Ann. For. Res. 51: 31-42, 2008

ANNALS OF FOREST RESEARCH
www.e-afr.org

Contrasting patterns of spatial genetic differentiation in two east asian five-needle pine species, *Pinus koraiensis* and *P. armandii*

M.M. Belokon, Y.S. Belokon, D.V. Politov

Belokon M.M., Belokon Y.S., Politov D.V. 2008. Contrasting patterns of spatial genetic differentiation in two east asian five-needle pine species, *Pinus koraiensis* and *P. armandii*. Ann. For. Res. 51: 31-42.

Abstract. Two species of East Asian five-needle pines of the section Strobus, Korean pine, *Pinus koraiensis* Sieb. et Zucc. and Armand's pine, *Pinus armandii* Franch are traditionally placed in two different subsections, *Cembrae* and *Strobi,* respectively. However, data on similarity of these pines in needle anatomy and pollen structure are reported. By means of starch gel electrophoresis we studied allozyme variation within populations of these species and between the two species. Among 30 studied loci we selected 17 polymorphic loci with reliable interpretation of allele correspondence between species. Allozyme patterns were similar in both species with respect to number of loci and predominant alleles at most loci. Intra-specific differentiation among 12 populations of *Pinus koraiensis* from Russia, Northeast China and South Korea was low, F_{ST} (proportion of among population variation in total variation) was as low as 0.019, D_N (Nei's genetic distance) varied between 0.001 and 0.006. This genetic uniformity can be explained by a common gene pool of this species within virtually continuous range under similar ecological conditions. Common vectors of selection, natural gene flow through pollen and seed dispersal as well as mixing of genetic material between regions by humans (at least in Russian forestry practice) are putative factors responsible for low differentiation in Korean stone pine. We estimated the same parameters in *Pinus armandii* from continental China (Guizhou, Shaanxi, Shenyan) where its range is highly fragmented, and found differentiation in this species to be much more pronounced (F_{ST} = 0.067, D_N = 0.011-0.029). By adding a sample from Taiwan both F_{ST} (0.338) and D_N (up to 0.232) were substantially higher indicating high level of differences between continental and Taiwanese *P. armandii*, putatively due to longer isolation time. Maximal contribution was from loci *Adh-1, Got-3, Mdh-1, 6-Pgd-3, Pgm-2* and *Skdh-2*. Differences were mainly caused by allelic frequencies but not by presence/absence of particular alleles, therefore observed allozyme patterns are not evidence for full species' status of Taiwan variety. In the two species, *P. koraiensis* and *P. armandii*, electrophoresis showed the same number of activity zones. In a combined set of *P. koraiensis* and *P. armandii* samples, the level of interspecific differences was high (F_{ST} = 0.477, D_N = 0.407÷0.457). Species had no common alleles or very little overlapping in allelic profiles at loci *Adh-1, Got-2,* and *Pgm-1*. Out of 61 alleles 35 were shared between species and 26 were found in only one of them. Cluster (UPGMA) and multivariate (PCA) analyses of allozyme variation showed good differentiation within *P. armandii,* and poor differentiation within *P. koraiensis*.

Keywords: *P. armandii, P. koraiensis*, allozymes, genetic differentiation, alleles, polymorphic loci, genetic distance.

Authors. Belokon M.-M., Belokon Y.-S., Politov D.-V.(dvp@vigg.ru) - Laboratory of Population Genetics, Vavilov Institute of General Genetics, Russian Academy of Sciences, 3 Gubkin str., GSP-1, Moscow, Russia 119991

Introduction

East Asia is one of the centers of species' diversity of soft pines (subgenus *Strobus,* or Haploxylon, genus *Pinus*) (Millar 1993, Millar 1998, Mirov 1967). Two species of East Asian five-needle pines of the section *Strobus*, Korean pine, *Pinus koraiensis* Sieb. et Zucc. and Armand's pine, *Pinus armandii* Franch. are traditionally placed in two different subsections, *Cembrae* and *Strobi*, respectively (Critchfield & Little 1966, Farjon 1984, Mirov 1967, Price et al. 1998). Korean pine is distributed in the Russian Far East, Northeast China, Korean Peninsula and Honshu and Shikoku Islands (Japan). This species is a key component of stone pine - broad-leaved forests occupying mainly elevations between 600 and 900 m (in Japan up to 2500 m) (Bobrov 1978, Farjon 1984, Mirov 1967). *P. koraiensis* prefers well-drained moderately moistened soil, in the mountains often growing in river valleys. In the northern part of the range it grows in the plains. No distinct varieties of taxonomic status are distinguished within Korean pine. *Pinus armandii* Franch. is distributed on a vast territory including south of continental China, Islands of Hainan and Taiwan, and two southern Japanese Islands, Yakushima and Tanegashima (Bobrov 1978, Critchfield & Little 1966, Mirov 1967). The range is discontinuous and its continental distributiont consists of several isolated parts. The main part of the range is spread along the Tsinlin Range in the Huang He River basin (Shaanxi and Gansu provinces). The isolated smaller parts of the range are located in southeastern Gansu and northern Sichuan provinces. One more range fragment encloses southern Sichuan and Yunnan provinces of China, northern Myanmar and northeastern India. *P. armandii* grows in middle and upper mountain belts between 1500 and 3600 m elevation. In its ecological preferences, *P. armandii* resembles *P. koraiensis*, being sensitive to draught, and growing better in moist habitats, but this species is definitely less tolerant to low winter temperatures. Within Armand's pine, varieties from the Japanese islands and Taiwan are described as subspecies *P. a. amamiana*, *P. a. mastersiana*, or even considered respectively as separate species *P. amamiana* Koidzumi, *P. mastersiana* Hayata by some taxonomists (Price et al. 1998).

The two species have large wingless seeds of high nutrition value. Seeds are harvested and dispersed mainly by the nutcracker (*Nucifraga caryocatactes* L.) (Lanner 1996). The principal morphological difference between Korean and Armand's pines is the construction and functioning of female cones. In *P. koraiensis* cones remain closed after seeds ripening to facilitate their harvesting by nutcrackers. In *P. armandii* cones open as in most pine species releasing seeds. However, winglessness of Armand's pine seeds also promoted dispersion and regeneration by animals.

Palynological data is evidence for high similarity in structure of pollen grains of these two species (Kuprianova & Litvintseva 1974). Anatomical studies also showed common traits in needle parenchyma cells structure (Litvintseva 1974). Based on these data, it was proposed to place them into a separate species raw *koraiensis-armandii* within the section *Strobus*, and similarity in morphological and anatomical traits was putatively attributed to hybridization between the ancestral species in the past (Bobrov 1978).

Allozyme analysis demonstrated that among stone pines *P. koraiensis* is most closely related to another Far Eastern and East Siberian species, *P. pumila* (Belokon et al. 1998, Goncharenko et al. 1991, Goncharenko et al. 1992, Krutovskii et al. 1990, Krutovskii et al. 1994, Krutovskii et al. 1995, Politov & Krutovskii 1994, Politov & Krutovskii 2004, Politov et al. 1992); however, these two species are quite different morphologically. Within the section *Strobus* Armand's pine belongs to the group of Asian species along with *P. pumila, P. parviflora* and *P. wallichiana* (Belokon et al. 1998). Analysis of phylogenetic relationship of Eurasian pines based on DNA sequences showed that *P. armandii* clusters together with *P. koraiensis* and other species of the section *Strobus* (Gernandt et al. 2005, Liston et al. 1999, Wang et al. 2000, Wang et al. 1999, Zhang & Li 2004); however, there is no strict evidence that Korean pine is the closest relative of Armand's pine. Positions of these species in the cladograms are variable.

Most frequently all pines of section *Strobus* (both stone pines and *Strobi* pines) form an undifferentiated group, and if some species tend to be distinct or form clusters to each other by one marker gene, this usually contradicts data by other markers. In the most recent system, a new subsection Strobus uniting former subsections *Cembrae* and *Strobi* was proposed within section *Quinquefolia* (Gernandt et al. 2005).

There is extensive data on the low genetic differentiation among populations of Korean pine assessed by allozyme loci variation (Belokon et al. 2007, Kim et al. 1994, Kim et al. 2005, Potenko 2004, Potenko & Velikov 1998, Potenko & Velikov 2001, Yang et al. 1989). Genetic differentiation among *P. armandii* varieties *armandii, mastersiana,* and *amamiana)* was studied by allozyme analysis using single sample per variety (Kanetani et al. 2004). To the best of our knowledge, genetic variation within continental *P. armandii* was not studied by means of molecular markers, but evaluation of geographic patterns of variation with respect to adaptive traits of genetic differentiation was shown to be substantial (Chang-Geng 1989). Since the two East Asian pine species have many common traits in their morphology and ecology, but substantially differ in range type (continuous in Korean pine and fragmented in Armand's pine) we undertook this comparative study of amount and distribution of allozyme variation within and between these species.

Material and methods

Plant material

We used 12 seed samples of *P. koraiensis* from Russia, northeast China and South Korea, and four samples of *P. armandii*, three from different isolated range fragments in continental China and one from Taiwan Island. Location of the sampling sites is given in table 1 and shown at figure 1. Most samples were bulked, representing a large amount of parental trees. For electrophoretic analysis of allozyme variation we used haploid tissues of endosperms and diploid tissues of embryos from mature seeds.

Electrophoresis

Conditions of seed storage and electrophoresis, as well as number of loci controlling each enzyme and the system of designations have been described earlier (Belokon et al. 2005, Krutovskii et al. 1987). In total, we studied 13 isozyme systems: alcohol dehydrogenase (ADH, 1.1.1.1); formate dehydrogenase (FDH, 1.2.1.2); fluorescent esterase (FE, 3.1.1.x); glutamate dehydrogenase (GDH, 1.4.1.2); glutamate oxaloacetate transaminase (GOT, 2.6.1.1); isocitrate dehydrogenase (IDH, 1.1.1.42); malate dehydrogenase (MDH, 1.1.1.37); phosphoenolpyruvate carboxylase (PEPCA, 4.1.1.31); 6-phosphogluconate dehydrogenase (6-PGD, 1.1.1.44); phosphoglucose isomerase (PGI, 5.3.1.9); phosphoglucomutase (PGM, 2.7.5.1); shikimate dehydrogenase (SKDH, 1.1.1.25) and superoxide dismutase (SOD, 1.15.1.1). Mendelian inheritance of the studied allozyme systems were earlier tested by segregation analysis in heterozygotes in both *P. koraiensis* (Politov 1989) and *P. armandii* (Lewandowski 2000).

Statistical analysis

Based on matrices of multi-locus genotypes of embryos and using computer programs BIOSYS-2 (Swofford & Selander 1981), POPGENE (Yeh et al. 1999) and GenAlEx V.6 (Peakall & Smouse 2006), we calculated allelic frequencies and standard parameters of

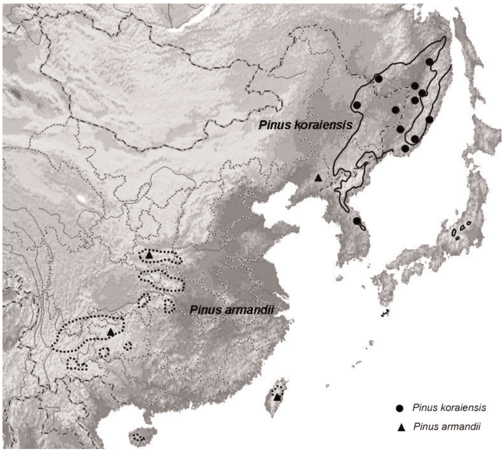

Figure 1 Location of sampling sites

Table 1 Location of *Pinus koraiensis* and *P. armandii* sampling sites

Sample	Code	Coordinates	Country	Location[1]
		Pinus koraiensis		
Obluchye	OB	48°45’N 131°15’E	Russia	Obluchye FMU, Jewish Autonomous Oblast
Lang Xiang	LX	47°00’ N 129°00’E	China	Lang Xiang, Heilongjiang Province, Lesser Hingan Range
East Laoyelin	EL	46°30’ N 133°00’E	China	East Laoyelin Range, Heilongjiang Province
Bektor	BK	49°35’ N 138°20’E	Russia	Bektor FD, Komsomolsk FMU, Khabarovski Krai
Malokhekhtsir	MH	48°20’ N 135°15’E	Russia	Malokhekhtsir FD, Khekhtsir EFMU Khabarovskii Krai
Lazo	LZ	47°30’ N 135°50’E	Russia	Lazo FD, Khor FMU, Khabarovski Krai
Podkhorenkovskoye	PH	47°15’ N 135°10’E	Russia	Podkhorenkovo FD, Vyazemsk FMU, Khabarovski Krai
Ozernoye	OZ	44°42’ N 133°35’E	Russia	Ozernoye FD, Arsenyev FMU, Primorski Krai
Chuguev	CHU	43°55’ N 134°06’E	Russia	Chuguev FD, Chuguev FMU, Primorski Krai
Sikhote-Alinskoye	SA	44°38’ N 135°05’E	Russia	Sikhote-Alin’ FD, Kavalerovo FMU, Primorski Krai
Tayozhnoye	TZ	45°15’ N 136°30’E	Russia	Tayozhnoe, Sikhote-Alin’ NR, Primorski Krai
Taebaek	TH	38°00’ N 128°00’E	Korea	Taebaek Range, Gangwon-do Province
		Pinus armandii		
Taiwan	TW	23°50’N 121°00’E	China	Lishan, Taiwan Island
Kaiyang	KAI	27° 00’N, 106°00’ E	China	Kaiyang, Guizhou Province
Xian	XI	34°00’ N, 108°00’ E	China	Xian, Shanxi Province
Shenyang	SY	41°40’ N 123°50’E	China	Shenyang, Liaoning Province[2]

[1] – For samples from Russian territory FD – Forest District, FMU – Forest Management Unit (includes several FD), EFMU – Experimental Forest Management Unit, NR – Natural Reserve

[2] – Artificial population

genetic variability and differentiation, including observed and expected (from Hardy-Weinberg equilibrium) heterozygosity, F-statistics (Nei 1977, Wright 1978), modified hierarchical F-statistics (Weir 1990, Weir & Cockerham 1984), and standard Nei's genetic distances D_N (Nei 1972). Bootstrap values for estimation of UPGMA-phenogram robustness (Felsenstein 1985) were computed using software package PHYLIP 3.6 (Felsenstein 1989, Felsenstein 1993).

Results

Genetic control of isozyme variation

Genetic control of isozyme systems was the same as previously described for pines of the section *Strobus* (Belokon et al. 2005, Belokon et al. 2007, Lewandowski 2000). The same number of activity zones was observed in both species: *Fdh, Gdh* and *Pepca* are controlled by a single locus each, *Adh, Idh, Pgi, Pgm* and *Skdh* by two loci, *Got* and *6-Pgd*, by three loci, and *Fest, Mdh* and *Sod* by four loci. For estimation of genetic subdivision and differentiation 17 more reliably scored loci allowing establishment of allelic correspondence between two species were selected (table 2).

Allelic composition and frequencies

At the majority of the studied loci most common alleles were the same in both species (table 2). At some loci (*Fest-2, Sod-2, Pgi-2*) all other alleles were also shared between the species and varieties. At some other loci less frequent alleles (mostly with frequencies below 0.05) were species-specific (Fdh^{190}, $Got\text{-}2^{123}$, $Got\text{-}3^{380}$, $Idh\text{-}1^{113}$, $Idh\text{-}1^{81}$, $Mdh\text{-}1^{null}$, $Mdh\text{-}2^{105}$, $6\text{-}Pgd\text{-}3^{90}$, $Pgm\text{-}2^{87}$, $Pgm\text{-}2^{null}$ for *P. armandii*; Gdh^{117}, Gdh^{87}, Gdh^{68}, $Got\text{-}1^{103}$, $Got\text{-}1^{null}$, $Got\text{-}2^{null}$, $6\text{-}Pgd\text{-}3^{null}$, $Pgm\text{-}1^{103}$, $Skdh\text{-}2^{80}$ for *P. koraiensis*). Differences in predominant alleles between the species of populations were observed in nine loci. At locus *Adh-1* there were virtually no alleles shared between the species (except for typical Korean pine allele Adh-1^{110} found in *P. armandii* sample TW at frequency as low as 0.021. The two species had almost completely different allelic composition at locus *Got-2*; alleles $Got\text{-}2^{100}$ and $Got\text{-}2^{115}$ were almost fixed in *P. koraiensis* and *P. armandii*, respectively. At *Mdh-2* most alleles were shared between the species, but predominant alleles were different. A similar situation was observed in locus *Pgm-1*, where three out of the four alleles were shared, with predominant alleles being different. At loci *Mdh-1* and *6-Pgd-3* most common alleles were the same in *P. koraiensis* and in continental *P. armandii* while alleles predominant in Taiwanese *P. armandii* were rare in other samples. In contrast, most common alleles at *Got-3* and *Pgm-2* were shared between Korean pine and Taiwanese *P. armandii* while continental Armand's pine had a different predominant allele. At locus *Pepca* two alleles were detected in both species, however, the predominant allele was different in each species.

Analysis of genetic subdivision by *F*-statistics

Intra-specific differentiation among 12 populations of *Pinus koraiensis* from Russia, Northeast China and South Korea was low, F_{ST} (proportion of variability among population) was as low as 0.019, D_N (Nei's genetic distance) varied between 0.001 and 0.006. The same parameters estimated in *Pinus armandii* from continental China (Guizhou, Shaanxi, Shenyan) showed that genetic differentiation is much more substantial (F_{ST}= 0.067, D_N=0.011-0.029). With adding a sample from Taiwan both F_{ST} (0.338) and D_N (up to 0.232) were substantially higher indicating high level of differences between continental and Taiwanese *P. armandii*, putatively due to longer isolation time. Maximal contribution to differentiation was from loci *Adh-1, Got-3, Mdh-1, 6-Pgd-3, Pgm-2* and *Skdh-2*. Differences were mainly caused by allelic frequencies but not by presence/absence of particular alleles, therefore observed allozyme patterns do not show evidence for species' status of Taiwan variety. However, based on total comparative level of allozyme differentiation, distinction of *P. armandii* var. *mastersiana* as a subspecies is supported.

Similar results were obtained by hierarchical Weir's F-statistics (table 3). At within-species level differentiation was relatively low F_{SP} but variable over loci (from 0.003 to 0.700). Averaged over loci among-species differentiation (F_{PT}=0.694) was high and this evidently accounts for most of the total high subdivision within overall set of samples (F_{ST}=0.714). There was substantial variance in by-loci values at among-species level (F_{PT} values from -0.001 (*Sod-2*) to 0.998 (*Got-2*). Nine loci where different alleles were predominant in different populations demonstrated highest overall F_{ST} values, from -0.002 to 0.998.

Patterns of intra-specific and inter-specific genetic differentiation UPGMA-clustering based on Nei's genetic distance matrix showed two main groups of OTUs corresponding to nominate species (figure 2). Multivariate (PCA) analysis also showed good clustering of samples onto species by first dimension (figure 3). However, within species patterns of differentiation contrasted between two species. Korean pine populations represent a tight cluster with hardly discernible within-species substructuring (figures 4, 5). The South Korean sample from Taebaek Range was most differentiated from other samples. Except for subdivision of total *P. koraiensis* onto this sample and all others, one cluster was supported by bootstrap (figure 4). In contrast, each Armand's

Table 2 Allelic frequencies within *Pinus koraiensis* and *P. armandii* populations

Locus/ Allele		*Pinus koraiensis*												*Pinus armandii*			
		OB	LX	BK	EL	MH	LA	PH	OZ	CHU	SA	TZ	TH	TW	KAI	SY	XI
Adh-1	*130*	0	0	0	0	0	0	0	0	0	0	0	0	0.277	0.019	0	0
	125	0	0	0	0	0	0	0	0	0	0	0	0	**0.702**	**0.981**	**1.000**	**1.000**
	120	0.012	0	0.006	0.044	0.001	0	0.008	0	0.018	0.014	0.012	0	0	0	0	0
	110	0.337	0.256	0.300	0.324	0.328	0.292	0.275	0.382	0.306	0.160	0.320	0.270	0.021	0	0	0
	100	**0.652**	**0.744**	**0.688**	**0.632**	**0.671**	**0.693**	**0.700**	**0.612**	**0.676**	**0.827**	**0.669**	**0.730**	0	0	0	0
	null	0	0	0.006	0	0	0.015	0.017	0.006	0	0	0	0	0	0	0	0
Fdh	*190*	0	0	0	0	0	0	0	0	0	0	0	0	0.129	0.154	0.333	0.091
	100	**1.000**	**1.000**	**0.988**	**1.000**	**1.000**	**1.000**	**1.000**	**0.982**	**1.000**	**1.000**	**1.000**	**1.000**	**0.871**	**0.846**	**0.667**	**0.909**
	null	0	0	0.012	0	0	0	0	0.018	0	0	0	0	0	0	0	0
Fest-2	*120*	0.004	0.015	0	0.020	0.004	0.004	0.034	0.012	0	0.003	0	0	0	0.019	0.100	0.198
	100	**0.990**	**0.980**	**1.000**	**0.974**	**0.994**	**0.967**	**0.963**	**0.988**	**0.994**	**0.993**	**1.000**	**1.000**	**0.870**	**0.962**	**0.900**	**0.791**
	null	0.006	0.005	0	0.007	0.003	0.028	0.003	0	0.006	0.003	0	0	0.130	0.019	0	0.012
Gdh	*117*	0.018	0	0.089	0	0.043	0.052	0	0.006	0	0.053	0	0	0	0	0	0
	100	**0.982**	**1.000**	**0.905**	**1.000**	**0.956**	**0.948**	**1.000**	**0.994**	**0.988**	**0.947**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
	87	0	0	0.006	0	0.001	0	0	0	0	0	0	0	0	0	0	0
	68	0	0	0	0	0	0	0	0	0.012	0	0	0	0	0	0	0
Got-1	*103*	0	0	0	0	0	0	0	0	0.006	0	0.017	0	0	0	0	0
	100	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.994**	**1.000**	**0.977**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
	null	0	0	0	0	0	0	0	0	0	0	0.006	0	0	0	0	0
Got-2	*123*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.029
	115	0	0	0	0	0	0	0	0	0	0	0	0	**0.988**	**1.000**	**1.000**	**0.971**
	100	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.994**	**1.000**	**1.000**	**1.000**	**1.000**	0.012	0	0	0
	null	0	0	0	0	0	0	0	0.006	0	0	0	0	0	0	0	0
Got-3	*380*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.111	0.044
	250	0.198	0.107	0.182	0.078	0.183	0.151	0.136	0.171	0.188	0.179	0.194	0.197	0.070	**0.950**	**0.889**	**0.956**
	100	**0.802**	**0.876**	**0.818**	**0.882**	**0.805**	**0.845**	**0.858**	**0.829**	**0.806**	**0.821**	**0.788**	**0.803**	**0.919**	0.050	0	0
	48	0	0.017	0	0.039	0.012	0.004	0.006	0	0.006	0	0.018	0	0.012	0	0	0
Idh-1	*113*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.045
	100	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.967**	**0.943**
	81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033	0.011
Mdh-1	*100*	**1.000**	**1.000**	**1.000**	**1.000**	**0.999**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	0.026	**0.739**	**0.833**	**0.938**
	90	0	0	0	0	0.001	0	0	0	0	0	0	0	**0.923**	0.261	0.167	0.063
	null	0	0	0	0	0	0	0	0	0	0	0	0	0.051	0	0	0
Mdh-2	*105*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033	0.244
	100	**0.788**	**0.858**	**0.729**	**0.857**	**0.733**	**0.795**	**0.836**	**0.806**	**0.688**	**0.839**	**0.802**	**0.754**	0.036	0.058	0	0.033
	95	0.017	0.005	0.047	0	0.011	0	0	0.029	0.065	0	0.041	0.205	0.012	0.442	0.133	0.122
	85	0.196	0.137	0.224	0.143	0.256	0.205	0.161	0.165	0.247	0.161	0.157	0.041	**0.952**	**0.500**	**0.833**	**0.589**
	75	0	0	0	0	0	0	0.003	0	0	0	0	0	0	0	0	0.011
Pepca	*136*	0.208	0.150	0.141	0.274	0.210	0.163	0.158	0.141	0.124	0.227	0.076	0.238	**0.824**	**0.980**	**0.967**	**0.989**
	100	**0.792**	**0.850**	**0.859**	**0.726**	**0.790**	**0.838**	**0.842**	**0.859**	**0.876**	**0.773**	**0.924**	**0.762**	0.176	0.020	0.033	0.011
6Pgd-3	*125*	0	0	0	0	0	0	0	0	0	0	0	0.008	0.023	0	0	0
	100	**0.930**	**0.959**	**1.000**	**0.994**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.992**	0.023	**0.540**	**0.633**	**0.678**
	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.044
	85	0.070	0.041	0	0	0	0	0	0	0	0	0	0	**0.955**	0.460	0.367	0.278
	null	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0
Pgi-2	*126*	0.002	0.005	0	0	0	0	0	0	0	0	0	0	0	0.019	0.167	0.216
	100	**0.998**	**0.995**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.992**	**1.000**	**0.981**	**0.667**	**0.773**
	84	0	0	0	0	0	0	0	0	0	0	0	0.008	0	0	0.167	0.011
Pgm-1	*103*	0.081	0.127	0.100	0.050	0.092	0.067	0.108	0.153	0.094	0.043	0.134	0.025	0	0	0	0
	100	**0.889**	**0.873**	**0.835**	**0.856**	**0.848**	**0.808**	**0.847**	**0.788**	**0.759**	**0.789**	**0.750**	**0.975**	0.022	0	0	0
	97	0	0	0	0	0.001	0	0	0.006	0	0	0	0	**0.978**	**1.000**	**0.944**	**0.944**
	92	0.030	0	0.065	0.094	0.059	0.125	0.045	0.053	0.147	0.168	0.116	0	0	0	0.056	0.056

Table 2 (continuation)

Pgm-2	*110*	0	0	0	0	0	0	0.003	0	0	0	0	0	0	**0.654**	**0.767**	**0.761**
	100	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.997**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	0.346	0.233	0.216
	87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.011
	null	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.011
Skdh-2	*120*	0.004	0	0	0	0	0	0	0	0	0	0.006	0	0	0.357	0.100	0.030
	100	**0.990**	**1.000**	**0.988**	**1.000**	**1.000**	**1.000**	**0.997**	**1.000**	**1.000**	**1.000**	**0.994**	**1.000**	**1.000**	**0.643**	**0.900**	**0.970**
	80	0.006	0	0.012	0	0	0	0.003	0	0	0	0	0	0	0	0	0
Sod-2	*100*	**0.974**	**0.974**	**0.976**	**0.981**	**0.997**	**0.994**	**0.966**	**1.000**	**0.976**	**0.992**	**1.000**	**0.967**	**0.987**	**1.000**	**1.000**	**0.987**
	65	0.026	0.026	0.024	0.019	0.003	0.006	0.034	0	0.024	0.008	0	0.033	0.013	0	0	0.013

Note. Most frequent alleles in the populations are boldfaced

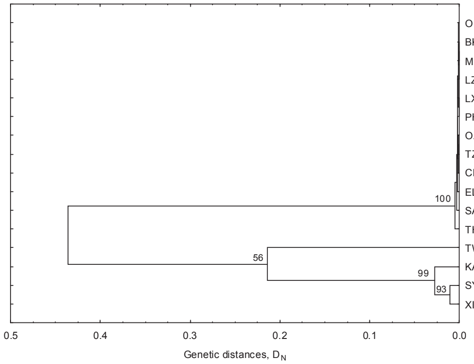

Figure 2 UPGMA dendrogram based on Nei's genetic distances by 17 allozyme loci for 12 *Pinus koraiensis* and 4 *P. armandii* samples (bootstrap values are at corresponding nodes)

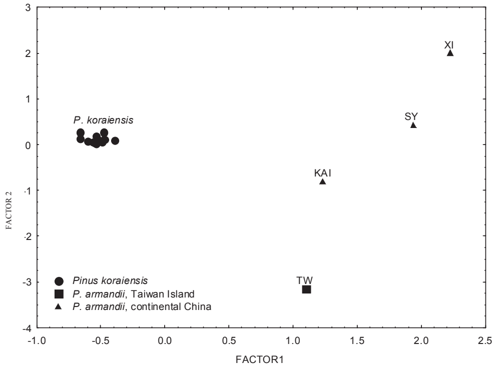

Figure 3 Ordination of 12 *Pinus koraiensis* samples and 4 *P. armandii* samples based of first two principal components by 17 allozyme loci frequencies

pine sample was distinct and well-differentiated from others (figures 2, 4). All *P. armandii* samples seem to be equally differentiated from *P. koraiensis*. Level of differentiation among continental *P. armandii* samples was about 10 times higher than that within *P. koraiensis*. Level of joining of Taiwanese Armand's pine (*P. armandii* var. *mastersiana*) was about 10 times higher than within continental samples of this species.

Discussion

There is no direct evidence in favor of close relatedness between *P. armandii* and *P. koraiensis,* however, numerous morphological, anatomical and palynological similarities (Bobrov 1978, Critchfield 1986, Kuprianova & Litvintetva 1974, Litvintseva 1974) create sound background for establishment of such relationships, and, therefore, for comparison of genetic variation patterns between the two species. The presented allozyme data clearly demonstrated that *P. koraiensis* and *P. armandii* have contrasting types of genetic differentiation. The extent of inter-population subdivision evidently correlates with degree of range fragmentation. Korean pine represents a morphologically and genetically uniform species homogeneous with respect to allozyme frequencies across at least the continental parts of its range and Sakhalin Island. Similar results were obtained for populations from the Russian Far East where F_{ST} varied among 1.5% (Potenko 2004, Potenko & Velikov 1998, Potenko & Velikov 2001) and 4% (Krutovskii et al. 1994, Krutovskii et al. 1995). The same level of subdivision was found in an allozyme study of 4 China populations (Yang et al. 1989)

Table 3 Hierarchical Weir's and non-hierarchical Wright's F-statistics

Locus	*F*-statistics (WEIR 1990) $\check{C}_S$ (F_{ST})	$\check{C}_P$ (F_{PT})	F_{SP}
Adh-1	0.634	0.628	0.016
Fdh	0.507	0.471	0.069
Fe-2	0.237	0.211	0.033
Gdh	0.032	0.009	0.023
Got-1	0.009	-0.004	0.013
Got-2	0.998	0.998	0.010
Got-3	0.475	0.419	0.097
Idh	0.124	0.101	0.025
Mdh-1	0.936	0.787	0.700
Mdh-2	0.568	0.554	0.031
Pepca	0.707	0.702	0.017
6Pgd-3	0.796	0.738	0.220
Pgi-2	0.437	0.374	0.101
Pgm-1	0.757	0.752	0.018
Pgm-2	0.935	0.868	0.504
Skdh-2	0.406	0.268	0.189
Sod-2	0.001	-0.002	0.003
Overall	0.714	0.694	0.066
	F_{ST} (WRIGHT 1978)		
P. koraiensis	0.019		
P. armandii continental	0.067		
P. armandii total	0.338		
Non-hierarchical total two species	0.477		

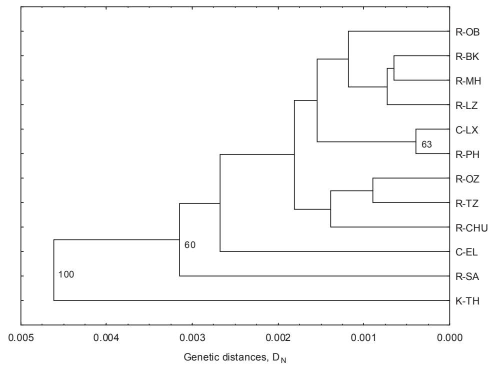

Figure 4 UPGMA dendrogram based on Nei's genetic distances by 17 allozyme loci for 12 *Pinus koraiensis* (bootstrap values are at corresponding nodes)

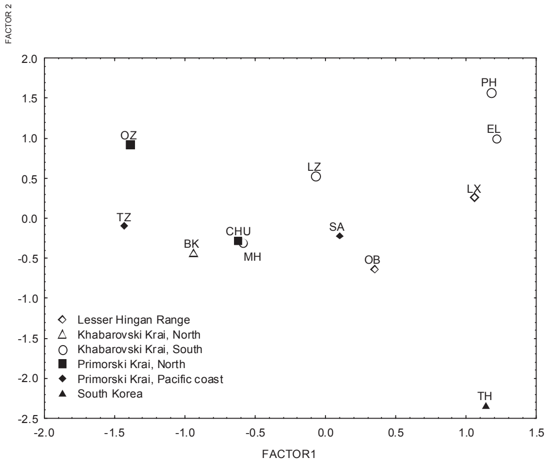

Figure 5 Ordination of 12 *Pinus koraiensis* samples based on first two principal components by 17 allozyme loci frequencies

where G_{ST} was 0.021. Populations of *P. koraiensis* in Korea were reported to be more genetically diverse (F_{ST}=6%) (Kim et al. 1994). Similar values (F_{ST}=7%) were observed for a combined set of samples from China, Korea and Russia (Kim et al. 2005). Homogeneity of allelic patterns at allozyme loci putatively reflects long and relatively undisturbed existence within a continuous area with little variation in edaphic and climatic conditions. Despite the fact that in the southern part of its range fragmentation takes place, this is the direct result of the last 100 years of anthropogenic pressure.

Due to difficulties in discrimination between pines of the section *Strobus* by fossil pollen, little is known about Tertiary and Pleistocene ranges of particular species including ones under consideration. However, the distribution of macrofossils made some researchers (Udra 1988) believe that Korean pine originated from Armand's pine during the Pliocene. During the Tertiary period Armand's pine was putatively distributed both within and far outside its modern range (Mirov 1967). Once continuous, its ancient range was then fragmented during the Quaternary period as a result of geological and climatic changes. Following this fragmentation, current geographical races were formed (Mirov 1967). Genetic differentiation was relatively high in *Pinus armandii* within continental China where its range is highly fragmented. In contrast to Korean pine, fragmentation of Armand's pine range is a much older phenomenon. Gene flow between the isolates ceased putatively in the Pleistocene in relation to glacial events. The territory of current China was outside the direct influence of the glaciation, but contributed to the general effect as the climate definitely became more arid. While for *P. koraiensis*, the Upper Pleistocene and Holocene were favorable eras when it was advancing to the north, for Armand's pine the dry and cold climate caused its disappearance from many regions. Retreat of the marine coast in the Pleistocene allowed Armand's pine to inhabit Islands of the South China Sea. The following rise of the sea level in the Holocene has lead to isolation of these island populations. Our results are very high for interspecific comparisons; genetic distance between continental and Taiwanese varieties corresponds well with earlier allozyme data (Kanetani et al. 2004); however, *P. armandii* var. *amamiana* from two small Japanese islands have much greater level of differences in allozyme loci. Moreover, its morphological specificity, especially with respect to female cone size and shape made some researchers treat is as a separate species *Pinus amamiana*. At this scale, Armand's pine from Taiwan deserves at least its most recognizable status of subspecies despite its genetic distance from continental variety of the same order than most closely related species known among soft pines (Krutovskii et al. 1990, Krutovskii et al. 1994, Krutovskii et al. 1995).

As to Korean pine, there is no strict evidence on existence of this species within the modern range before or during glacial time. According to pollenological data (Neischtadt 1952, Neischtadt 1957), during the Pleistocene *P. koraiensis* existed to the south of Lake Hanka and advanced to the north relatively recently, in the Holocene period. Therefore, *P. armandii* and *P. koraiensis* might have connected in the relatively recent past. Both genetic data (Belokon et al. 1998, Goncharenko et al. 1992, Krutovskii et al. 1990, Krutovskii et al. 1994, Krutovskii et al. 1995, Wang et al. 1999), and anatomical features of needles (Critchfield 1986, Litvintseva 1974) and pollen structure (Kuprianova & Litvintseva 1974) indicate substantial similarity.

Greater fragmentation of Armand's pine range is caused to a large extent by higher human impact. However, differences in species' ecology should be taken into account as well, especially more tight interaction of Korean pine with nutcrackers putatively allowing this species to withstand both Pleistocene cataclysms and raising anthropogenic pressure.

Acknowledgements

The authors are grateful to Prof. Reiner Finkeldey, Dr. Huoran Wang, Dr. Sergey N. Goroshkevich, Dr. Anatoly V. Gromadin, Pavel Razumov, and staff of Khabarovsk Forest Seed Station who kindly provided the seeds used in this study. The study was supported by the Russian Academy of Sciences through several research programs and by the grant offered by the Russian Federation President as Support of Scientific Schools NSh-8596.2006.4.

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Rezumat. Belokon M.M., Belokon Y.-., Politov D.V., 2008. Modele contrastante ale diferențierii genetice spațiale la două specii de pin cu cinci ace, est asiatice, *Pinus koraiensis* şi *P. armandii*. Ann. For. Res. 51: 31-42.

Pinul coreean (*Pinus koraiensis*) şi Pinul armand (*P. armandii*) sunt două specii cu arealul în estul Asiei aparținând Secției Strobus şi subsecțiilor *Cembrae* şi respectiv *Strobi*. Deşi fac parte din subsecții diferite, există o similaritate a acestor specii în privința structurii anatomice a acelor şi polenului; respectiva similaritate se prezintă în comunicarea de față. Cu ajutorul electroforezei în suspensie de amidon a fost studiată variația aloenzimatică atât în interiorul celor două specii cât şi între ele. Dintre cei 30 loci studiați, au fost selectați 17 loci polimorfici care au prezentat interpretări credibile în privința alelelor dintre specii. Cu privire la numărul de loci şi la alelele predominante a majorității locilor, s-a constatat că modelele aloenzimatice au fost similare pentru ambele specii. Diferențierea intraspecifică între 12 populații de *P. koraiensis* din Rusia, Nord-estul Chinei şi Sudul Coreei a fost mică, adică proporția variabilității între populații F_{ST} a avut valoarea 0,019 iar distanța genetică a lui Nei a variat între 0,001 şi 0,006. Această uniformitate genetică poate fi explicată printr-un fond comun de gene prezent în populațiile studiate aflate într-un areal continuu situat în condiții ecologice similare. Vectorii comuni ai selecției cum ar fi: migrația naturală a genelor prin polen şi semințe precum şi amestecarea materialului genetic între regiuni de către factorul antropic (valabil cel puțin în practica silvică din Rusia) sunt factorii prezumtivi responsabili pentru diferențierea mică la pinul coreean. Se estimează prezența aceloraşi parametri şi la *P. armandii* răspândit în China continentală (Guizhou, Shaanxi, Shenyan) unde arealul speciei este foarte fragmentat; totuşi, în acest caz s-a constatat că diferențierea la această specie este mult mai pronunțată, adică F_{ST}=0,067, D_N=0,011-0,029). Prin includerea în analize a unui eşantion din Taiwan, atât F_{ST} (0,338), cât şi D_N (cu valoarea de până la 0,232) au devenit semnificativ mai mari ceea ce indică prezența unui nivel ridicat de diferențiere între *P. armandii* continental şi taiwanez; probabil, aceasta se datorează unei lungi perioade de izolare în timp. Contribuția maximă este atribuibilă locilor *Adh-1, Got-3, Mdh-1, 6-Pgd-3, Pgm-2* şi *Skdh-2*. În principal, diferențele se datorează frecvențelor alelice şi nu prezența sau absența anumitor alele; prin urmare, modelele aloenzimatice observate nu oferă suportul necesar pentru a denumi varietatea taiwaneză ca subspecie distinctă. Datele rezultate din electroforeză la *P. koraiensis* şi *P. armandii* au indicat prezența aceluiaşi număr al zonelor de activitate. Din analiza combinată a mostrelor de *P. koraiensis* şi *P. armandii* a rezultat că niveul diferențelor interspecifice a fost mare, respectiv F_{ST}=0,477, D_N =0,407

- 0,457. Speciile nu au avut alele comune şi nici mici suprapuneri al profilele alelice la locii *Adh-1, Got-2,* şi *Pgm-1*. Din 61 alele, 35 au fost împărţite între diferite specii, iar 26 au fost găsite doar la una dintre ele. Analiza grupelor de arbori (cluster analysis) (UPGMA) şi analiza multivariată (PCA) a variaţiei aloenzimelor au arătat existenţa unei mari variaţii în interiorul speciei *P. armandii* precum şi o mică diferenţiere în interiorul speciei *P. koraiensis*.

Cuvinte cheie: *Pinus koraiensis, Pinus armandii,* aloenzime, diferenţiere genetică, alele, loci polimorfici, distanţă genetică.

(Tradus de I. Blada)