

## Genetic Diversity and Relationships Among Siberian and Far Eastern Larches Inferred from RAPD Analysis

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**Abstract**—Genetic diversity of larches from six geographically distant regions, Tomsk, Irkutsk, Ulan-Ude (Siberia), and Blagoveshchensk, Khabarovsk, Yuzhno-Sakhalinsk (Far East) was examined by means of RAPD analysis. Tree DNA samples were compared using 457 RAPD loci (97% of which were polymorphic), identified with 17 primers of random sequences. In the samples examined, 32 to 49% of the genes were in heterozygous state, mean expected heterozygosity ( $H_{exp}$ ) varied from 0.1373 to 0.1891, and the genetic distances ( $D_N$ ) for different sample pairs varied from 0.0361 to 0.1802. The main population parameters were determined for *Larix sibirica* Ledeb., *L. gmelinii* (Rupr.) Rupr., and *L. kamtschatica* (Rupr.) Carr. Analysis of the genetic relationships showed that *L. kamtschatica* was characterized by highest genetic differentiation from the other larches examined, while larches from Primorskii krai were genetically close to *L. sibirica*.

### INTRODUCTION

The genus *Larix* Mill. contains from 10 to 15 (according to some authors, more than 20) larch species [1–4], distributed in cold and temperate parts of the Northern Hemisphere. Larch species are distinguished by their high plasticity and the absence of the mechanisms of reproductive isolation, which can favor the processes of introgressive hybridization and the formation of hybrid populations, characterized by high variability of phenotypic characters. This pattern of the population structure can often lead to false identification of taxonomic units and also to taxon fragmentation and artificial complexity.

Larch species from the Ural, Siberia, and Far East have been studied rather extensively. Species *L. sibirica* Ledeb. [1, 5–8], *L. × czekanovskii* Szaf. [5], *L. gmelinii* (Rupr.) Rupr. [9, 10], *L. cajanderi* Mayr [9], and *L. sukaczewii* Dyl. have been characterized in most detail [7, 11–13]. The opinions on the number of larch species in the Russian Far East are controversial. According to Voroshilov [14], the Far Eastern larch is represented by only one species, *L. gmelinii* (Rupr.) Rupr. (the area encompasses Primorye, Amur, Okhotiya, Kamchatka, and the Kuril Islands), along with its main varieties, between which there are many intermediate forms. Regional reports, however, contain other data on the larch species composition. For instance, Starikov [15] thinks that there are four larch species in Khabarovsk krai, *L. gmelinii* (Rupr.) Rupr., *L. cajanderi* Mayr, *L. ochotensis* Kolesn., and *L. maritima* Sukacz. In Primorskii krai Gukov [16] distinguishes six larch species, *L. olgensis* A. Henry, *L. komarovii* Kolesn., *L. amurensis* Kolesn., *L. ochotensis* Kolesn., *L. maritima* Sukacz., and *L. lubarskii* Sukacz. Multivolume edition *Vascular*

*Plants of the Soviet Far East* [17] contains the descriptions of the three larch species, *L. gmelinii* (Rupr.) Rupr., *L. cajanderi* Mayr, and *L. olgensis* A. Henry. Furthermore, it is noted in this edition that species status of *L. olgensis* is rather doubtful, while *L. kamtschatica* (Rupr.) Carr., *L. komarovii* Kolesn., *L. amurensis* Kolesn., *L. ochotensis* Kolesn., *L. maritima* Sukacz., *L. lubarskii* Sukacz., and *L. middendorffii* Kolesn. are not considered isolated species.

Scarce knowledge on the Far Eastern representatives of the genus *Larix* has a negative effect on the resolution of practical issues, since larch is one of the main woody plant species of Siberia and the Far East. Intensive and sometimes uncontrolled timber-harvesting operations, fires, pests, continuous increase of the human pressure on the biosphere, along with many other reasons lead to the dramatic reduction of forest areas, resulting in the loss of genetic and species diversity. Elaboration of measures aimed at the gene pool and species diversity preservation along with the *Larix* resources conservation require the data on the genetic structures of the populations and species.

In recent decades, population and evolutionary studies of some members of the family Pinaceae have been performed using molecular genetic markers, including allozymes [6–8, 10–13, 18–21], randomly amplified polymorphic DNAs (RAPDs) [22–27], microsatellites (SSRs) [28–31], and others. In our earlier study, using RAPD method we have examined five larch samples from Primorskii krai and determined their genetic parameters [32].

The goal of the present study was to examine genetic diversity, to evaluate the level of genetic differ-

**Table 1.** Larch samples from Siberia and the Far East studied

Sample	Code	Location, coordinates
1. Ulan-Ude	ULAN	20 km northern Ulan-Ude, southern ridges of the Ulan-Burgasy range, coordinates: 52° N, 107°35' E (11 trees)
2. Tomsk	TOM	Tomsk oblast, Timiryazev forestry. Tree age, 30–40 years, coordinates: 56°30'39" N, 84°49'45" E (11 trees)
3. Irkutsk	IRK	100 km southern Irkutsk, 10 km from the settlement of Kultuk, southern part of the Lake Baikal, lakeside declination of the Primorskii range (12 trees)
4. Sakhalin	SAKH	Sakhalin Island, Aniva raion, 1 km northwestern the settlement of Peschanskoe, 1–1.5 km from the seashore, swamped larch forest, coordinates: 46°43'54" N, 142°36'02" E (7 trees)
5. Khabarovsk	KHAB	Khabarovsk krai, Lazo raion, the settlement of Kiya, Bichevskoe peat bed, ledum–peatmoss larch forest (3 trees)
6. Blagoveshchensk	BLAG	Amur oblast, Blagoveshchensk raion, tree plantations, tree age, approximately 20 years (11 trees)
7. Komarova-1	KOM-1	Primorskii krai, head water of the Lagernaya river, coordinates: 45°70' N, 136°35' E (5 trees)
8. Komarova-2	KOM-2	Primorskii krai, Irtysh River, coordinates: 45°05' N, 135°80' E (9 trees)
9. Amurskaya	AMUR	Primorskii krai, Khreimanov brook, coordinates: 45°46' N, 134°14' E (9 trees)
10. Ol'ginskaya	OLG	Primorskii krai, head water of the Arzamasovka River, Vymoinaya fold, coordinates: 43°90' N, 135°21' E (3 trees)
11. Okhotskaya	OCH	Primorskii krai, Veselyi pass, coordinates: 45°48' N, 137° E (6 trees)

entiation, and to analyze the relationships among the larch species from Siberia and the Far East.

## MATERIALS AND METHODS

*Experimental material* consisted of young needles of 55 trees from six samples of Siberian and the Far Eastern larches (Table 1, nos. 1–6). The sample group included five samples, representing three species, *L. sibirica* (ULAN, TOM, and IRK), *L. kamtschatica* (SAKH), and *L. gmelinii* (KHAB), all collected from natural populations. The sixth sample, BLAG, was taken from tree plantation and its species affiliation was uncertain.

*Genomic DNA* was extracted from 300–500 mg of fresh needles according to the method described in [22].

*Polymerase chain reaction* was conducted in a thermal cycler UNO II 48 (Biometra, Germany) using the 10-mer primer of random sequence (Operon Technologies Inc., United States). The reaction mixture and temperature conditions were as described earlier [33]. Twenty-five microliters of amplification buffer contained 0.125 units of *Taq* polymerase and 12.5 ng of genomic DNA. PCR products were fractionated by means of electrophoresis in 1.4% agarose gel in the presence of ethidium bromide and photographed in the UV light.

*Statistical analysis of RAPD phenotypes* was carried out via comparison of the bands in RAPD profiles of the samples tested using the RFLPscanPlus 3.12 software program. Note that only the bands that were reproduced in repeated experiments were scored (the intensity polymorphism was not taken into account). For each primer, binary matrices were constructed.

RAPD loci were named after the primers in the presence of which they were obtained with the addition of their sizes in base pairs.

Experiments were carried out using diploid samples, and because of this, in order to obtain unbiased estimates of the RAPD allele frequencies, the later were calculated by use of indirect method [34] based on the frequencies of the individuals lacking the given fragment.

Quantitative estimates of RAPD polymorphism ( $P_{95}$ ), mean expected heterozygosity ( $H_{exp}$ ), Nei genetic distances ( $D_N$ ), as well as the construction of the dendrograms of genetic relationships between the samples based on the matrices of the ( $D_N$ ) values by use of the unweighted pair-grouped method of cluster analysis (UPGMA), were performed using the TFPGA applied software package [35]. Calculations of the mean allele number per locus ( $A$ ), effective allele number per locus ( $A_e$ ), mean sample gene diversity ( $H_S$ ), total gene diversity in the total sample ( $H_T$ ), subdivision index ( $G_{ST}$ ), gene flow value ( $N_m$ ), coefficient of pairwise similarity between the individuals ( $S$ ), and the mean within-group similarity ( $S_{pop}$ ) were carried out using the POPGENE software package [36]. All values of population parameters, except the proportion of polymorphic loci and the genetic distances, are theoretically expected.

Dendrogram of genetic relationships between the individuals was constructed using the TREECON software package [37]. The test for population differentiation was conducted using the TFPGA software package.

To estimate the relationships between the Siberian and Far Eastern larches, binary matrices were con-

**Table 2.** Genetic variability detected with the primers used

Primer	Nucleotide sequence (5' → 3')	Number of loci in samples						Total number of loci scored
		ULAN	TOM	IRK	SAKH	KHAB	BLAG	
*OPA-01	CAGGCCCTTC	9/14	14/18	10/14	8/11	5/8	11/15	26
OPA-03	AGTCAGCCAC	4/11	3/10	7/10	7/14	7/16	5/10	18
OPA-04	AATCGGGCTG	17/22	17/23	21/26	23/25	20/25	31/32	33
OPA-07	GAAACGGGTG	13/15	9/12	12/14	5/6	7/11	17/17	31
*OPA-09	GGGTAACGCC	15/19	13/17	14/17	11/14	9/15	19/21	31
OPA-10	GTGATCGCAG	13/16	15/17	13/17	16/20	12/20	20/22	27
*OPA-11	CAATCGCCGT	16/17	14/16	17/19	10/12	4/6	15/16	28
*OPA-12	TCGCGATAG	12/15	9/12	8/12	14/17	9/11	16/17	31
OPA-15	TTCCGAACCC	8/10	4/7	4/6	7/10	10/12	14/15	20
*OPA-19	CAAACGTCGG	10/12	12/14	11/13	15/17	10/14	21/21	29
*OPA-20	GTTGCGATCC	14/18	12/15	13/15	12/16	6/13	18/19	29
OPB-07	GGTGACGCAG	13/18	14/17	13/15	13/15	9/15	20/22	26
*OPB-10	CTGCTGGGAC	8/14	8/15	10/13	15/18	11/19	21/24	26
*OPB-11	GTAGACCCGT	10/12	13/14	8/11	9/11	5/8	16/17	21
*OPC-02	GTGAGGCGTC	13/16	16/17	16/17	12/15	7/14	13/15	28
OPC-06	GAACGGACTC	18/18	14/16	17/19	21/23	9/13	24/24	31
*OPC-19	GTTGCCAGCC	9/14	9/12	13/15	12/15	4/11	12/16	22
Total		202/261	196/252	207/253	210/259	145/231	293/323	457

Notes: In the numerator, the number of polymorphic loci; in the denominator, the number of scored loci for each sample. ULAN, TOM, IRK, SAKH, KHAB, and BLAG: population codes (see Table 1).

\* Primers used for RAPD analysis of 87 DNA specimens from 11 larch samples from Siberia and the Far East.

structured and statistically analyzed. The matrices were based on the RAPD profiles for 55 trees from six samples examined in the present study (Table 1, nos. 1–6), and on those for 32 trees from five populations of Primorskii krai (Table 1, nos. 7–11), obtained earlier [32]. Dendrograms were constructed using neighbor-joining method (NJ) with the bootstrap estimates the reliability of the branching order (1000 replications) within the TREECON software package. As the root, the *L. decidua* DNA sample was taken.

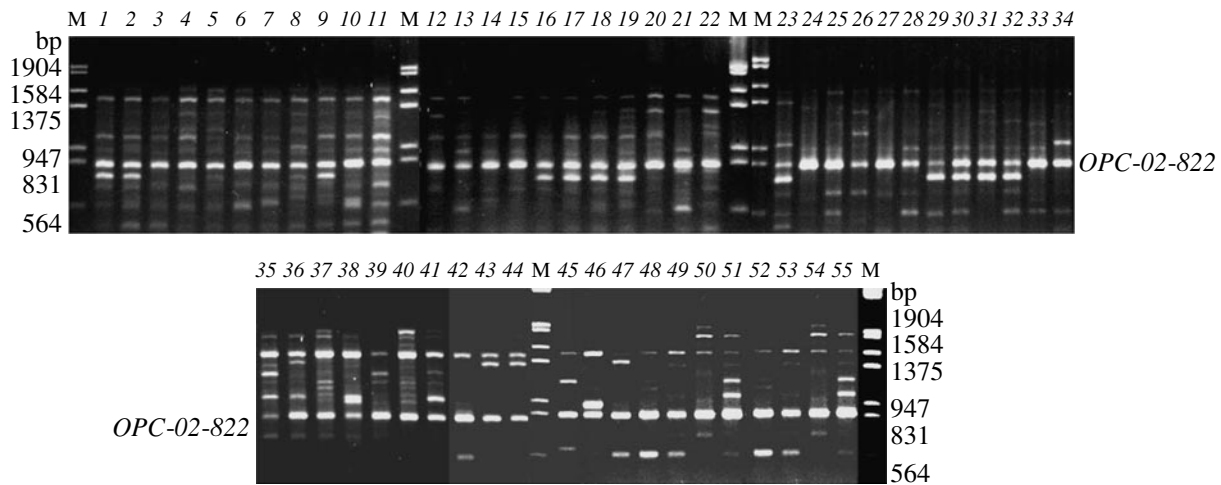
## RESULTS AND DISCUSSION

RAPD analysis was carried out using 17 primers (Table 2) chosen from a set of primers, which were effective in the PCR reactions with the larch DNA templates [38]. Each primer was characterized by a specific pattern, differing from the others by the number of fragments, their sizes, and by the level of their expression. As an example, Fig. 1 presents the amplification profiles obtained for 55 DNA specimens from six population samples with primer OPC-02.

Comparison of the RAPD profiles provided estimation of 457 RAPD fragments variability. As a result, 445 of these fragments (97%) were found to be polymorphic. Eleven monomorphic loci, including OPA-01-935, OPA-10-1197, OPA-11-1286, OPA-12-1023, OPA-20-1057,

OPA-03-603, OPA-03-786, OPA-03-1123, OPB-10-849, OPC-19-797, and OPC-02-822 were common for all samples. The number of amplicons in the RAPD profiles varied from 18 to 33 depending on the primer used (Table 2), constituting on average 26.9 loci per primer. The samples examined displayed remarkable differences relative to the allelic frequencies at most of the RAPD loci.

Table 3 presents the values of the main genetic polymorphism estimates calculated based on the allelic frequencies for each population sample examined. The proportion of polymorphic loci in the individual samples at the significance level of 95% ( $P_{95}$ ) varies from 32 to 49%, the mean number of alleles per locus ( $A$ ) constitutes from 1.3173 to 1.6411, effective allele number ( $A_e$ ) is from 1.2093 to 1.2919, and mean expected heterozygosity ( $H_{exp}$ ) is 0.1373 to 0.1891. The mean within-group similarity ( $S_{pop}$ ) index in individual populations varied from 0.7570 to 0.8286. Sample BLAG was characterized by the highest values of  $P_{95}$ ,  $A$ ,  $A_e$ , and  $H_{exp}$  along with the lowest value of  $S_{pop}$ . The mean values of the main genetic variability parameters for six larch samples ( $P_{95} = 39.53\%$ ;  $A = 1.457$ ;  $A_e = 1.2486$ ;  $H_{exp} = 0.1564$ ) were lower than those also based on the RAPD data for the Primorskii krai population (without OLG) ( $P_{95} = 57.84\%$ ;  $A = 1.5784$ ;  $A_e = 1.3016$ ;  $H_{exp} = 0.1943$ ). Based on the isozyme data, Potenko and Velikov showed that Far Eastern populations of *Abies*



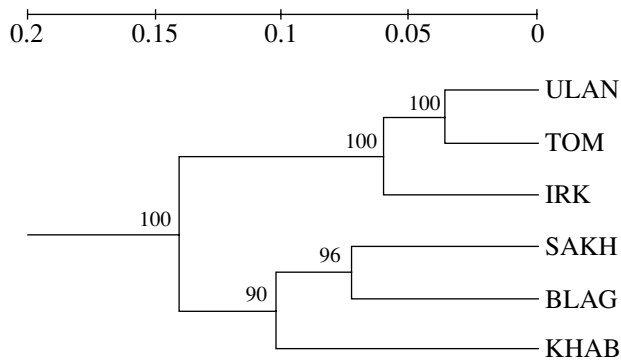
**Fig. 1.** RAPD profiles of 55 larch DNA samples obtained with primer OPC-02. M, (*EcoRI* + *HindIII*)-digested DNA of phage lambda. Lanes 1–11, sample ULAN; 12–22, TOM; 23–34, IRK; 35–41, SAKH; 42–44, KHAB; 45–55, BLAG. For sample code see Table 1.

*nephrolepis* and *A. sakhalinensis*, *Larix gmelinii*, *Picea jezoensis*, *P. glehnii*, and *P. koraiensis*, *Pinus koraiensis* and *Taxus cuspidata* were characterized by high values of polymorphism, heterozygosity, mean allele number per locus, and effective allele number [39]. These populations possess the reserve of genetic variability, and they form the “variability centers,” located in the Sikhote-Alin’ and in Lower-Amur mountain chain [39]. It is suggested that larch populations of Primorskii krai [32] also form such center, where active forming and speciation are taking place.

The main genetic parameters determined for larch samples from Siberia and the Far East examined were close to those for some Siberian and Far Eastern *Larix* species (Table 3), and also to the mean genetic polymorphism estimates calculated for 102 other Gymnospermae species ( $P = 53.4\%$ ;  $A = 1.83$ ;  $A_e = 1.20$ ;  $H_{exp} = 0.151$ ) from the allozyme data [40]. The genetic distances ( $D_N$ ) calculated over 457 RAPD loci for differ-

ent pairs of samples varied reaching sixfold difference (Table 4). This observation can be explained by their belonging to different taxonomic units (population, species, subspecies, hybrid forms, etc.). It was demonstrated that sample KHAB was most genetically distant from the samples ULAN, TOM, and IRK, while samples ULAN and TOM were closest to each other. Figure 2 presents the dendrogram of genetic relationships between the samples examined. Grouping was performed with high degree of reliability (bootstrap support of 90% and higher). The samples formed two clusters; the first of them included the Siberian samples (ULAN, TOM, and IRK), and the second was composed of the Far Eastern samples (SAKH, KHAB, and BLAG).

Based on morphological characters of reproductive organs used in species diagnostics, all Siberian samples were attributed to one species, *L. sibirica*. Analysis of the frequencies of 445 RAPD fragments provided identification of the RAPD marker sets typical of Siberian samples. Group ULAN–TOM–IRK shared 12 RAPD loci, ULAN–TOM had 31 loci in common, and groups ULAN–IRK and TOM–IRK possessed 19 and 18 common loci, respectively. In addition, RAPD profiles of ULAN and TOM demonstrated the presence of the marker allele OPA-07-521, absent in all other Siberian and Far Eastern samples. The between-population differentiation index ( $G_{ST}$ ) and the value of gene flow ( $N_m$ ) calculated for the three samples were 0.1864 and 2.18, respectively. The test for differentiation revealed the heterogeneity of the total sample ULAN–TOM–IRK and substantial differences in the RAPD fragment frequencies within the pairs TOM–IRK ( $\chi^2 = 1033.23$ ;  $d.f. = 914$ ;  $P = 0.0036$ ) and ULAN–IRK ( $\chi^2 = 999.0724$ ;  $d.f. = 914$ ;  $P = 0.0258$ ). Only the sample pair ULAN–TOM represented a group of closely related individuals ( $\chi^2 = 580.3697$ ;  $d.f. = 914$ ;  $P = 1.0000$ ). Coefficients of genetic differences of sample IRK with any of Siberian



**Fig. 2.** UPGMA dendrogram of genetic relationships between six larch samples from the Siberia and Far East. For sample code see Table 1. Numbers indicate clustering reliability values (bootstrap support).

**Table 3.** Main parameters of genetic variability in larches from Siberia and the Far East

Sample (species)	A	A <sub>e</sub>	P <sub>95</sub> , %	H <sub>exp</sub>	S <sub>pop</sub>	Source
RAPD marker data						
ULAN	1.4420	1.2543	36.54	0.1553	0.8286	Personal data
TOM	1.4289	1.2420	37.85	0.1491	0.8252	
IRK	1.4530	1.2450	35.89	0.1493	0.8248	
SAKH	1.4595	1.2490	45.95	0.1584	0.7964	
KHAB	1.3173	1.2093	31.73	0.1373	0.7885	
BLAG	1.6411	1.2919	49.23	0.1891	0.7570	
KOM-1	1.5042	1.2763	50.42	0.1796	0.6412	[32]
KOM-2	1.6017	1.2938	60.17	0.1910	0.6791	
AMUR	1.6737	1.3348	67.37	0.2113	0.6194	
OLG	1.3220	1.2001	32.2	0.1117	0.6778	
OCH	1.5339	1.3016	53.39	0.1955	0.6710	
Allozymic marker data						
<i>L. sibirica</i>	1.71	1.21	57.0	0.129		[6]
	1.5–2.1		46.7–73.3	0.130–0.195		[19, 20]
<i>L. gmelinii</i>	2.46		60.1	0.129		[10]
	2.0–2.5		60.0–73.3	0.140–0.186		[19, 20]
	1.955	1.199	61.9	0.129		[39]
<i>L. cajanderi</i>	2.0		53.3	0.120		[19]
<i>L. czekanowskii</i>	2.1		73.3	0.167		[19]
<i>L. amurensis</i>	2.3		66.7	0.178		[19]
<i>L. olgensis</i> (Olga bay)	1.5–2.1		40.0–66.7	0.088–0.108		[19]
	1.4		37.5	0.082		[20]
<i>L. ochotensis</i>	2.1		66.7	0.181		[19]
<i>L. kamtschatica</i>	1.7		50.0	0.166		[18]
	1.8		73.3	0.160		[19]

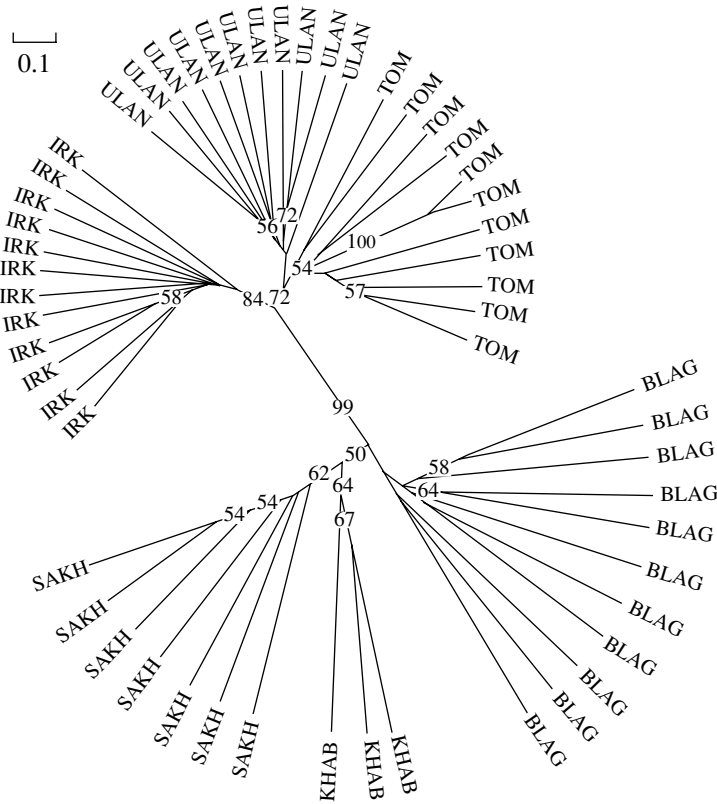
samples (Table 4) were considerably higher than the within-species difference values for *L. sibirica* (0.008–0.025), obtained using allozyme markers [19]. These data also point to the genetic differentiation of sample IRK from the two others. It can be suggested that IRK larches are either hybrids between *L. sibirica* and *L. gmelinii*, or a variety of *L. sibirica*. A definite answer to this question requires a complex population study of larches from this geographical region using biochemical, cytological, and other molecular genetic methods.

The main genetic variability parameters for *L. sibirica* calculated from RAPD markers of the total sample ULAN–TOM, were as follows: P<sub>95</sub> = 45.08%; A = 1.5427; A<sub>e</sub> = 1.2791; H<sub>exp</sub> = 0.1698. The mean sample genetic diversity (H<sub>S</sub>) constituted 0.1457; total gene diversity in the combined sample (H<sub>T</sub>) was 0.1644; overall between-sample diversity (D<sub>ST</sub>), 0.0187; genetic

differences (D<sub>N</sub>), 0.0353; and the between-population differentiation (G<sub>ST</sub>), 0.1139. Based on the allozyme loci variability in populations of western (from Ural and Western Siberia) and eastern (Eastern Siberia and the north of Western Siberia) races of *L. sibirica*,

**Table 4.** Genetic differences D<sub>N</sub> between the larch samples examined inferred from 457 RAPD loci

Sample	ULAN	TOM	IRK	SAKH	KHAB
TOM	0.0361				
IRK	0.0598	0.0602			
SAKH	0.1369	0.1299	0.1290		
KHAB	0.1792	0.1802	0.1753	0.1086	
BLAG	0.1157	0.1140	0.1089	0.0729	0.0972



**Fig. 3.** Unrooted UPGMA tree showing genetic relationships between the individuals in the samples studied. Numbers indicate clustering reliability values (bootstrap support). For sample code see Table 1.

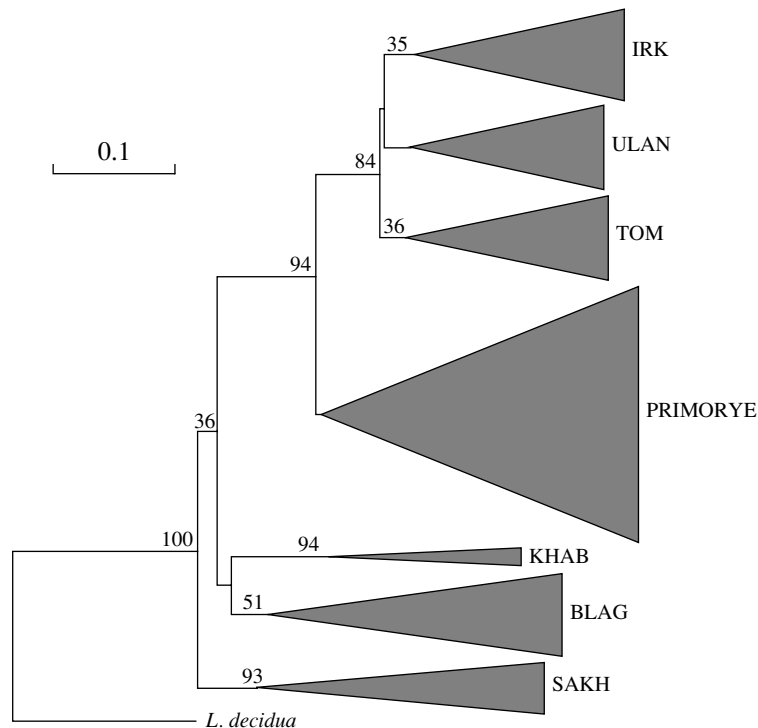
Semerikov *et al.* [8, 19, 20] showed substantial genetic subdivision between them ( $D_N = 0.025$ ;  $F_{ST} = 0.079$ ). High values of genetic differentiation ( $D_N$  and  $G_{ST}$ ) obtained for samples ULAN and TOM can be explained precisely by their belonging to different races: sample TOM represents the populations of the western group, while ULAN is composed of the representatives of the eastern population group. The  $G_{ST}$  and  $D_N$  values calculated from RAPD markers were approximately 1.4 times higher than those based on the allozyme data. This observation can be explained by the fact that allozyme markers detect variation only at the expressed genes, which are not always selectively neutral, while RAPD markers also enable examination of the noncoding DNA sequences, which comprise the major proportion of eukaryotic genome and are under lower selective pressure. An increase in the number of RAPD markers enables investigation of larger proportion of genomic DNA, resulting in more complete species variability pattern. The mean value of the gene flow between the two samples of *L. sibirica*, calculated as  $N_m = 0.5(1 - G_{ST})/G_{ST}$  [36], constituted 3.89. At the same time, the value of this parameter calculated with the different formulas and using the allozymic marker data [19] varied in the range from 2.91 to 5.57.

The mean within-population differentiation calculated for each of the six Siberian and Far Eastern sam-

ples examined varied from 0.1714 to 0.243. This parameter represents the estimation of the genetic difference levels between the trees in the sample. The highest within-population differentiation was typical of sample BLAG, where genetic differences between the trees were on average 24%. Figure 3 presents an unrooted tree, which clearly demonstrates the within-population relationships. The trees examined were highly reliably (bootstrap support over 50%) grouped in accordance with their growing sites.

To establish genetic relationships among the larches from Siberia and the Far East, RAPD analysis of 87 DNA specimens from 11 population samples (see Materials and Methods) was carried out using 10 primers (Table 2). Comparison of the RAPD profiles enabled examination of the genetic variability of 261 RAPD fragments. Only four monomorphic loci, OPA-01-935, OPB-10-849, OPC-19-797, and OPC-02-822 were shared by all 11 samples. The test for differentiation carried out for the populations of Primorskii krai revealed no statistically significant differences relative to the RAPD fragment frequencies in the total population sample. Based on these data, all populations from Primorskii krai were considered as one sample, PRIMORYE.

Figure 4 presents the NJ dendrogram of genetic relationships between the studied larch samples from Sibe-



**Fig. 4.** Rooted NJ tree showing the relationships between the Siberian and Far Eastern larches. Numbers indicate clustering reliability values (bootstrap support). For sample code see Table 1. PRIMORYE, population group of Primorskii krai. As a root, the *L. decidua* DNA sample was used.

ria and the Far East. The reliable tree topology was obtained for two groups. The first group was formed by the three Siberian samples, ULAN, TOM, and IRK (bootstrap support 84%), while the second group comprised the first group together with PRIMORYE (bootstrap support 94%). The *L. kamtschatica* sample SAKH forms an individual branch (bootstrap support 93%), pointing to substantial differentiation of this sample from the other larch samples studied.

Grouping of the Siberian samples (ULAN, TOM and IRK) in one cluster with the total sample of Primorskii krai (PRIMORYE) points to more close genetic relationships of the larches from Primorskii krai and *L. sibirica* compared to the other species examined. These data are in line with the idea of Dylis [1] on the closeness of Olginskaya section larches (*L. olgensis* with a number of ecotypes) to *L. sibirica*.

All samples, except SAKH, form a single cluster, but the reliability of this grouping is low (bootstrap support, 36%). For this reason, establishing of the genetic relationships at this level requires further study using other, probably, molecular genetic methods. Nevertheless, based on the NJ tree topology (Fig. 1), it can be concluded that *L. sibirica* (samples ULAN, TOM, and IRK) and *L. gmelinii* (sample KHAB) are genetically closer to each other than to *L. kamtschatica* (sample SAKH). According to Vas'kovskii [41], Siberian and Daurian larches appeared in Pleistocene, and probably, represented close lineages. New paleobotanical findings introduce substantial corrections to the geological

age of the genus *Larix*. For instance, based on the macroremains found in the Upper Oligocene deposits of the Siziman's bay (Khabarovsk krai), an extinct species, *Laricioxylon sichotealinense* Blokh., displaying similarities to the modern species of *L. sibirica* and *L. gmelinii* was described leading to a suggestion on the existence of the Sikhote-Alin' common ancestor of these two species [42].

The data on the genetic relationships among the Siberian and Far Eastern larches obtained in the present study using RAPD markers are consistent with the evidence inferred from allozyme analysis [19].

This study is the first analysis of the genetic variability in the Siberian and Far Eastern larches carried out by RAPD method. Based on the RAPD marker data, the main population parameters were determined for three species: *L. sibirica* ( $P_{95} = 45\%$ ,  $A = 1.54$ ,  $A_e = 1.28$ ,  $H_{exp} = 0.1698$ ,  $G_{ST} = 0.1139$ ,  $N_m = 3.89$ ), *L. gmelinii* ( $P_{95} = 32\%$ ,  $A = 1.32$ ,  $A_e = 1.21$ ,  $H_{exp} = 0.1373$ ), and *L. kamtschatica* ( $P_{95} = 46\%$ ,  $A = 1.46$ ,  $A_e = 1.25$ ,  $H_{exp} = 0.1584$ ). Analysis of the genetic relationships showed that *L. kamtschatica* was characterized by highest genetic differentiation from the other larches examined, while larches from Primorskii krai were genetically close to *L. sibirica*.

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