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HOKKAIDO UNIVERSITY
Genetic Diversity, Structure and Differentiation of Gmelin Larch (Larix gmelinii (Ruopr.) Ruopr.) 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 (Ruopr.) Ruopr.) 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 (Fis = 0.2672). More than 92% of total genetic variability was within the population and only 7.89% (Fst = 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 (Ruopr.) Ruopr.) 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 km² 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 Yakutia, 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% β-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.

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![Fig. 1. Geographical locations of *L. gmelinii* populations under study.](image-url)
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 9S ) 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-I was monomorphic in all populations. The others loci (Est-I, 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-I, 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 9S ) 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
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

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<tr>
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<td>130</td>
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population from Eastern Zabaikalje (\(H_e = 0.092; H_s = 0.128; n_e = 1.18\) and \(H_e = 0.122; H_s = 0.140; n_e = 1.22\), 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 (\(F_{in}\)) was equal to 22.88%. Value of \(F_{in}\) 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.

Table 4. Estimates of the fixation index \(F_{is}\) for four \(L. gmelinii\) populations.

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<th>Populations</th>
<th>Evenkia</th>
<th>Eastern Zabaikalje</th>
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<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
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<td>G-6pd</td>
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<td>-0.0204</td>
<td>-0.08</td>
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<tr>
<td>Got-3</td>
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<td>0.6454</td>
<td>0.3462</td>
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<tr>
<td>Idh</td>
<td>0.6444</td>
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<td>-0.08</td>
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- Locus is monomorphic

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

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<td>0.0104</td>
</tr>
<tr>
<td>Got-3</td>
<td>0.3651</td>
<td>0.4066</td>
<td>0.0654</td>
</tr>
<tr>
<td>Idh</td>
<td>0.2229</td>
<td>0.2429</td>
<td>0.0258</td>
</tr>
<tr>
<td>Lap-1</td>
<td>-0.0332</td>
<td>-0.0295</td>
<td>0.0036</td>
</tr>
<tr>
<td>Lap-2</td>
<td>-0.0385</td>
<td>-0.0093</td>
<td>0.0280</td>
</tr>
<tr>
<td>Mdh-1</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Mdh-2</td>
<td>-0.0201</td>
<td>-0.0099</td>
<td>0.0100</td>
</tr>
<tr>
<td>Mdh-3</td>
<td>0.1455</td>
<td>0.3728</td>
<td>0.2660</td>
</tr>
<tr>
<td>Mdh-4</td>
<td>-0.0556</td>
<td>-0.0441</td>
<td>0.0109</td>
</tr>
<tr>
<td>Me-1</td>
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<td>-0.0204</td>
<td>0.0368</td>
</tr>
<tr>
<td>Me-2</td>
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<td>0.1699</td>
<td>0.0212</td>
</tr>
<tr>
<td>6-Pgd</td>
<td>0.1962</td>
<td>0.2055</td>
<td>0.0115</td>
</tr>
<tr>
<td>Skdh</td>
<td>-0.0483</td>
<td>-0.0299</td>
<td>0.0175</td>
</tr>
<tr>
<td>Mean</td>
<td>0.2288</td>
<td>0.2897</td>
<td>0.0789</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Populations</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>I – Evenkia</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II – Evenkia</td>
<td>0.0042</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III – Evenkia</td>
<td>0.0025</td>
<td>0.0037</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>IV – Eastern Zabaikalje</td>
<td>0.0343</td>
<td>0.027</td>
<td>0.0291</td>
<td>-</td>
</tr>
</tbody>
</table>

Our value of $F_{st}$ for *L. gmelinii* populations from Evenkia and Eastern Zabaikalje was in excess of the mean values of this index ($F_{st} = 0.038$) for *L. gmelinii* populations studied from the Khabarovsk territory (Potenko and Razumov 1996) and from other regions ($F_{st} = 0.021$) of the species range (Semerikov et al. 1999), but it was within the limits of $F_{st}$ values ($F_{st} = 0.020 - 0.086$) 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 $F_{st}$ 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 $F_{st}$ 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 ($F_{st} = 0.0166$).

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.

**Acknowledgements**

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**References**


