'S (1978) genetic distances.

Population		1	2	3	4	5	6	7	8	9
1	Khorogochi	***	.009	.015	.008	.010	.016	.018	.026	.079
2	Dipkun		***	.000	.001	.000	.002	.004	.006	.079
3	Snezhnyi			***	.000	.000	.001	.004	.005	.083
4	Burga				***	.001	.002	.004	.008	.080
5	Tukhala					***	.001	.002	.005	.083
6	Khekhtsir						***	.002	.004	.084
7	Dolmi							***	.000	.081
8	Uglekamensk								***	.077
9	Atlasovo									***

Aat-2, Fl-Est, *Hk*, *Idh-1*, and *Lap-2* loci were negative and at 15 loci were positive, reaching 0.191 at *Adh*. Mean F_{IT} was 0.030. Positive F_{IS} and F_{IT} values indicate a slight deficiency of heterozygotes relative to the Hardy-Weinberg equilibrium for the populations investigated and for *P. jezoensis* in total. The F_{ST} value at 20 loci was 0.024. Thus, about 98% of the total genetic variability resided within populations and only 2.4% among populations (Table 4).

Chi-square tests of homogeneity for allele frequencies in the mainland populations showed that there are no significant differences at most of the loci. Among the populations the most heterogenous allele frequencies were revealed at the *Adh*, *Me* and *6-Pgd-1* loci (Table 4).

Unbiased Nei's genetic distance values (D_N) were low between the mainland populations (0.000–0.026) of *P. jezoensis* and averaged 0.006. The largest values (0.077–0.084, at average, D_N = 0.081) were detected between these populations and the Atlasovo population (Table 5).

UPGMA cluster analysis (Fig. 3) split the mainland populations into two clusters, a cluster of the central and northern populations and a cluster of the southern populations. These clusters on the phylogenetic tree were confirmed by bootstrap values. The marginal Khorogochi population is close to these clusters. The Atlasovo population from the Kamchatka Peninsula is rather distant from these clusters.

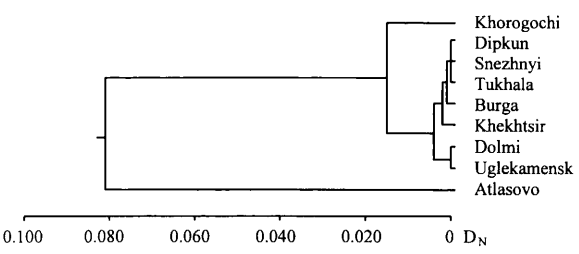

Figure 3. Dendrogram of UPGMA cluster analysis based on NEI's (1978) genetic distances between the nine populations of Yeddo spruce. Numbers within the phylogenetic tree represent bootstrap numbers based on 1000 replicates.

DISCUSSION

P. jezoensis exhibited levels of genetic variation that were similar to those reported for predominantly outcrossed, wind-pollinated species with a regional distribution (HAMRICK *et al.* 1992). Genetic diversity maintained by the mainland populations of *P. jezoensis* (H_e = 0.189) was slightly higher than the average value for a number of spruce species analyzed at more than 18 loci (H_e = 0.180) (GONCHARENKO & PADUTOV 2001). Our estimates for Yeddo spruce were higher than those for *P. engelmannii* (Parry) Engelm. (H_e = 0.152, SHEA 1990), *P. glauca* (Moench.) Voss. (H_e = 0.174, YEH & ARNOTT 1986), *P. mariana* (Mill.) B. S. P. (H_e = 0.107, YEH *et al.* 1986), *P. omorika* (Panè.) Purk. (H_e = 0.130, KUITTINEN *et al.* 1991), *P. rubens* Sarg. (H_e = 0.079, HAWLEY & DEHAYES 1994), and *P. sitchensis* (Bong.) Carr. (H_e = 0.150, YEH & EL-KASSABY 1980). The Atlasovo population exhibited a much higher level of genetic variation (H_e = 0.241), similar to those reported for widespread Eurasian spruces such as *P. abies* (L.) Karst. and *P. obovata* Ledeb. (H_e = 0.252 and H_e = 0.213, respectively, KRUTOVSKII & BERGMANN 1995, H_e = 0.193 and H_e = 0.257, respectively, GONCHARENKO & PADUTOV 2001).

The values of expected heterozygosity of the northern and central mainland populations are higher than those of the southern ones, as for red spruce (HAWLEY & DEHAYES 1994). It is possible that the higher genetic variability evident in Yeddo spruce from the northern and central mainland regions and lower genetic variability in the south could reflect differences resulting from past migrating patterns in the late Pleistocene - Holocene. Studies of fossil conifer pollen (KOROTKII *et al.* 1997) indicate that 18–20 thousand years ago the vegetation of the Sikhote Alin was similar

to that of the contemporary north-west coast of the Sea of Okhotsk. The latitudinal shift to the south was almost 10°. Thus, the modern counterpart of natural vegetation for the south Sikhote-Alin in that time is now roughly situated at the northern limit of the *P. jezoensis* natural range where it forms isolated stands (MAŃ'KO 1987). After the climate had cooled Yeddo spruce expanded into northern Sikhote-Alin, where in the middle Holocene period a zone of Korean pine-spruce-broadleaf and fir-spruce mixed forests formed.

During this period in the south Sikhote-Alin, warmer temperatures caused an upward elevational shift of the Yeddo spruce populations to the altitude about 1500 meters above sea level. The rapid population growth in the south Sikhote-Alin in altitudes between 700 and 1200 meters above sea level was a result of a decrease in temperatures and an increase of relative humidity in the late Holocene (GOLUBEVA & KARAULOVA 1983, KOROTKII *et al.* 1997).

Levels of genetic variability may have been reduced in the south where warmer temperatures caused an upward elevational shift of the spruce populations, resulting in small, isolated populations that were possibly subjected to increased levels of inbreeding and increased genetic drift. Theoretical studies of the genetic effects of population bottlenecks have shown that if the population expands rapidly after a bottleneck, an increased number of rare alleles due to new mutations will be found until equilibrium heterozygosity has been attained, with the discrepancy being most pronounced for rare alleles (MARUYAMA & FUERST 1984). This can explain higher values of the mean number of alleles per locus in the most mainland population (A = 2.63) in comparison with Sakhalin Island (A = 2.21) (GONCHARENKO & PADUTOV 2001) and the Kamchatka Peninsula (A = 1.85) populations that were likely to be not subjected to fluctuation of population during the Pleistocene and Holocene periods. The relatively low value of the mean number of alleles per locus (A = 2.20), the high level of expected heterozygosity (H_e = 0.211) and the mean number of unrare alleles per locus ($A_{5\%}$ = 1.85) in the Khorogochi population may be a result of genetic drift occurring in a quite small population (< 100 trees) isolated from the other mainland populations. Loss of alleles occurs more rapidly than loss of heterozygosity after severe restrictions in the population size as shown in studies of MARUYAMA & FUERST (1985).

Positive F_{IS} and F_{IT} values indicate that deficiency of heterozygotes was low for the populations investigated and for *P. jezoensis* in total. It should be noted that an excess of heterozygotes was found in the Sakhalin Island and Shikotan Island populations (GONCHARENKO & POTENKO 1992, GONCHARENKO & PADUTOV 2001).

The rapid expansion of Yeddo spruce to the north in the late Pleistocene can explain the low level of interpopulation differentiation between the mainland populations (F_{ST} = 0.024), and it is similar to the average value for the island populations of *P. jezoensis* (F_{ST} = 0.030) (GONCHARENKO & PADUTOV 2001), although lower than in several other spruce species (G_{ST} = 0.055) (HAMRICK *et al.* 1992).

Low estimates of NEI's genetic distances confirm the close genetic relationship between most of the mainland populations (D_N = 0.006). The genetic drift occurring in the isolated Khorogochi population could explain the higher level of differentiation from the other mainland populations (D_N = 0.015). The mean value of genetic differentiation was slightly lower than those between the five populations of Yeddo spruce from the mainland, Sakhalin Island and Shikotan Island (D_N = 0.009–0.012) studied earlier (GONCHARENKO & POTENKO 1992, GONCHARENKO & PADUTOV 2001). The largest value (D_N = 0.081) was revealed between the mainland populations and the Atlasovo population from the Kamchatka Peninsula. This should not be considered as biased sampling from the Kamchatka Peninsula, because similar allele frequencies were found in six loci (*Gdh, Aat-1, Aat-2, Lap-1, Lap-2* and *Mdh-1*) studied here and in the work of GÖMÖRY & PAULE (1990).

In 1934 KOMAROV (1934) defined the taxonomic status of several stands in the Kamchatka Peninsula as a separate species *P. kamtchatkensis* Lakas. Later, VASIL'EV (1950) subdivided *P. jezoensis* into three species: *P. ajanenesis* Fish., *P. microsperma* (Lindl.) Carr. and *P. komarovii* V. Vassil., but the Kamchatka Peninsula stands were described as *P. ajanenesis* populations isolated from the main range. Independent taxonomic status of *P. ajanenesis*, *P. microsperma* and *P. komarovii* was widely accepted (VOROB'EV 1968, USENKO 1969, URUSOV 1995). However, recent research on the morphological diversity in the native Russian populations on the Sikhote-Alin mountain range does not support the subdivision of *P. jezoensis* into a number of distinct species (FROLOV 1993, POTEMKIN 1994).

According to the descriptions of the species ranges made by VASIL'EV (1950), and URUSOV (1995) the Khorogochi, Dipkun and Snezhnyi populations represent *P. ajanenesis,* Burga, Tukhala, Khekhtsir and Dolmi populations - *P. microsperma,* and Uglekamensk population - *P. komarovii.* The low genetic differentiation between those mainland populations observed in this study does not support the subdivision of *P. jesoensis*. Meanwhile, higher genetic differentiation between the mainland populations and the Atlasovo population, the existence of a clear range border of

spruce in Kamchatka Peninsula and the absence of introgressive hybridization between mainland and Kamchatka's populations allowed us to consider the spruces from mainland and Kamchatka Peninsula as distinct taxa. The observed level of genetic differentiation between the mainland and the Kamchatka populations was similar to those between closely related coniferous species such as *Picea abies* and *P. obovata* (D_N = 0.072, KRUTOVSKII & BERGMANN 1995, D_N = 0.083, GONCHARENKO & PADUTOV 2001), *Pinus virginiana* Mill. and *P. clausa* (Champ. ex Engelm.) Vasey ex Sarg. (D_N = 0.071, PARKER *et al.* 1997), and *P. taeda* L. and *P. echinata* Mill. (D_N = 0.074, EDWARDS-BURKE *et al.* 1997).

The time of geographic isolation of the Kamchatka Peninsula vegetation since the mid-Quaternary (KOLESNIKOV 1961) is in accordance with the time calculated on the basis of the mean D_N value between the mainland and the Kamchatka Peninsula populations (t = 405,000 years) using the NEI's (1975) equation: $t = 5 \times 10^6 D_N$.

Further studies of genetic differentiation between the mainland population of Yeddo spruce and the population of *P. jezoensis* var. *hondoensis* (Mayr) Rehd. from Honshu Island (Hondo spruce) would make a considerable contribution to the investigation of the species.

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