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Allozyme Variation and Phylogenetic Relationships in *Picea jezoensis* (Pinaceae) Populations of the Russian Far East

V. V. Potenko[1,2,3]

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Genetic variation and differentiation of 12 populations of Picea jezoensis *from the Russian Far East were studied using 20 allozyme loci. The mean number of alleles per locus was 2.63, the percent of polymorphic loci was 88.1%, the observed heterozygosity was 0.181, and the mean value of expected heterozygosity amounted to 0.189. The values of expected heterozygosity of the northern and central mainland populations were higher than in the southern part of the natural range. A significant bias of Hardy–Weinberg heterozygosity to equilibrium heterozygosity* (H_{eq}) *suggests that most of the mainland populations have recently experienced a severe expansion in population size while populations from Kamchatka Peninsula have undergone a reduction in population size. Unbiased Nei's genetic distance values were low within and between the mainland and Sakhalin Island populations* ($D_N = 0.008$)*. The largest values* ($D_N = 0.063$) *were found between the mainland/Sakhalin and Kamchatka Peninsula populations. Based on genetic distance,* P. jezoensis *and* P. kamtschatkensis *could be considered as distinct taxa, but* P. ajanensis*,* P. microsperma*, and* P. komarovii *do not warrant taxonomic recognition.*

KEY WORDS: *Picea jezoensis*; isozymes; genetic variation; phylogeny.

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[1] Institute of Biology and Soil Sciences, Russian Academy of Sciences, Far East Branch, 690022, Vladivostok, Russia.

[2] Present address: Cathedra of Medical Biology and Genetics, Gomel State Medical University, 5 Lange Street, Gomel 246000, Republic of Belarus.

[3] To whom correspondence should be addressed; e-mail: vpotenko@mail.ru.

INTRODUCTION

Yeddo or Yezo spruce, *Picea jezoensis* (Sieb. et Zucc.) Carr., occurs in natural and artificial stands in Russia, China, Korea, and Japan (Fig. 1). In Russia, *P. jezoensis* is distributed in the Maritime Territory, the central and southern parts of Khabarovsk Territory, the Jewish Autonomous Region, Amur Territory, the southeast part of the Republic of Sakha (Yakutia), Sakhalin Island, the southern Kuriles, and the central part of Kamchatka Peninsula. The spruce is a dominant tree species in the Russian Far East. It forms the mountain spruce-fir forests, mainly with *P. obovata* Ledeb., *P. koraiensis* Nakai, and *Abies nephrolepis* (Trautv.) Maxim. on the mainland and with *Picea glehnii* Mast. and *Abies sachalinensis* (Schmidt) Mast. on Sakhalin Island (Man'ko, 1987). Komarov (1934) defined the taxonomic status of spruce stands on Kamchatka Peninsula as a separate species, *Picea kamtschatkensis* Lacas. Later, Vasil'ev (1950) subdivided *P. jezoensis* into three species: *P. ajanensis* Fish., *P. microsperma* (Lindl.) Carr., and *P. komarovii* V. Vassil., but the Kamchatka Peninsula stands were described as *P. ajanensis* populations, thereby isolated from the main range. Until now, *P. ajanensis*, *P. microsperma*, and *P. komarovii* have been widely accepted as independent species (Urusov, 1995; Usenko, 1969; Vorob'ev, 1968). Recent research on morphological diversity in the native spruce populations of the Sikhote-Alin mountain range, however, does not support the subdivision of *P. jezoensis* into a number of distinct species (Frolov, 1993; Potemkin, 1994), at least in the mainland part of the natural range.

To date, only two allozyme studies of *P. jezoensis* populations have been conducted: one on a population in the Kamchatka Peninsula (Gömöry and Paule, 1990), and the other on three populations on Sakhalin Island and one population in Khabarovsk Territory (Goncharenko and Potenko, 1992). High levels of genetic variation were revealed within the populations, but the taxonomic status of *P. kamtschatkensis*, *P. ajanensis*, *P. microsperma*, and *P. komarovii* appeared to be unjustified. In addition, almost all previously analyzed populations were geographically isolated and represented marginal parts of *P. jezoensis* natural range. Assessments of allozyme variation of *P. jezoensis* from a wide range of mainland areas have not yet been reported.

The aims of this study were to investigate the levels of genetic variation and differentiation of 12 *P. jezoensis* populations representing a rangewide collection from the mainland, Sakhalin Island, and Kamchatka Peninsula and to infer the genetic relationships between *P. ajanensis*, *P. microsperma*, *P. komarovii*, and *P. kamtschatkensis*.

MATERIALS AND METHODS

Seeds were collected from 377 Yeddo spruce trees in 12 native populations from Amur Region, Khabarovsk Territory, Maritime Territory, Sakhalin Island, and

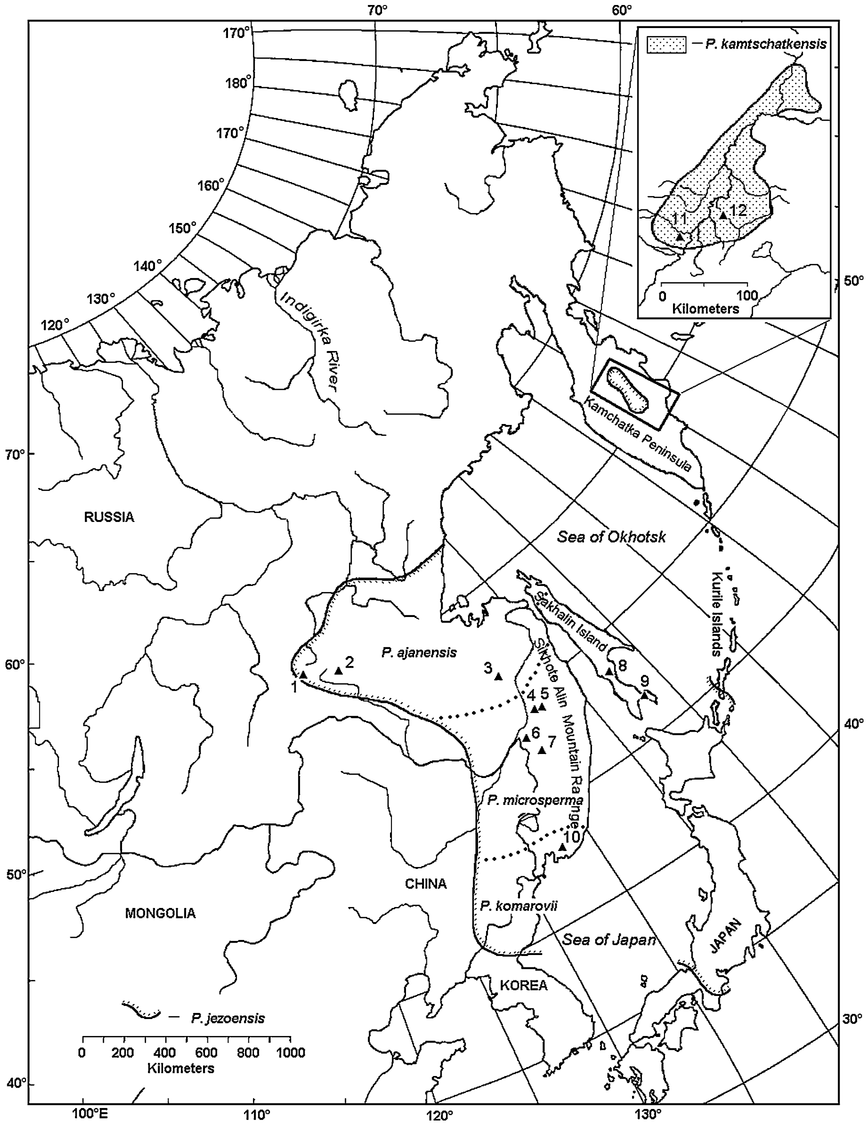

Fig. 1. Range of *Picea jezoensis* (Man'ko, 1987) and the locations of 12 populations sampled for electrophoretic analysis. *Dotted line*: Limits of the natural range of *P. microsperma* (modified from Vasil'ev, 1950). Sampling sites: 1, Khorogochi. 2, Dipkun. 3, Snezhnyi. 4, Burga. 5, Tukhala. 6, Khekhtsir. 7, Dolmi. 8, Makarov. 9, Beregovoe. 10, Uglekamensk. 11, Milkovo. 12, Schapina.

Table I. Locations of Populations Sampled and Number of Trees Analyzed

Population No.	Location	Latitude (°N)	Longitude (°E)	No. trees
1	Khorogochi, Amur Region	55.3	123.8	28
2	Dipkun, Amur Region	55.1	126.8	28
3	Snezhnyi, Khabarovsk Territory	50.8	136.3	34
4	Burga, Khabarovsk Territory	49.1	136.9	33
5	Tukhala, Khabarovsk Territory	49.1	137.3	54
6	Khekhtsir, Khabarovsk Territory	48.3	135.0	37
7	Dolmi, Khabarovsk Territory	47.4	135.7	26
8	Makarov, Sakhalin Island	48.6	142.7	27
9	Beregovoe, Sakhalin Island	46.6	143.3	26
10	Uglekamensk, Maritime Territory	43.4	133.2	42
11	Milkovo, Kamchatka Peninsula	54.9	158.7	20
12	Schapina, Kamchatka Peninsula	55.3	159.6	22

Kamchatka Peninsula (Fig. 1). The populations from these areas correspond to the putative species *Picea kamtschatkensis*, *P. ajanensis*, *P. microsperma*, and *P. komarovii*, according to morphological classification (Komarov, 1934; Vasil'ev, 1950). Table I presents the population locations and the number of trees analyzed.

Six megagametophytes per tree were subjected to horizontal starch gel electrophoresis. Seed tissues were analyzed for 14 enzyme systems (abbreviation 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 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). The enzymes AAT, ADH, GDH, HK, ME, PGM, and SDH were run using a Tris-EDTA-borate buffer; FL-EST, LAP, and SkDH were run using Tris citrate/Tric-HCl buffer; and GPI, IDH, MDH, and 6-PGD were run using Tris citrate buffer. The buffer recipes and details of laboratory procedures are described in Potenko and Velikov (1998). Enzyme staining followed standard methods (Cheliak and Pitel, 1984) with minor modifications. The inheritance of allozyme polymorphisms in spruces was previously described by Goncharenko and Potenko (1991a).

Allele frequencies were analyzed using the Biosys-1 computer program (Swofford and Selander, 1989). For each population, the mean number of alleles per locus (A), the percentage of polymorphic loci (P and P_{95}), the observed heterozygosity (H_o), the expected heterozygosity (H_e), and Wright's fixation index (F) were calculated.

We conducted the sign test, the standardized differences test, and the Wilcoxon sign-rank test to determine whether each population showed a significant bias of Hardy–Weinberg heterozygosity to equilibrium heterozygosity under the infinite allele model of Maruyama and Fuerst (1985). In a recently bottlenecked population, the Hardy–Weinberg heterozygosity should be higher than the expected equilibrium gene diversity ($H_e > H_{eq}$), which is computed from the observed number of alleles. Also, in a recently expanded population, H_e should be lower than H_{eq} (for more details, see Luikart and Cornuet, 1998). To perform this test, we used the Bottleneck computer program (Cornuet and Luikart, 1996).

The analysis of gene diversity followed Nei (1973). Data on total genetic diversity (H_T), genetic diversity within populations (H_S), and proportion of genetic variation found among populations (G_{ST}) were calculated. Levels of genetic differentiation were assayed on the basis of Nei's unbiased genetic distances (Nei, 1978). The relationships among the populations were visualized by constructing phylogenetic trees using the unweighted pair group (UPGMA) method (Sneath and Sokal, 1973). The reliability of the phylogenetic trees was tested using bootstrap estimates (Felsenstein, 1985).

RESULTS

Parameters of genetic variation were calculated on the basis of allele frequencies of 20 loci (Table II). For 12 populations, the mean number of alleles per locus ranged from 1.65 to 3.05, and averaged 2.42. Allelic richness was dependent on sample size ($r = 0.77$). The percentage of polymorphic loci ranged from 60.0 to 95.0%, with an average of 83.8%. The observed heterozygosity was between 0.136 and 0.241, with an average of 0.193. The mean value of expected heterozygosity

Table II. Genetic Variability at 20 Loci in 12 Populations of Yeddo Spruce

Population	A	P_{95}	P	H_e[a]	H_o	F
1. Khorogochi	2.20 (0.26)	55.0	65.0	0.211 (0.051)	0.176 (0.042)	0.085
2. Dipkun	2.55 (0.18)	55.0	95.0	0.203 (0.039)	0.188 (0.045)	0.026
3. Snezhnyi	2.85 (0.17)	75.0	95.0	0.210 (0.034)	0.204 (0.044)	– 0.007
4. Burga	2.55 (0.20)	65.0	90.0	0.201 (0.040)	0.207 (0.041)	– 0.036
5. Tukhala	2.85 (0.21)	65.0	85.0	0.188 (0.037)	0.181 (0.033)	0.000
6. Khekhtsir	3.05 (0.20)	70.0	95.0	0.184 (0.034)	0.185 (0.039)	0.021
7. Dolmi	2.35 (0.15)	55.0	90.0	0.171 (0.039)	0.169 (0.040)	– 0.001
8. Makarov	2.25 (0.18)	60.0	80.0	0.204 (0.044)	0.217 (0.046)	– 0.055
9. Beregovoe	2.25 (0.14)	65.0	90.0	0.207 (0.041)	0.194 (0.048)	– 0.006
10. Uglekamensk	2.65 (0.17)	50.0	90.0	0.145 (0.037)	0.136 (0.033)	– 0.017
11. Milkovo	1.80 (0.14)	60.0	70.0	0.251 (0.050)	0.241 (0.054)	0.007
12. Schapina	1.65 (0.13)	55.0	60.0	0.231 (0.051)	0.214 (0.048)	0.036
Mean	2.42 (0.42)	60.8 (7.3)	83.8 (12.3)	0.201 (0.027)	0.193 (0.027)	0.004

Note. A, average number of alleles per locus; P, percentage of polymorphic loci; P_{95}, percentage of polymorphic loci at the 95% level; H_o, observed heterozygosity; H_e, expected heterozygosity; F, Wright's fixation index. Standard errors in parentheses.

[a] Unbiased estimate (see Nei, 1978).

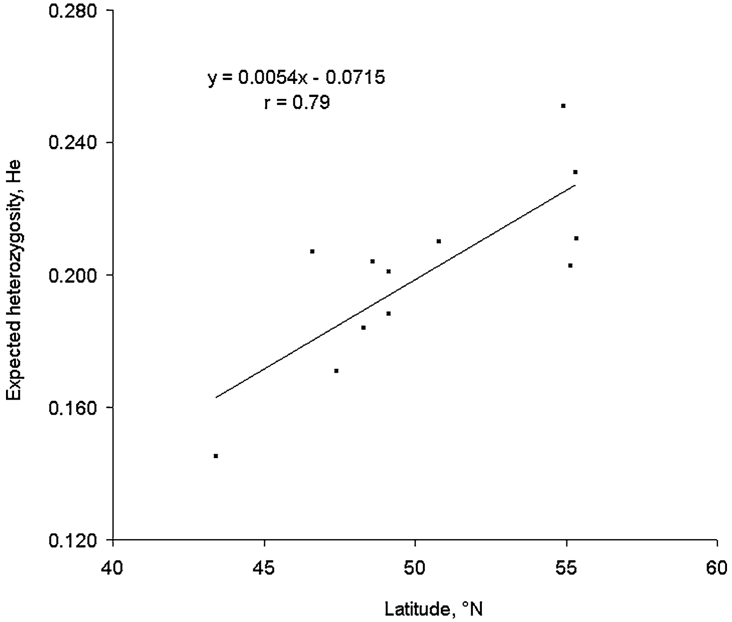

Fig. 2. The correlation of expected heterozygosity (H_e) with latitude for 12 natural populations of *Picea jezoensis*.

amounted to 0.201, with variation from 0.145 to 0.251. Expected heterozygosity of the Kamchatka Peninsula and the northern mainland populations was higher than in the southern part of the natural range. Actually, expected heterozygosity tended to increase with the increase of latitude ($r = 0.79$; Fig. 2). For individual populations, the mean F values varied from -0.036 to 0.085 (Table II), indicating little deviation from Hardy–Weinberg expectations.

The sign test, the standardized differences test, and the Wilcoxon sign-rank test indicate that all the mainland populations of *P. jezoensis*, except the Khorogochi population, had a significant heterozygosity deficit, and the two Kamchatka Peninsula populations had a significant heterozygosity excess (Table III).

P. jezoensis exhibited high differentiation values ($G_{ST} = 0.076$), with the most pronounced differences revealed at loci *Skdh*-1, *Aat*-1, *Idh*-2, *Lap*-1, *Adh*, and *Pgm*-2 (Table IV).

Unbiased Nei's genetic distance values were low within and between the mainland and the Sakhalin Island populations (0.000–0.029; averaging $D_N = 0.008$), and high between the mainland/Sakhalin and the Kamchatka Peninsula populations (0.055–0.071; averaging $D_N = 0.063$) (Table V). UPGMA cluster analysis split the *P. jezoensis* populations into two main clusters: the Kamchatka Peninsula populations (11 and 12) and the mainland/Sakhalin Island populations

Table III. Bias of heterozygosity of 12 Spruce Populations

Population	Sign test	Standardized differences test	Wilcoxon sign-rank test
1. Khorogochi	+0.285	+0.472	+0.500
2. Dipkun	−0.049*	−0.013*	−0.005**
3. Snezhnyi	−0.002**	−0.002**	−0.001***
4. Burga	−0.023*	−0.019*	−0.013*
5. Tukhala	−0.001***	−0.005**	−0.005**
6. Khekhtsir	−0.002**	−0.000***	−0.000***
7. Dolmi	−0.007**	−0.005**	−0.013*
8. Makarov	−0.149	−0.171	−0.116
9. Beregovoe	−0.196	−0.127	−0.084
10. Uglekamensk	−0.001***	−0.000***	−0.002**
11. Milkovo	+0.010**	+0.006**	+0.012*
12. Schapina	+0.007**	+0.006**	+0.017*

Note. Significance of heterozygosity deficit (−) or excess (+) in sign test, standardized differences test, and Wilcoxon sign-rank test under the infinite allele model of Maruyama and Fuerst (1985).
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table IV. Gene Diversity for 20 Loci in Populations of *P. jezoensis*

Locus	H_T	H_S	G_{ST}
Aat-1	0.214	0.168	0.215
Aat-2	0.065	0.060	0.073
Adh	0.386	0.351	0.092
Gdh	0.018	0.018	0.018
Idh-1	0.227	0.218	0.041
Idh-2	0.090	0.078	0.132
Mdh-1	0.049	0.048	0.034
Mdh-2	0.096	0.093	0.033
Mdh-3	0.646	0.624	0.035
Sdh	0.435	0.410	0.059
Skdh-1	0.266	0.186	0.302
6-Pgd-1	0.106	0.101	0.045
Lap-1	0.186	0.163	0.127
Lap-2	0.102	0.101	0.017
Hk	0.306	0.293	0.042
Pgm-1	0.282	0.271	0.039
Pgm-2	0.250	0.227	0.092
Me	0.315	0.294	0.068
Gpi	0.087	0.085	0.027
Fe	0.106	0.103	0.026
Mean	0.212	0.195	0.076

Note. H_T, total genetic diversity; H_S, genetic diversity within populations; G_{ST}, proportion of genetic variation among populations.

Table V. Estimates of Genetic Distances[a] Between *Picea jezoensis* Populations Based on 20 Allozyme Loci

Population	1	2	3	4	5	6	7	8	9	10	11	12
1. Khorogochi	*	0.009 (0.007)	0.015 (0.008)	0.008 (0.003)	0.010 (0.006)	0.016 (0.012)	0.018 (0.010)	0.028 (0.015)	0.029 (0.013)	0.026 (0.016)	0.071 (0.028)	0.068 (0.028)
2. Dipkun		*	0.000 (0.001)	0.001 (0.001)	0.000 (0.001)	0.002 (0.001)	0.004 (0.003)	0.013 (0.003)	0.015 (0.007)	0.006 (0.005)	0.064 (0.026)	0.061 (0.026)
3. Snezhnyi			*	0.000 (0.001)	0.000 (0.000)	0.001 (0.001)	0.004 (0.004)	0.014 (0.004)	0.016 (0.009)	0.005 (0.006)	0.067 (0.024)	0.061 (0.025)
4. Burga				*	0.001 (0.001)	0.002 (0.003)	0.004 (0.003)	0.015 (0.005)	0.015 (0.007)	0.008 (0.006)	0.065 (0.025)	0.061 (0.027)
5. Tukhala					*	0.001 (0.001)	0.002 (0.002)	0.013 (0.003)	0.015 (0.007)	0.005 (0.006)	0.068 (0.027)	0.063 (0.027)
6. Khekhtsir						*	0.002 (0.002)	0.012 (0.003)	0.008 (0.007)	0.004 (0.003)	0.067 (0.023)	0.061 (0.024)
7. Dolmi							*	0.003 (0.001)	0.007 (0.002)	0.000 (0.004)	0.067 (0.025)	0.061 (0.025)
8. Makarov								*	0.004 (0.004)	0.006 (0.005)	0.062 (0.023)	0.057 (0.024)
9. Beregovoe									*	0.009 (0.002)	0.066 (0.021)	0.059 (0.022)
10. Uglekamensk										*	0.061 (0.021)	0.055 (0.024)
11. Milkovo											*	0.000 (0.001)
12. Schapina												*

[a] Nei (1978). Standard errors in parentheses.

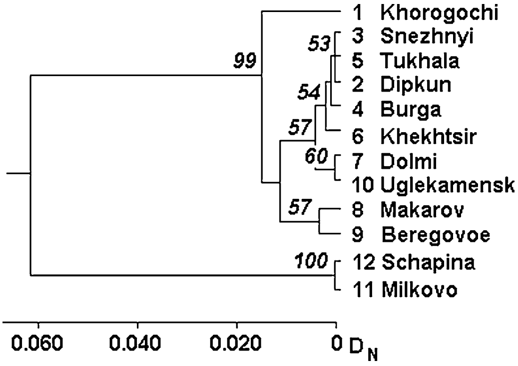

Fig. 3. UPGMA phylogenetic tree based on Nei's (1978) genetic distances calculated for 20 isozyme loci in *Picea ajanensis*, *P. microsperma*, *P. komarovii*, and *P. kamtschatkensis*. Numbers near nodes are percentage of 1000 bootstrap replicates. Only bootstrap values higher than 50% are shown.

(Fig. 3). The mainland/Sakhalin cluster is divided into three clusters: the central and northern mainland populations (2–6), the southern mainland populations (7, 10), and the Sakhalin Island populations (8, 9), but these are not well supported by bootstrap analysis. The marginal Khorogochi population (1) is close to the mainland/Sakhalin Island populations.

DISCUSSION

Yeddo spruce populations exhibited levels of genetic variation similar to those reported for populations of long-lived woody species with a regional geographic range ($H_e = 0.194$) and with a boreal-temperate distribution ($H_e = 0.204$) (Hamrick *et al.*, 1992). The estimates of H_e were higher than those for some North American boreal spruce species analyzed at more than 18 loci: *P. engelmannii* (Parry) Engelm. ($H_e = 0.152$, Shea, 1990), *P. glauca* (Moench.) Voss. ($H_e = 0.174$, Yeh and Arnott, 1986), *P. mariana* (Mill.) B.S.P. ($H_e = 0.107$, Yeh *et al.*, 1986), and *P. rubens* Sarg. ($H_e = 0.079$, Hawley and DeHayes, 1994); but close to those for some Eurasian boreal spruce species: *P. abies* (L.) Karst. ($H_e = 0.165$, Giannini *et al.*, 1991; $H_e = 0.185$, Goncharenko and Potenko, 1991a; $H_e = 0.252$, Krutovskii and Bergmann, 1995), *P. obovata* ($H_e = 0.186$, Goncharenko and Potenko, 1991a; $H_e = 0.213$, Krutovskii and Bergmann, 1995), and *P. glehnii* ($H_e = 0.185$, Goncharenko and Potenko, 1991b; $H_e = 0.213$, Wang and Nagasaka, 1997).

The values of expected heterozygosity of the northern populations were higher than those of the southern populations (Table II). It is possible that the higher genetic variability observed in *P. jezoensis* from the northern regions and the lower genetic variability observed in the south could reflect differences originating from past migration patterns in the late Pleistocene-Holocene. Studies of fossil conifer pollen (Korotkii *et al*., 1997) indicate that 18,000–20,000 years ago the vegetation of the Sikhote Alin mountain range was similar to that of the contemporary northwest 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 the late Pleistocene is now roughly situated at the northern limit of the *P. jezoensis* natural range where it forms isolated stands (Man'ko, 1987). After the climate had cooled, *P. jezoensis* expanded into the northern Sikhote-Alin, where in the middle of the Holocene period a zone of Korean pine-spruce-broadleaf and spruce-fir mixed forests was formed. Simultaneously, in the south Sikhote-Alin, warmer temperatures caused an upward elevational shift of *P. jezoensis* populations to an altitude of about 1500 m, where it formed small, isolated populations. Thus, levels of genetic variability may have been reduced in the south because populations were possibly subjected to increased levels of inbreeding and increased gene drift. The rapid population growth in the south Sikhote-Alin at altitudes ranging between 700 and 1200 m was a result of some decrease in temperatures and an increase in relative humidity at the end of the Subboreal period and the beginning of the Subatlantic period of the Holocene (Golubeva and Karaulova, 1983; Korotkii *et al*., 1997).

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 arising due to novel mutations will be detected until equilibrium heterozygosity has been attained, with the discrepancy being most pronounced for rare alleles (Maruyama and Fuerst, 1984). The loss of alleles occurs more rapidly than loss of heterozygosity after severe restrictions in population size (Maruyama and Fuerst, 1985). The results of the sign test, the standardized differences test, and the Wilcoxon sign-rank test allow us to deduce that most *P. jezoensis* populations have recently experienced a severe expansion in population size, but the spruce populations from Kamchatka Peninsula have experienced a reduction in population size (a bottleneck) and Sakhalin populations and the Khorogochi population of *P. jezoensis* are in heterozygosity equilibrium (Table III).

Surprisingly, the Kamchatka Peninsula populations exhibited a similar or higher level of heterozygosity (Table II), compared to the levels reported for widespread Eurasian spruces such as *P. abies* (L.) Karst. ($H_e = 0.165$, Giannini *et al*., 1991; $H_e = 0.185$, Goncharenko and Potenko, 1991a; $H_e = 0.252$, Krutovskii and Bergmann, 1995) and *P. obovata* ($H_e = 0.186$, Goncharenko and Potenko, 1991a; $H_e = 0.213$, Krutovskii and Bergmann, 1995).

Records regarding historical population sizes are important for explaining the high level of heterozygosity of spruce on Kamchatka Peninsula. Palynology studies show that in the late Pliocene-early Pleistocene, spruce forests were dominant on Kamchatka Peninsula (Neishtadt, 1957). Moreover, Vas'kovskii (1959) found fossils of *P. praeajanensis* in a basin of the Elgi River (a tributary of the Indigirka River) dating to the first Quaternary interglacial. *P. praeajanensis* is considered a possible ancestor of the modern *P. jezoensis* (Vas'kovskii, 1959). Therefore, the natural range of *P. praeajanensis* likely included the Kamchatka Peninsula. It appears that Kamchatka Peninsula populations only recently experienced a severe reduction in population size, as supported by a significant excess of Hardy–Weinberg heterozygosity to equilibrium heterozygosity (Table III).

The G_{ST} value obtained in this study was of the same order of magnitude as those observed in other gymnosperms ($G_{ST} = 0.073$, Hamrick *et al.*, 1992). Nevertheless, the level of differentiation maintained by *P. jezoensis* populations was higher than the value for the genus *Picea* in general ($G_{ST} = 0.055$, Hamrick *et al.*, 1992). Only for one Eurasian spruce, *P. schrenkiana* Fisch. et Mey. ($G_{ST} = 0.118$, Goncharenko *et al.*, 1992a,b), and two North American spruces, *P. chihuahuana* Martinez ($F_{ST} = 0.248$, Ledig *et al.*, 1997) and *P. breweriana* S. Wats. ($F_{ST} = 0.152$, Ledig *et al.*, 2005), were the reported estimates of G_{ST} (F_{ST}) greater than in *P. jezoensis*. However, after excluding the outlier Milkovo and Schapina populations from Kamchatka Peninsula, G_{ST} was 0.036 among the other 10 populations, which is low for spruces. The present range excludes gene exchange between populations from the mainland and Kamchatka Peninsula, and the possible bottleneck in the Kamchatka populations can bias allele frequencies, both of which are possible causes of the observed genetic differentiation.

The allozyme data provided evidence of a close relationship between *P. ajanensis*, *P. microsperma*, and *P. komarovii* populations. The levels of genetic differentiation between these taxa are limited ($D_N = 0.029$, Table V) and do not exceed the among-populations values found in conifers of the same species (Goncharenko *et al.*, 1992a,b, 1995; Ledig *et al.*, 2004). Moreover, the UPGMA cluster analysis did not group populations of *P. ajanensis*, *P. microsperma*, and *P. komarovii* into their own clusters (Fig. 3). Therefore, the subdivision of *P. jezoensis* into three putative species appears unjustified. Meanwhile, the *P. kamtschatkensis* populations form their own cluster on the phylogenetic tree. Higher genetic differentiation between the mainland populations and populations from Kamchatka Peninsula, and the possible absence of gene exchange between the mainland and Kamchatka populations, allow us to conclude that the spruces from the mainland and Kamchatka Peninsula are distinct taxa, *P. jezoensis* and *P. kamtschatkensis*. The observed level of genetic differentiation between these taxa was similar to the genetic differentiation reported for closely related conifers, i.e., spruces *P. abies* and *P. obovata* ($D_N = 0.102$, Goncharenko and Potenko, 1991a; $D_N = 0.072$, Krutovskii and Bergmann, 1995), pines *Pinus virginiana* Mill. and *P. clausa* (Champ. ex Engelm.) Vasey ex Sarg. ($D_N = 0.071$, Parker *et al.*, 1997), *P. sibirica* Du Tour and *P. cembra* L. ($D_N = 0.065$, Krutovskii *et al.*, 1995), *P. taeda* L. and *P. echinata* Mill. ($D_N = 0.074$, Edwards-Burke *et al.*, 1997), and firs *Abies balsamea* (L.) Mill. and *A. fraseri* (Pursh.) Poir. ($D_N = 0.06$, Jacobs *et al.*, 1984)). Thus, our data support Komarov's (1934) classification of a distinct spruce taxon in the Kamchatka Peninsula.

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