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## Genetic Diversity, Structure and Differentiation of Gmelin Larch (*Larix gmelinii* (Rupr.) Rupr.) Populations from Central Evenkia and Eastern Zabaikalje

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### Abstract

On the basis of 17 loci, coding allozyme diversity G-6-PD, 6-PGD, IDH, MDH, SKDH, GDH, GOT, LAP, EST, ME, the values of the main parameters of genetic variability, structure and differentiation of Gmelin larch (*Larix gmelinii* (Rupr.) Rupr.) populations from Central Evenkia and Eastern Zabaikalje were established. The studies were conducted by method of horizontal starch-gel electrophoresis. 76.5 % of gene loci assayed were revealed to be polymorphic at 95 % and 94.1 % at 99 % criteria. The mean number of alleles per locus, the mean observed heterozygosity as well as the mean expected heterozygosity and the effective number of alleles were equal to 2.59, 0.100, 0.141 and 1.21, respectively. All populations of *L. gmelinii* examined showed a deficit of heterozygous genotypes. The highest deficit of heterozygotes was characteristic of *L. gmelinii* populations from Evenkia ( $F_{is} = 0.2672$ ). More than 92% of total genetic variability was within the population and only 7.89% ( $F_{st} = 0.0789$ ) was among the populations. The mean genetic distance D (Nei 1972) between populations ranged from 0.0025 to 0.0343 and averaged 0.0168. The most substantial differences were revealed between populations from Evenkia and Eastern Zabaikalje (D changed from 0.0270 to 0.0343). The populations of *L. gmelinii* in Evenkia were slightly differentiated (D changed from 0.0025 to 0.0042). The data obtained suggest that geographically distant from each other populations of *L. gmelinii* from Central Evenkia and Eastern Zabaikalje have more significant level of genetic differentiation in comparison with geographically close populations from Evenkia. The mean value of genetic distance D between populations from these regions is equal to 0.03. Analysis of data obtained as part of the study of genetic variability and differentiation a great variety of conifer species showed that such extent of genetic differences is usually observed between populations belonging to different geographical races of a single species (Millar *et al.* 1988, Conkle *et al.* 1988, Krutovskii *et al.* 1989, Hamrick and Godt 1989 and etc.). On this basis the larch populations from Evenkia and Eastern Zabaikalje can be regarded as geographic races of *L. gmelinii*. Results of our studies confirm the opinion of Abaimov and Milyutin (1995) about heterogeneity of *L. gmelinii* in various regions of its range.

*Key words:* Eastern Zabaikalje, Evenkia, genetic diversity, *Larix gmelinii*, structure and differentiation of populations

### Introduction

Gmelin larch (*Larix gmelinii* (Rupr.) Rupr.) is one of the main forest-forming coniferous species at high latitudes of the Asian part of Russia. The range of this species almost completely coincides with the permafrost zone and makes up about 1.9 million km<sup>2</sup> in Siberia (Abaimov *et al.* 1980). The northern boundary of the range extends eastwards from lower flow of the Lena River to the Pyasina River basin and goes westward at 70-71° N. Throughout its length, the boundary coincides with the climatic limit of the range of woody plants (Abaimov *et al.* 1984). The western boundary of the *L. gmelinii* range goes southwards from the basin of upper flow of the Kheta River, crosses the basins of the Lama and Khantaiskoe lakes of the Taimyr Peninsula, meets the Nizhnyaya Tunguska River close to its left tributary Uchami, goes

along the dividing ridge of the Nizhnyaya Tunguska and Podkamennaya Tunguska Rivers to the northeastern shore of the Baikal Lake, and passes into Mongolia at 110° E in the south. The eastern boundary of the *L. gmelinii* range crosses the left part of the Lena River basin at approximately 120° - 122° E and, in the south, goes near the border of the Chita region toward Great Khingan Mountains of China (Abaimov *et al.* 1998).

There is no doubt that *L. gmelinii* is heterogeneous within the limits of its vast area. Growing in such different on natural conditions regions as the Taimyr, the Evenkia, the West Yakutiya, the Zabaikalje and etc. *L. gmelinii* undoubtedly has to differentiate into more small intraspecific taxons distinguishing at morphological and other features as well as ecological peculiarities (Abaimov and Milyutin, 1995).

Unfortunately genetic diversity, structure and intraspecific differentiation of *L. gmelinii* populations are still poorly studied. Recently, genetic data have been reported only for a few *L. gmelinii* populations of Eastern Siberia (Semerikov *et al.* 1999) and the Russian Far East (Potenko and Razumov 1996). However it would be noted that species status of some populations studied in these regions was still unclear.

Lack of data for the other regions of the natural species range does not permit to estimate the genetic potential and intraspecific differentiation of *L. gmelinii* while the knowledge of the level and distribution of genetic variation through the species range is necessary to elaborate the concepts for the exploitation and restoration of *L. gmelinii* forests as well as for the conservation of their genetic diversity. The objective of this work was to study the genetic diversity, structure and differentiation of *L. gmelinii* populations from Evenkia and Eastern Zabaikalje.

## Materials and Methods

### Plant materials

Seeds material for electrophoretic studies were collected from 106 trees in four natural populations of *L. gmelinii*. Three populations (I, II, III) are located in the central part of the Central Siberian Plateau within a radius of 25 km from the site where the Kochechum River meets Nizhnyaya Tunguska (near the settlement of Tura, Evenkia). One population (IV) is situated in the Chita region of Eastern Zabaikalje (Fig. 1). Population I is located on the right bank of the Kochechum River, near the mouth of the Bazhenovskii Stream. The geographical coordinates are 64°19' N, 100°07' E. The average age of the trees is 174 years. Population II is situated on the left bank of the

Kochechum River. The geographical coordinates are 64°19' N, 100°13' E. The age of the trees in this population varies from 36 to 73, averaging 50. Population III is located on the left bank of the Nizhnyaya Tunguska River 25 km upstream of the mouth of the Kochechum River. The geographical coordinates are 64°17' N, 100°14' E. Overmature trees prevail in this population. The average age of the trees is 204 years. Population IV of *L. gmelinii* is situated in 40 km south-westward from Chita city. The geographical coordinates are 51°51' N, 113°10' E. The mean age of the trees in this population is 50 years.

### Isoenzyme and Statistical Analysis

Seeds for electrophoretic analysis were sampled randomly from a set of not less than 50 seeds extracted from 10 to 20 cones from each of 106 trees. Previously the seeds were soaked in distilled water during for 24 hours. The megagametophyte of seed was separated from the embryo and then ground in one or two drops of an extracting buffer (0.1M Tris-HCl pH 7.5%, 0.15%  $\beta$ -mercaptoethanol, 1% triton X-100). To determine the genotype of the tree 6-8 megagametophytes were examined. The extracts were absorbed onto filter-paper wicks and these were inserted into a 13-14 % starch gel. Electrophoretic separation of extracts was conducted in horizontal chambers. Two buffer systems were used for electrophoresis: I - Tris-citric acid, pH 6.2 (Adams and Joly 1980), II - Tris-citric acid, pH 8.5 / Lithium hydroxide-boric acid, pH 8.1 (Ridgway *et al.* 1970). Gel and electrode buffers were as recommended for these systems. Ten enzyme systems were assayed per each tree. Standart methods of histochemical enzyme staining (Brewer 1970, Vallejos 1983, Cheliak and Pitel 1984, Goncharenko and Padutov 1988) were followed

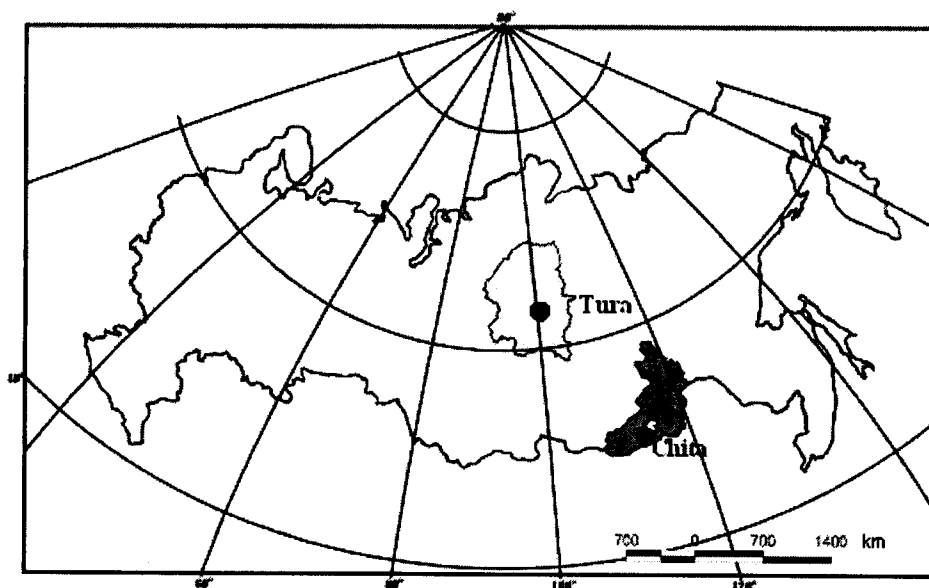


Fig. 1. Geographical locations of *L. gmelinii* populations under study.

■ - Chita region    □ - Evenkia

with minor modifications. The enzymes assayed, their abbreviations, the buffer systems used are shown in Table 1.

Enzymes, loci, and alleles were designated as described by Prakash *et al.* (1969). Loci were numbered according to the electrophoretic mobility of their products. Thus, the locus coding the most mobile enzyme was labelled 1 and locus coding an enzyme with more low mobility was labeled 2, etc. Within each locus, the most frequent allozyme and the corresponding allele were designated as 100. Other allozymes of the locus were designated according to their electrophoretic mobility with reference to allozyme 100. Alleles that coded phenotypically undetectable allozymes were designated as n (null alleles).

To estimate the levels of genetic variability in the populations studied the main genetic parameters such as percentage of polymorphic loci at 95% ( $P_{95}$ ) and at 99% ( $P_{99}$ ) criteria, mean number of alleles per locus

(A), the mean observed heterozygosity ( $H_o$ ), the mean expected heterozygosity ( $H_e$ ) and the effective number of alleles ( $n_e$ ) were calculated. The chi-square "goodness-of-fit" tests were used to determine if observed genotype frequencies were in accordance with expectations under Hardy-Weinberg equilibrium. The distribution of genetic variation within and between populations was evaluated using Wright's F-statistics (Guries and Ledig 1982). The genetic distance (D) among the populations was estimated by Nei's method (Nei 1972). Unweighted pair-group method (UPGMA) of cluster analysis (Sneath and Sokal 1973) was used to visualize differences in genetic distance among populations. Calculations based on genetic data were performed using the PopGen computer program (Yeh *et al.* 1999).

## Results and Discussion

### Genetic Variability within Population

Forty four allelic variants at the 17 loci were revealed in the course of the study of the 10 enzyme systems in natural populations of *L. gmelinii* from Evenkia and Eastern Zabaikalje. Locus Mdh-1 was monomorphic in all populations. The others loci (Est-1, Gdh, G-6pd, Got-1, Got-2, Got-3, Idh, Lap-1, Lap-2, Mdh-2, Mdh-3, Mdh-4, Me-1, Me-2, 6-Pgd, Skdh,) were polymorphic at least in one population. Fourteen loci had two (Got-2 G-6pd, Idh, Lap-1, Lap-2, Mdh-2, Me-2,) or three (Gdh, Got-1, Got-3 Mdh-3, Me-1, 6-Pgd, Skdh,) alleles. The loci of Est-1, Mdh-4 loci had the highest number of alleles (4 alleles). The allele frequencies are listed in Table 2. The most common alleles of the polymorphic loci were as a rule the same in all populations.

On the basis of allelic frequencies the main parameters of genetic variability were calculated (Table 3). As seen in table 3 the values of all parameters varied among the populations. The percentage of polymorphic loci at 95 % criterion ( $P_{95}$ ) ranged from 41.06% to 58.82% and at 99% criterion ( $P_{99}$ ) from 70.59% to 88.24%. The mean number of alleles per locus (A) ranged from 1.88 to 2.24. The mean value of observed heterozygosity ( $H_o$ ) varied from 0.078 to 0.122. The

Table 1. Enzymes, their Abbreviations (Abbr.), Enzyme Commission numbers (EC) and buffer systems used for electrophoresis.

Enzyme	Abbr.	EC	Buffer
Glucose-6-phosphate dehydrogenase	G-6-PD	1.1.1.49.	I
Isocitrate dehydrogenase	IDH	1.1.1.42.	I
Malate dehydrogenase	MDH	1.1.1.37.	I
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44.	I
Shikimate dehydrogenase	SKDH	1.1.1.25.	I
Esterase	EST	3.1.1.1.	II
Glutamate dehydrogenase	GDH	1.4.1.2.	II
Glutamate-oxaloacetate transaminase	GOT	2.6.1.1.	II
Leucine aminopeptidase	LAP	3.4.11.1.	II
Malic enzyme	ME	1.1.1.40.	II

Table 3. Genetic variability at 17 loci in populations studied of *L. gmelinii* (standart deviation in parentheses).

Populations	Percentage of polymorphic loci		Mean number of alleles per locus	Mean heterozygosity		Effective number of alleles
	$P_{95}$	$P_{99}$		Observed	Expected	
			A	$H_o$	$H_e$	$n_e$
I – Evenkia	47.06	76.47	1.88	0.086	0.111	1.15
II – Evenkia	47.06	70.59	1.88	0.078	0.115	1.18
III - Evenkia	58.82	88.24	2.24	0.111	0.147	1.21
IV – Eastern Zabaikalje	58.82	70.59	1.88	0.122	0.14	1.22
In total for the species	76.47	94.12	2.59 (0.79)	0.1 (0.08)	0.141 (0.141)	1.21 (0.28)

mean expected heterozygosity ( $H_e$ ) was higher than the mean observed heterozygosity and ranged from 0.111 to 0.147. The effective number of alleles ( $n_e$ ) ranged from 1.15 to 1.22. As a whole for four populations studied of *L. gmelinii* the percentage of polymorphic loci at 99% polymorphism criterion composed 94.12%, the mean number of alleles per locus, the mean observed heterozygosity, the mean expected heterozygosity and the effective number of alleles were equal to 2.59, 0.100, 0.141 and 1.21, respectively. The effective number of alleles per locus was much lower than the actual number of alleles. This means that in the populations there were a lot of genes in which the frequencies were low and not contributing much to the population genetic variability.

The mean value of expected heterozygosity ( $H_e = 0.141$ ) obtained in our study was close to those

computed for the genus *Larix* ( $H_e = 0.142$ ) on the basis of published data for 13 *Larix* species (Fins and Seeb 1986, Cheliak *et al.* 1988, Shurkhal *et al.* 1989, Lewandowski *et al.* 1991, Liu and Knowles 1991, Ying and Morgenstern 1991, Semerikov and Matveev 1995, Potenko and Razumov 1996, Goncharenko and Silin 1997, Shigapov *et al.* 1998, Semerikov *et al.* 1999) and for 42 conifer species ( $H_e = 0.145$ ) (Krutovskii *et al.* 1989). The mean observed heterozygosity ( $H_o = 0.100$ ) was somewhat lower than average estimates of this parameter both for the 13 *Larix* species ( $H_o = 0.138$ ) and for 42 species of conifer ( $H_o = 0.152$ ) reviewed of Krutovskii *et al.* (1989).

Populations of *L. gmelinii* from Central Evenkia had lower as a whole the average estimates the observed heterozygosity, the expected heterozygosity and the effective numbers of alleles in comparison with the

Table 2. Allele frequencies for 17 loci in *L. gmelinii* populations studied.

Locus	Allele	Populations			
		Evenkia		Eastern Zabaikalje	
		I	II	III	IV
Est-1	95	0.041	-	-	-
	100	0.688	0.580	0.593	0.767
	108	-	0.060	0.037	-
	115	0.271	0.360	0.370	0.233
Gdh	81	0.042	0.020	-	0.017
	100	0.958	0.980	1.000	0.950
	125	-	-	-	0.033
G-6pd	1.88	0.146	0.060	0.185	0.333
	100	0.854	0.940	0.815	0.667
Got-1	89	-	0.020	0.056	-
	100	1.000	0.940	0.926	1.000
	108	-	0.040	0.018	-
Got-2	100	0.917	0.980	0.926	0.933
	109	0.083	0.020	0.074	0.067
Got-3	71	0.042	0.060	0.148	0.167
	100	0.937	0.940	0.833	0.683
	137	0.021	-	0.019	0.150
Idh	100	0.937	1.000	0.926	0.983
	130	0.063	-	0.074	0.017
Lap-1	100	0.979	0.960	0.963	0.983
	Null	0.020	0.040	0.037	0.017
Lap-2	100	1.000	1.000	0.963	1.000
	Null	-	-	0.037	-
Mdh-1	100	1.000	1.000	1.000	1.000
Mdh-2	90	0.021	-	0.018	-
	100	0.979	1.000	0.982	1.000
Mdh-3	93	-	-	-	0.016
	100	0.854	0.700	0.796	0.217
	112	0.146	0.300	0.204	0.767
Mdh-4	67	-	-	0.037	-
	100	0.958	0.940	0.908	0.950
	122	-	0.040	0.018	0.050
	Null	0.042	0.020	0.037	-
Me-1	94	-	-	-	0.067
	100	1.000	1.000	0.963	0.933
	109	-	-	0.037	-
Me-2	100	0.917	0.960	0.944	1.000
	118	0.083	0.040	0.056	-
6-Pgd	86	-	-	0.018	-
	100	0.938	0.880	0.945	0.933
	121	0.062	0.120	0.037	0.067
Skdh	90	-	-	-	0.050
	100	0.979	0.940	0.982	0.950
	130	0.021	0.060	0.018	-

population from Eastern Zabaikalje ( $H_0 = 0.092$ ;  $H_e = 0.128$ ;  $n_e = 1.18$  and  $H_0 = 0.122$ ;  $H_e = 0.140$ ;  $n_e = 1.22$ , respectively). At the same time the mean number of alleles per locus in total for larch populations from Evenkia was significantly higher than those in the population from Eastern Zabaikalje ( $A = 2.35$  and  $A = 1.88$ , respectively) mostly at the expense of rare alleles. The mean values  $P_{95}$  and  $P_{99}$  for three larch populations from Evenkia were 64.70% and 94.12%, respectively, for the population from Eastern Zabaikalje the values of these parameters were 58.82% and 70.59%, respectively.

### Analysis of genetic structure

In all *L. gmelinii* populations examined the observed heterozygosity was lower than expected heterozygosity under Hardy-Weinberg conditions. In each population substantial deviations from Hardy-Weinberg proportions of genotypes were observed for no less than three or four loci at various significance levels. The most significant deficit heterozygous genotypes was revealed in *L. gmelinii* populations from Evenkia. The value of inbreeding coefficient  $F_{is}$  (averaged over all gene loci) which estimates the deviations from Hardy-Weinberg equilibrium of individuals in each population varied among populations studied in this region from 0.2291 to 0.3269 and averaged 0.2672 (Table 4). The highest deficit of heterozygotes was observed in the population II which is the youngest (mean age 50 years) and lacks trees of older generations. The two other populations were older and included trees of several after-fire generations. The  $F_{is}$  estimates for these populations were substantially lower, 0.2291 in population I (mean age 174 years) and 0.2457 in population III (mean age 204 years). In the population

of *L. gmelinii* from Eastern Zabaikalje the deficit of heterozygotes amounted to only 12.96% ( $F_{is} = 0.1296$ ). It is more than two times less as for Evenkian populations as a whole. On average for *L. gmelinii* populations studied, the inbreeding of individual within the population ( $F_{is}$ ) was equal to 22.88%. Value of  $F_{it}$ , which characterizes individual inbreeding relative to the species, was 0.2897 on average (Table 5). In other words, each *L. gmelinii* tree in the region proved to be 28.97% deficit of heterozygous genotypes.

According to published data a deficit of heterozygotes in *L. gmelinii* populations of the Eastern Siberia and the Russian Far East (Semerikov *et al.* 1999) was no more than 5% and even a slight excess of heterozygotes was characteristic of the populations of the Khabarovsk territory (Potenko and Razumov 1996).

One of the most probable causes of significant deficit heterozygous genotypes in *L. gmelinii* populations from Evenkia is higher than in other populations of the species the proportion of trees resulting from self-pollination or inbreeding. All three Evenkian populations had been affected by fires, and their after-fire restoration was due to a few trees that remained intact or were only slightly damaged. This is especially true for population II, in which not a single tree of an older generation was found. A higher frequency of homozygous genotypes in the population consisting mostly of young trees as compared with the populations that had older trees prevailing may be attributed to natural selection, which eliminates a fraction of the inbred progeny from a population.

A substantial deficit of heterozygous genotypes caused by high level inbreeding have been reported for many predominantly outcrossing species, including other larches: *L. sukaczewii* populations from the Urals (24.5%) (Shigapov *et al.* 1998), several *L. sibirica* populations (10.4 – 30%) (Semerikov and Matveev 1995) and *L. laricina* (27.1%) from Northern Ontario (Knowles *et al.* 1987). A higher heterozygote deficiency in younger populations has been observed in *L. laricina* (Ying and Morgenstern 1991) and *Thuja occidentalis* L. (Perry and Knowles 1990, Matthers-Sears *et al.* 1991). It is noted that species of the genus *Larix* and especially their northern populations have a considerably higher inbreeding rate as compared with most conifers.

### Genetic Variability among Populations

The subdivision of genetic structure among *L. gmelinii* populations studied was estimated using index  $F_{st}$  (Table 5). The value  $F_{st}$ , which was obtained as a weighted mean of  $F_{st}$  for all the populations investigated, ranged among polymorphic loci from 0.0036 to 0.2660. The highest contribution to the among-population variability was made by the Mdh-3, G-6pd and Got-3 loci, while the contribution of the Lap-1 locus was the smallest. The average value of  $F_{st}$  for all loci assayed was equal to 0.0789. This means that about 92% of the total genetic diversity resided within the population, while only 7.89% was distributed between the populations.

Table 4. Estimates of the fixation index  $F_{is}$  for four *L. gmelinii* populations.

Locus	Populations			
	Evenkia			Eastern Zabaikalje
	I	II	III	IV
Est-1	0.3551	0.6229	0.5645	0.2547
Gdh	-0.0435	-0.0204	-	-0.0405
G-6pd	0.4983	-0.0638	0.5091	0.25
Got-1	-	0.6503	-0.064	-
Got-2	-0.0909	-0.0204	-0.08	-0.0714
Got-3	0.6496	0.6454	0.3462	0.2405
Idh	0.6444	-	-0.08	-0.0169
Lap-1	-0.0213	-0.0417	-0.0385	-0.0169
Lap-2	-	-	-0.0385	-
Mdh-2	-0.0213	-	-0.0189	-
Mdh-3	0.1638	0.1429	0.2008	0.0868
Mdh-4	-0.0435	-0.049	-0.0672	-0.0526
Me-1	-	-	-0.0385	-0.0714
Me-2	-0.0909	-0.0417	0.6471	-
6-Pgd	-0.0667	0.6212	-0.0452	-0.0714
Skdh	-0.0213	-0.0638	-0.0189	-0.0526
Mean	0.2291	0.3269	0.2457	0.1296

- Locus is monomorphic

Table 5. Estimates of Wright's F-statistics calculated for each locus for all *L. gmelinii* populations studied.

Locus	F <sub>is</sub>	F <sub>it</sub>	F <sub>st</sub>
Est-1	0.4702	0.4815	0.0214
Gdh	-0.0379	-0.0226	0.0148
G-6pd	0.3444	0.3876	0.0659
Got-1	0.2582	0.2801	0.0296
Got-2	-0.0762	-0.0650	0.0104
Got-3	0.3651	0.4066	0.0654
Idh	0.2229	0.2429	0.0258
Lap-1	-0.0332	-0.0295	0.0036
Lap-2	-0.0385	-0.0093	0.0280
Mdh-1	0.0000	0.0000	0.0000
Mdh-2	-0.0201	-0.0099	0.0100
Mdh-3	0.1455	0.3728	0.2660
Mdh-4	-0.0556	-0.0441	0.0109
Me-1	-0.0594	-0.0204	0.0368
Me-2	0.1519	0.1699	0.0212
6-Pgd	0.1962	0.2055	0.0115
Skdh	-0.0483	-0.0299	0.0175
Mean	0.2288	0.2897	0.0789

Table 6. Genetic distance D (Nei, 1972) between *L. gmelinii* populations based on data from the 17 loci.

Populations	I	II	III	IV
I – Evenkia	-			
II – Evenkia	0.0042	-		
III – Evenkia	0.0025	0.0037	-	
IV – Eastern Zabaikalje	0.0343	0.027	0.0291	-

Our value of F<sub>st</sub> for *L. gmelinii* populations from Evenkia and Eastern Zabaikalje was in excess of the mean values of this index (F<sub>st</sub> = 0.038) for *L. gmelinii* populations studied from the Khabarovsk territory (Potenko and Razumov 1996) and from other regions (F<sub>st</sub> = 0.021) of the species range (Semerikov *et al.* 1999), but it was within the limits of F<sub>st</sub> values (F<sub>st</sub> = 0.020 – 0.086) established for other species of *Larix*: *L. occidentalis* (Fins and Seeb 1986), *L. laricina* (Cheliak *et al.* 1988, Liu and Knowles 1991, Ying and Morgenstern 1991), *L. sibirica* (Semerikov and Matveev 1995, Semerikov *et al.* 1999), *L. decidua* (Lewandowski and Meinartowicz 1991, Maier 1992), *L. sukaczewii* (Shigapov *et al.* 1998).

The obtained estimate of F<sub>st</sub> in our study indicate to more significant subdivision of *L. gmelinii* populations analysed in comparison with populations of this species studied previously. However it will be noted that higher mean F<sub>st</sub> value was due to differences between *L. gmelinii* populations from Evenkia on the one hand and Eastern Zabaikalje on the other hand. The populations from Evenkia were slightly differentiated (F<sub>st</sub> =

0.0166).

The degree of genetic differentiation among *L. gmelinii* populations studied was quantified using Nei's genetic distance D (Nei 1972). Estimated from the allele frequencies of the 17 loci, genetic distances D between populations are listed in the Table 6. As the Table 6 demonstrates, the most significant differences were observed between populations from Evenkia and Eastern Zabaikalje. Genetic distance D between populations from these regions ranged from 0.0270 to 0.0343 and averaged 0.03. Genetic differences between tightly associated, geographically close populations of *L. gmelinii* from Evenkia were far less. Genetic distance D among Evenkia populations ranged from 0.0025 to 0.0042 with a mean of 0.0035.

The dendrogram constructed by an unweighted pair-group method (UPGMA) of cluster analysis and D values (Fig. 2) depicts a fairly distinct division between populations studied. Thus *L. gmelinii* populations from Evenkia clustered together while Eastern Zabaikalje population remain ungrouped. This population had the highest mean genetic distance values with the other

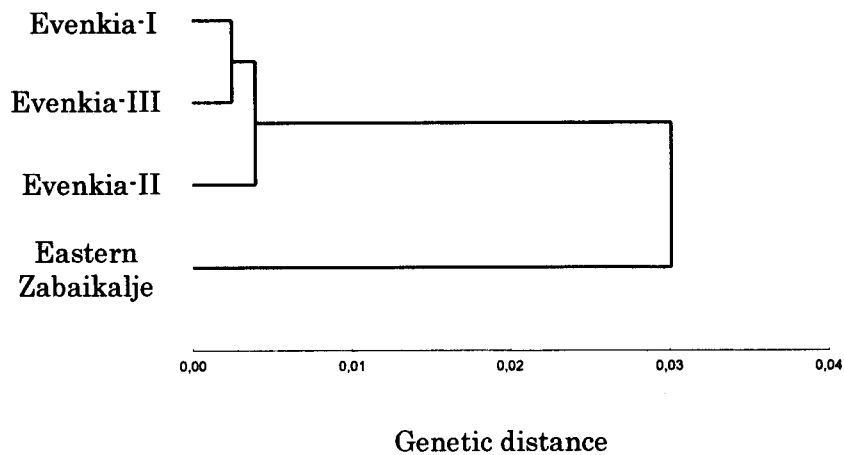


Fig. 2. UPGMA dendrogram based on genetic distances between *Larix gmelinii* populations.

populations, which were approximately nine as great as those between Evenkian populations.

### Conclusion

As a result of studies conducted it was established that genetic diversity and structure of *L. gmelinii* populations in Central Evenkia and in Eastern Zabaikalje are significantly distinguished. The mean genetic distance  $D$  (Nei 1972) between populations from these regions calculated on the basis of allelic frequencies 17 isoenzyme loci is equal to 0.03. The review of data at genetic diversity and intraspecific differentiation of conifer species (Millar *et al.* 1988, Conkle *et al.* 1988, Krutovskii *et al.* 1989, Hamrick and Godt 1989 and etc.) showed that such extent of genetic differentiation is characteristic of geographical races of a single species. On this basis we concluded that *L. gmelinii* populations from Central Evenkia and Eastern Zabaikalje are genetically isolated and can be regarded as geographical races. The data obtained confirm the supposition of Abaimov and Milyutin (1995) that *L. gmelinii*, growing in various on natural conditions regions within the limits of its vast area, has to subdivide into more small intraspecific taxons. Results of our studies represent only a part of the total work that is necessary to fully assess the variability of this species.

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### References

Abaimov A.P., Karpel B.A. and Koropachinskii I.Yu.

(1980) About the boundaries of species ranges of Siberian larch species. Botanical J., 5 (1): 118 – 120 (In Russian).

Abaimov A.P., Lesinski J.A., Martinsson O. and Milyutin L.I. (1998) Variability and ecology of Siberian larch species. Umea: Swedish Univ. Agric. Sci., Report 43.

Abaimov A.P. and Milyutin L.I. (1995) Recent ideas about Siberian larches and problems of their study. Proc. XII Sukachev Memorial Conf., Novosibirsk: RAS. 41 - 60 (In Russian).

Adams W.T. and Joly R.I. (1980) Genetics of Allozyme Variants in Loblolly Pine. Heredity, 71 (1): 33 - 40.

Brewer G. J. (1970) Introduction to isozyme techniques. N.Y., L.: Acad. Press. 186 p.

Cheliak W.L. and Pitel J.A. (1984) Technique for starch gel electrophoresis of enzyme from forest tree species. Petawawa Nat. For. Inst., Infor. Report PI-X-42.

Cheliak W.L., Wang J. and Pitel J.A. (1988) Population structure and genetic diversity in tamarack (*Larix laricina* (Du Roi) K. Koch.). Can. J. Forest Res., 18: 1318 - 1324.

Conkle M.T., Schiller G. and Grunwald C. (1988) Electrophoretic analysis of diversity and phylogeny of *Pinus brutia* Ten. and closely related taxa. System. Bot., 13: 411 - 424.

Goncharenko G.G. and Padutov V.E. (1988) Studies of tree species by isozyme electrophoresis: A manual. Gomel': Bel. Inst. Lesn. Khoz.: 68 p. (in Russian).

Goncharenko G.G. and Silin A.E. (1997) To the question about genetic variation and differentiation of Kuril larch (*Larix kurilensis* Mayr.) and Japanese larch (*Larix kaempferi* Sarg.). Dokl. Acad. Nauk, 354 (6): 835 – 838 (in Russian).

Guries R.P. and Ledig F.T. (1982) Genetic diversity



- and population structure in Pitch pine (*Pinus rigida* Mill.). *Evolution*, 36: 387 - 402.
- Hamrick J.L. and Godt M.J. (1989) Allozyme diversity in plant species. In *Plant population genetics, breeding and genetic resources*, Brown H.D. et al. (eds.). Sinauer Associates Inc., Sunderland, 43 - 63
- Fins L. and Seeb L.W. (1986) Genetic variation in Allozyme of Western larch. *Can. J. Forest.*, 16: 1013 - 1018.
- Knowles P., Furnier G.R., Aleksiuk M.A. and Perry D.G. (1987) Significant levels of self-fertilization in natural populations of tamarack. *Can. J. Bot.*, 65 (6): 1087 - 1091.
- Krutovskii K.V., Politov D.V., Altukhov Yu.P., Milyutin L.I., Kuznetsova G.V., Iroshnikov A.I., Vorob'ev V.N. and Vorob'eva N.A. (1989) Genetic variation of Siberian cedar (*Pinus sibirica* Du Tour). IV. Genetic diversity and genetic differentiation of populations. *Genetica*, 25 (11): 2009 - 2032 (in Russian with English summary).
- Lewandowski A., Berczyk J. and Meinartowicz L. (1991) Genetic structure and the mating system in an old stand of Polish larch. *Silvae Genet.*, 40: 75 - 79.
- Lewandowski A. and Meinartowicz L. (1991) Levels and patterns of allozyme variation in some European larch (*Larix decidua*) populations. *Hereditas*, 115 (3): 221 - 226.
- Liu Z. and Knowles P. (1991) Patterns of allozyme variation in tamarack (*Larix laricina*) from Northern Ontario. *Can. J. Bot.*, 69: 2468 - 2474.
- Maier J. (1992) Genetic variability in European larch (*Larix desidua* Mill.). *Ann. Sci. For.*, 49: 39 - 47.
- Matthes-Sears U., Stewart S.C. and Larson D.W. (1991) Sources of allozymic variation in *Thuja occidentalis* in Southern Ontario, Canada. *Silvae Genet.*, 40 (3 - 4): 100 - 105.
- Millar C.J., Strauss S.H., Conkle M.T. and Westfall K.D. (1988) Allozyme differentiation and biosystematics of the Californian closed-cone pines (*Pinus* subsect. *Oocarpea*). *System.Bot.*, 13: 351 - 370.
- Nei M. (1972) Genetic distance between populations. *Amer. Naturalist*, 106: 283 - 291.
- Perry D.J. and Knowles P. (1990) Evidence of high self-fertilization in natural populations of eastern white cedar (*Thuja occidentalis*). *Can. J. Bot.*, 68: 663 - 668.
- Potenko V.V. and Razumov P.N. (1996) Genetic variability and population structure of Dahurian larch in the Khabarovsk Krai. *Lesovedenie*, 5: 11 - 18 (in Russian with English summary).
- Prakash S., Lewontin R.C. and Hubby J.L. (1969) A molecular approach to the study of genetic heterozygosity in natural populations. IV Patterns of genic variation in central, marginal, and isolated populations of *Drosophila pseudoobscura*. *Genetics*, 61: 841 - 858.
- Ridgway G.J., Sherburne S.W. and Lewis R.D. (1970) Polymorphism in the Esterases of Atlantic Herring. *Trans. Am. Fish. Soc.*, 99: 147 - 151.
- Semerikov V.L. and Matveev A.V. (1995) Investigation of genetic variability in Siberian larch (*Larix sibirica* Ldb.) at isozyme loci. *Genetica*, 31 (8): 1107 - 1113 (in Russian with English summary).
- Semerikov V.L., Semerikov L.F. and Lascoux M. (1999) Intra- and interspecific allozyme variability in Eurasian *Larix* Mill. species. *Heredity*, 82: 193 - 204.
- Shigapov Z.Kh., Putenikhin V.P., Shigapova A.Sh. and Urazbakhtina K.A. (1998) Genetic structure of the Ural populations of *Larix sukaczewii* Dyl. *Genetica*, 34 (1): 65 - 74 (in Russian with English summary).
- Shurkhal A.V., Podogas A.V., Semerikov V.L. and Zhivotovskii L.A. (1989) Allozyme polymorphism in Siberian larch (*Larix sibirica* Ledeb.). *Genetica*, 25 (10): 1899 - 1901 (in Russian with English summary).
- Sneath P.M. and Sokal R.R. (1973) *Numerical taxonomy*. San Francisco: Freeman. 573 p.
- Vallejos G.E. (1983) *Enzyme activity staining. Isozymes in plant genetics and breeding*. Amsterdam :Elsevier Sci. Publ. B.V., 469 - 516.
- Yeh F.C.H., Yang R. and Boyle T. (1999) POPGENE Version 1.32: Microsoft Windows - based Freeware for population genetic analysis.
- Ying L. and Morgenstern E.K. (1991) Inheritance and linkage relationships of some isozymes of *Larix laricina* in New Brunswick, Canada. *Silvae Genet.*, 39 (5-6): 245 - 251.