Genetic structure of ural populations of *Larix sibirica* Ledeb. on the base of analysis of nucleotide polymorphism

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Abstract

Research on the state and dynamics of the gene pool (an important natural resource that determines the potential fitness of living organisms and, ultimately, their long-term survival) becomes an important problem in the context of increased anthropogenic environmental impact. They are especially important for key species of ecosystems of a global scale importance. *Larix sibirica* Ledeb., which spreads from the Western Siberia to the Russian North-West, is one of such forest tree species. We identified patterns of genetic structure of populations on the example of the species' Western race on the Middle and Northern Urals. The analysis of nucleotide polymorphism of genes of *ABA-inducible protein*, *MADS-box-transcription factor* and of *4-kumarat: CoA ligase* (a part of the gene) was used as a method. Evidences were obtained that a part of the populations previously formed a single large population. At the same time, populations with different gene pools were found. As a result, differences between populations within the region were more pronounced (fixation index $F_{ST} = -0.021 - 0.260$, total haplotype diversity $Hd = 0.636 - 0.911$; nucleotide diversity $\pi = 0.005 - 0.009$; number of mutations $\theta_w = 0.005 - 0.012$) than in other parts of the race. Causes of this phenomenon are discussed. It was concluded that the larch forests with a unique gene pool and/or high genetic diversity should be objects of population-oriented forestry and conservation.

Keywords: : *Larix sibirica* Ledeb., Urals, genetic structure, gene pool, populations, nucleotide polymorphism

Introduction

The genus *Larix* Mill. includes a group of coniferous tree species of Russia which occupy wide areas from coasts of the Barents Sea to Kamchatka peninsula. Stands with their domination cover about 2.80 million square km (37 % of the Russian forest area and 54 % of its Siberian part) and have more than 25 billion cubic meters of timber (Martinsson and Lesinski, 2007). These estimates clearly prove a great economic, social and ecological importance of the Russian larch on the whole planet scale. Eurasian *Larix sibirica* Ledeb. has the largest area and spreads from the Western Siberia to the Russian North-West. Two genetic population groups revealed in the species (Semerikov and Lascoux, 2003). One of them is identified as the Eastern race (Semerikov et al., 2007). It represents forests with domination of the larch in the eastern part of the Western Siberia and all the Eastern Siberia. The Western race, named also earlier as *L. sukaczewii* Dyl. (Dylis, 1947), occupies especially extensive areas, including the European part of Russia, the Urals and a part of West Siberian lowlands. The race differs from the eastern group of populations by a very small involvement in the stand composition. Its proportion in a stand vary from 0.2 to 1.1 % of forest areas within Russian regions (Martinsson and Lesinski, 2007), although this parameter increases towards the eastern race. Usually the race occurs in mixed stands of spruce (*Picea obovata* Ledeb.), birch (*Betula* spp.), aspen (*Populus tremula* L.), pine (*Pinus sylvestris* L.), etc. Unfortunately, information about genetic structure within the race is insufficient because the most of mtDNA markers already used on these territories were not polymorphic enough to reveal geographical structure on regional scale (Semerikov et al., 2013) and reference samples seem not be enough.

The western macro-slope of the Urals (this mountain system extends to more than 2000 km as a border between Europe and Asia) is particularly interesting for research of gene pool of the western race of *L. sibirica*. Climatic and environmental factors change gradually in the East European and West Siberian plains. In comparison to this fact, a great variation of natural conditions exists in the Urals in climate, relief, soils, hydrologic features, etc. These factors, together with succession processes after last glaciation and human impact caused fragmentation, geographic isolation and small population sizes of larch stands. A role of the gene drift is more significant under such conditions in the formation of the gene pool and relatively irregular distribution of allele frequencies is expected. Indeed, it was revealed that the western race of *L. sibirica* has here the most genetically diverse populations according to phenotypic, karyological and isozyme data (Putenikhin et al., 2004). Population, associative and comparative genomic analysis of forest tree species under such conditions allows to single out the adaptive structure of populations, understand the molecular evolution and detect genes affected by various forms of selection (Krutovsky, 2006). Research on the state, reproduction and dynamics of the gene pool (as an important natural resource that determines the potential fitness of living organisms and, ultimately, their long-term survival) becomes an important scientific problem in the context of global climate change and increased anthropogenic environmental impact (Rellstab et al., 2016). The knowledge about patterns of local population differentiation of forest tree species are important to develop scientifically based forest protection, forest-seed and reforestation programs (Petit et al., 1998) which are necessary in forestry mostly on the local level.

Despite the potentially great scientific interest, investigations of the gene pool of larch populations in the region, based on new DNA techniques have not been carried out. Thus, the aim of this research is to identify patterns of genetic structure of populations and differentiation among them by using the analysis of nucleotide polymorphism.

Materials and Methods

Population sampling and DNA extraction

Material for the research was collected in 9 natural populations of the western race of *Larix sibirica* Ledeb. in the Northern and Middle Urals (Table 1). The samples were collected regarding the latitudinal gradient along the mountain system and altitudes of stands. Geographic distances between stands averaged 279 ± 21 km and vary within 30–189 km (pairs *Ls1/Ls2*, *Ls1/Ls3*, *Ls1/Ls4*, *Ls2/Ls3*, *Ls2/Ls4*, *Ls3/Ls4*, *Ls4/Ls5*, *Ls6/Ls7*, *Ls7/Ls8* and *Ls8/Ls9*), 189–349 km (*Ls1/Ls5*, *Ls1/Ls6*, *Ls1/Ls7*, *Ls2/Ls5*, *Ls2/Ls6*, *Ls3/Ls5*, *Ls3/Ls6*, *Ls3/Ls7*, *Ls4/Ls6*, *Ls4/Ls7*, *Ls5/Ls7*, *Ls6/Ls8*, *Ls6/Ls9* and *Ls7/Ls9*) and 349–508 km (other 12 pairs). The needle samples of the larch were taken in 270 adult trees (Table 1). Distances between neighboring trees were at least 50 m to reduce the effect of family clustering of individuals on the results.

The DNA isolation was carried out using the technique (Rogers, Bendich, 1985) modified by the addition of PVPP (polyvinylpyrrolidone) as a sorbent. The weight of the plant material per a tree was 20 mg. The concentration and spectral characteristics of DNA were examined on [®] Nano-Drop2000 (Thermo scientific, USA). The DNA concentration of each sample was adjusted for PCR to 10 ng/μL.

DNA amplification and sequencing

We studied three loci to analyze the population nucleotide polymorphism. The locus *ABA-WDS* (it is designated as *Locus 1*) was sequenced for the first time in the genome of *L. sibirica*. The sequences of this locus were determined in *Pseudotsuga menziesii* var. *menziesii* and are represented on the GenBank NCBI (<http://www.ncbi.nlm.nih.gov/>) under the numbers AY832389 – AY832416. We also re-sequenced two loci, *sSPcD-FD040B03103* and *4CL1-363* (designated as *Locus 2* and *Locus 3*, respectively), the sequences of which are presented in GenBank NCBI under the numbers KC213987 – KC214265 (Table 2). This allowed us to use for the analysis data on additional larch populations from another part of the species range (Semerikov et al., 2013). All the loci belong to the nuclear genome; they are parts of the protein coding genes. *Locus 1* is a part of the gene *ABA-WDS* which encodes ABA-inducible dehydrin protein. *Locus 2* (it belongs to the *MADS-box* transcription factor gene) encodes the DNA-binding *MADS* domain. *Locus 3* belongs to the *4CL* gene, which encodes the enzyme 4-coumarate: CoA ligase (Dixon, 2010).

Table 1
Geographic coordinates, sample sizes and average number of analyzed sequences in *L. sibirica* populations

Abbreviation	Location	Coordinates	N ₁	N ₂
Ls1	Perm region, Natural reserve „Vishersky“	N 61.1130 E 58.8899	30	20
Ls2	Perm region, Natural reserve „Vishersky“	N 61.1178 E 59.1537	30	10
Ls3	Perm region, Krasnovishersk	N 60.3264 E 57.0931	30	8
Ls4	Perm region, Cherdyn	N 60.5147 E 55.9203	30	18
Ls5	Perm region, Gainy	N 60.1739 E 53.6213	30	16
Ls6	Sverdlovsk region, Kachkanar	N 58.7824 E 59.4068	30	16
Ls7	Perm region, Dobryanka	N 58.2998 E 56.3664	28	12
Ls8	Perm region, Suksun	N 57.0688 E 57.5653	30	10
Ls9	Sverdlovsk region, Pervouralsk	N 56.9499 E 59.8483	30	16

N₁ – sample size; N₂ – the average number of analyzed sequences

Amplification of *Locus 1* was carried out as described in our previous studies (Nechaeva et al., 2017). For amplification of other two loci, the nested PCR method was used (Semerikov et al., 2013). The BigDye® Terminator v3.1 Cycle Sequencing Kit was used for the sequencing reaction. Product purification was carried out using the BigDye® XTerminator™ Purification Kit (Applied Biosystems, USA). Capillary electrophoresis was performed on a Genetic Analyzer 3500xL (Applied Biosystems, USA) in two directions (forward and reverse). The editing of nucleotide sequences was carried out in SequenceScanner v2.0 (Applied Biosystems, USA). The location of the polymorphic positions was determined by multiple alignment in the Unipro UGENE v1.29 computer program (Okonechnikov et al., 2012) using the MAFFT algorithm (Kato and Standley, 2013).

Table 2
Description of the analyzed loci

Loci	Product of gene, locus size	Source
Locus 1	ABA-inducible protein (a part of the gene), 344 bp	Krutovsky and Neale, 2005; Eckert et al., 2009,
Locus 2	MADS-box-transcription factor (complete gene), 1518 bp	Semerikov et al., 2013
Locus 3	4-kumarat: CoA ligase (a part of the gene), 1236 bp	Semerikov et al., 2013

bp - base pairs

The data on sequenced locus sequences was compared to the existing sequences in the NCBI genetic database (National Center for Biotechnology Information, <http://www.ncbi.com>) using the automatic on-line alignment system BLAST+ v2.3.0 (Camacho et al., 2009). The structural analysis (analysis of the intron-exon structure) of the sequences was carried out in the DnaSP v6 program (Rozas et al., 2017). In this program, polymorphic sites were also analyzed, heterozygous positions were established and haplotypes were reconstructed in DnaSP v6 program (Rozas et al., 2017). Indels were not taken into account. The resulting nucleotide sequences of three loci of *L. sibirica* with a total length of 250.391 nucleotides (for all the trees studied) are included in the world genetic database of GenBank NCBI under the numbers: KT364889-KT365131.

Data analysis

The following nucleotide polymorphism indices were calculated based on the comparison of their nucleotide sequences: number of variable sites (S), number of haplotypes in a population (h_n), total haplotypic diversity (H_d) (Nei, 1973), nucleotide diversity (π), which estimates the average number of pair differences between two sequences per site, nucleotide diversity parameter calculated based on the number of mutations θ_w or the Watterson estimate (Nei, 1987). The Arlequin v3.5.2.2. program (Excoffier and Lischer, 2010) was used to calculate these parameters, as well as to estimate the haplotype diversity (average numbers pairwise differences between haplotype - k) and the fixation index (F_{ST}), to analyze molecular variance (AMOVA). A cluster analysis was carried out using the

STRUCTURE v2.3.4 program (Hubisz et al., 2009). The number of clusters (K) ranged from 1 to 12. The program STRUCTURE Harvester (Earl, vonHoldt, 2012) was used to determine the most probable number of genetic groups based on the LnPD log likelihood by Evanno's methods (Evanno et al., 2005). This web-program allows detect the appropriate number of clusters by their individual search. Five analysis runs were performed, each included a data acquisition phase consisting of 100000 iterations. To visualize the results, the CLUMPAK (Kopelman et al., 2015) service was used. Principal Component Analysis is performed in PAST v3 program (Hammer et al., 2001). Program PopART v1.7 (Leigh, Bryant, 2015) was used to build haplotype networks.

Results

The characteristics of the sequences and nucleotide polymorphism

As a result of sequencing, 243 nucleotide sequences of three gene loci were obtained (68, 85, 90 sequences for *loci* 1, 2, and 3, respectively). The total length of the sequences of the three loci of all individuals studied was 250391 nucleotides. The alignment length varies from 360 to 1395 bp (Table 1). Structural analysis of the sequences showed that the studied loci contained both coding and non-coding elements of the genes. About half of the analyzed sequence (55.2 %) was exons, and the rest was introns and non-coding elements (Table 3). Moreover, the largest number of polymorphic positions was found in non-coding parts of loci. Of all the substitutions in coding elements (exons), only 2.5 % were non-synonymous (i.e., potentially exposed to natural selection). A total of 200 polymorphic positions were detected and analyzed in sequences of three loci. The smallest number of polymorphic positions was detected at *locus* 3, and the most polymorphic was *locus* 2, containing 133 substitutions and two deletions (Table 3).

The parameters of population genetic variability are presented in Table 4. The most part of populations are characterized by relatively high and comparatively similar genetic diversity with some exceptions (Ls8: $H_d = 0.636$; Ls7: $\pi = 0.009$; $\theta_w = 0.012$).

The haplotype composition

In total 27 haplotypes were detected in the *Locus 1*, with an average number of paired differences of 3.668 ± 0.457 (varies from 1 to 16). Haplotype aba-2 was found in all the populations. It unites 23.8 % of all the sequences obtained. *Locus 2* has 68 haplotypes, differing from 1 to 54 nucleotides ($0.71 - 3.87$ %). The average number of pair differences (k) is 10.647 ± 4.475 . Almost half (49 %) haplotypes were unique for a population. The most common haplotype sSPs-2 (it was found in eight of nine populations) combines 26.5 % of all sequences. Thirty haplotypes were identified in the *Locus 3*. Differences between them vary from 1 to 8 nucleotides ($0.09 - 0.72$ %). The average number of pair differences was 2.317 ± 0.563 . The most part of haplotypes (18 or 60 %) were unique. Haplotype Hap-3 is found in all the stands and it unites 23.8 % of the sequences. Some

populations were characterized by especially high haplotypic diversity (Table 5: the highest values of the parameters in a population are in bold).

Table 3
Structural elements of three loci of *L. sibirica* populations

Structural element, bp/locus	Locus 1	Locus 2	Locus 3	In total
Exons	360	195	1028	1583
Introns and non-coding	-	1200	82	1282
Total length	360	1395	1110	2865

Bp – length of the locus structural element in base pairs

Table 4
Genetic diversity of *L. sibirica* populations for three studied loci

Population	$Hd \pm sd$	$\pi \pm sd$	θ_w
Ls1	0.872 ± 0.020	0.006 ± 0.002	0.006
Ls2	0.871 ± 0.039	0.006 ± 0.002	0.006
Ls3	0.887 ± 0.003	0.006 ± 0.002	0.006
Ls4	0.857 ± 0.038	0.005 ± 0.002	0.005
Ls5	0.836 ± 0.077	0.006 ± 0.001	0.007
Ls6	0.859 ± 0.016	0.007 ± 0.002	0.006
Ls7	0.883 ± 0.030	0.009 ± 0.003	0.012
Ls8	0.636 ± 0.059	0.006 ± 0.002	0.008
Ls9	0.911 ± 0.011	0.007 ± 0.002	0.007
Pooled sample	0.896 ± 0.010	0.007 ± 0.002	0.015

Hd – total haplotypic diversity; π – nucleotide diversity; θ_w – nucleotide diversity parameter calculated based on the number of mutations; sd – standard deviation

According to previous our studies (Nechaeva et al., 2017), Locus 3 among the three studied loci has the most selectively neutral polymorphism, so a median haplotype network has been shown on Figure 1 as an example of identification of links between haplotypes. Some dominant haplotypes are well traced in their median network (see an example of Hap-1, Hap-3 and Hap-9 of Locus 3). They combine the majority of populations that form stellate structures indicating the common origin of the constituent haplotypes. At the same time, the common haplotypes occupy both central and distal positions on branches, and the presence of possible alternative links does not allow establish any unequivocal relationship between haplotypes. This structure of the median network suggests that a part of the populations studied previously forms a single large population. The genetic structure of the distribution of haplotypes noted on the median network of haplotypes is well traced on the topology of the distribution of haplotypes in geographic space (Figure 2). It is noticeable that the spatial distribution of haplotypes and haplotypic network does not show consistency with the location of trees that bear these haplotypes. At the same time, attention is drawn to the fact that the most numerous haplotype Hap-3, which unites all populations, is practically absent in the sample Ls8. This population is characterized by the smallest haplotypic diversity with

a predominance of Hap-9. This haplotype is relatively rare for other populations and almost absent in the next sample Ls9. Interesting enough are differences in the composition of haplotypes of Ls1 and Ls2, separated only by a distance of about 30 km and locating on the neighboring ridges of the Urals. The median networks of the other two loci also have a similar complex branched structure with several dominant haplotypes, and haplotypes that differ in several mutational positions. Such picture is characteristic for populations that existed for a long time in a sufficiently high abundance in the non-isolated environment (Svetlakova et al., 2014).

Figure 1
Median network of the haplotypes of Locus 3. Circles represent individual haplotypes, the diameter of a circle corresponds to the number of samples with the haplotype and reflects its approximate frequency. The length of the branches corresponds to the number of mutations between haplotypes. Cross lines denote the number of mutations between individual haplotypes.

Figure 2
Spatial distribution of haplotypes in the populations of *L. sibirica*.

Table 5
The number of haplotypes (h_n) and average number of pairwise differences between haplotypes (k) of *L. sibirica* populations

Populations	Locus 1		Locus 2		Locus 3	
	h_n	k	h_n	k	h_n	k
Ls1	9	3.34	12	9.97	8	1.88
Ls2	5	4.49	14	6.84	6	3.00
Ls3	5	3.25	11	10.69	9	2.09
Ls4	7	3.27	12	7.29	6	2.07
Ls5	5	3.20	8	9.23	13	2.98
Ls6	8	3.77	11	11.62	7	1.93
Ls7	6	4.12	14	21.09	9	3.16
Ls8	5	3.96	6	6.43	6	1.73
Ls9	10	3.62	13	12.66	11	1.99
Average	6.7	3.67	11.2	10.65	8.3	2.32

The genetic differentiation

According to the results of the AMOVA analysis of all three loci, the bulk of the entire genetic variability (91.4 %) account for its within-population component ($F_{ST} = 0.086$, $p < 0.001$). This index for each of three loci varies from 0.050 (*Locus 1*) to 0.101 (*Locus 3*) and 0.160 (*Locus 2*). F_{ST} values vary significantly between pairs of populations (Table 6). *Locus 2* contributes the largest part to the differentiation among populations. The parameter ranges from -0.001 to 0.487. In general population pairs had statistically relevant differences ($p < 0.050$) in values of F_{ST} . *Locus 1* has a relatively small contribution to differences between populations ($F_{ST} = -0.002 - 0.144$). Significant differences are found only in a few pairs and almost exclusively with participation of Ls7. The pairwise values of F_{ST} in *Locus 3* change from -0.001 up to 0.250. Significant differences were revealed between many pairs of populations. Ls7, Ls8 and Ls2 differ from other stands on the highest level. Relatively high values of the fixation index are also revealed between the pair Ls7 and Ls8. According to our previous studies (Nechaeva et al., 2017), *Locus 3* has the most selectively neutral polymorphism among

the studied loci. Therefore, its F_{ST} values (Table 6) can more correctly reflects the gene flow between populations. It is noteworthy that the differentiation of populations at this locus is expressed at much higher levels of significance in comparison to all the loci. When using F_{ST} values, the Principal Component Analysis demonstrated unevenly distribution of populations (Figure 3). Samples Ls2, Ls5, Ls7 and Ls8 were significantly differentiated from the others independently on a locus studied.

Figure 3
Results of the Principal Component Analysis using the F_{ST} values in three loci of *L. sibirica*.

The analysis of the population structure using the STRUCTURE program (Figure 4) was carried out to determine the optimal number of genetic clusters. It is established that the most probable is the structure that is detected at $K = 4$. Evanno analysis using the $LnPD$ logarithm of the likelihood revealed that the average value of the logarithm of the probability function $LnP(D)$ increases from $K = 2$ to $K = 4$, and then decreases sharply; the dispersion also increases significantly. The maximum value of $LnP(D)$ with low dispersion is observed at $K = 4$. The value of the ΔK coefficient is also maximal at $K = 4$. The genotypic profiles of most samples are characterized by a mixed structure, with the exception of Ls2 and Ls8 (Figure 4), which contain haplotypes that are rare in other populations; these populations also show comparatively large pairwise F_{ST} values.

To determine the relation of the population genetic structure with the geographical location of the stands, the correlation analysis (Mantel's test) was carried out on the base F_{ST} . The analysis on three loci indicates the absence of a correlation between these parameters. However, the analysis of the most selectively neutral *Locus 3* revealed a weak correlation ($r = 0.158$, $p = 0.010$). When most genetically differentiated populations (Ls7 and Ls8) were excluded from the analysis, a more significant positive correlation was revealed ($r = 0.282$, $p = 0.060$). This parameter reaches the largest value ($r = 0.761$, $p = 0.040$) when analyzing populations (Ls1, Ls2, Ls6, Ls9) located along the ridges of the Ural Mountains.

Table 6

Pairwise F_{ST} values for all the loci (below the diagonal) and *Locus 3* (above the diagonal)

Populations	Ls1	Ls2	Ls3	Ls4	Ls5	Ls6	Ls7	Ls8	Ls9
Ls1		0.079*	0.040	0.055	0.022	0.053	0.160***	0.194***	-0.008
Ls2	0.127***		0.104**	0.131**	0.086*	0.094*	0.198***	0.164***	0.090**
Ls3	0.063**	0.108***		0.041	0.074*	0.022	0.212***	0.126***	-0.001
Ls4	0.042	0.140***	0.048		0.022	-0.014	0.095*	0.084*	0.077*
Ls5	0.007	0.078***	0.024	0.014		0.009	0.070	0.104***	0.050
Ls6	0.012	0.097***	0.030	-0.001	-0.020		0.153***	0.042	0.060*
Ls7	0.017	0.131***	0.084**	0.106**	0.013	0.080**		0.250***	0.207*
Ls8	0.254***	0.220***	0.142***	0.218***	0.194***	0.214***	0.260***		0.160***
Ls9	-0.021	0.084***	0.023	0.028	-0.009	0.007	0.028	0.213***	

* $p < 0.050$, ** $p < 0.020$, *** $p < 0.010$. Level of significance determined based on 1023 permutations

Figure 4

Results of the analysis of the structure of *L. sibirica* populations using the STRUCTURE v. 2.3 program (the number of clusters $K = 4$).

We additionally analyzed the nucleotide sequence of the *Locus 3* (4CL1-363) in other *L. sibirica* populations (Semerikov et al., 2013) by using the genetic database of the GenBank NCBI. Thus, we added to the analysis a set of eight populations of the species' western race (Figure 5). They are located on the northern and southern parts of area of distribution of the race, including the eastern macro-slope of the Middle Urals. When studying the correlation of genetic and geographical distances, no significant correlation was found ($r = 0.001$, $p = 0.480$ at three loci and $r = 0.158$, $p = 0.010$ for *Locus 3*). About 30 % (11 of 37) of revealed haplotypes were found to be common in these populations of *L. sibirica* in the Western Siberia, and 19 haplotypes (51 %) were identified only in our study. Hap-7 is represented in four populations (Ls1, Ls2, Ls4, Ls7), Hap-13 in two populations (Ls2 and Ls9), the rest are singularly present in all populations except Ls6. The joint median network reveals again the same haplotype structure of *Locus 3* with dominant haplotypes forming stellate structures and apparently ancestral for the entire population. At the same time, they have a large share in comparison with the populations of *L. sibirica* in the

Western Siberia (Figure 5). It is noticeable that some of the haplotypes found in this study have a higher frequency of occurrence than in populations previously studied (Semerikov et al., 2013), for example, Hap-7 and Hap-13.

Figure 5

Median network of the haplotypes of *Locus 3*. Samples of this study are indicated by red shades, blue shades represent samples of *L. sibirica* of the Western Siberia (Semerikov et al., 2013). Others comments are given in Figure 1.

Discussion

Relatively high nucleotide diversity of the western race of *L. sibirica* of the Middle and Northern Urals was established in this study. This result is confirmed by results of morphological and allozyme investigations (Putenikhin et al., 2004). They have been allowed to identify so called "Perm-Kama pre-Urals" larch populations with the highest level of variation in Urals. The polymorphism of larch populations of the Middle and Northern Urals ($Hd = 0.846 \pm 0.027$; $\pi = 0.0064 \pm 0.0004$; $\theta_w = 0.0070$

± 0.0007) are higher of values typical for other parts of the species area of distribution. For example, total haplotype and nucleotide diversities for 8 candidate genes in 11 populations of the both races of *L. sibirica* were $Hd=0.822$; $\pi = 0.0054$ and $\theta_w = 0.0067$ (Semerikov et al., 2013).

Differences between populations within the studied relatively small region are more pronounced in these parameters ($Hd = 0.636 - 0.911$; $\pi = 0.0050 - 0.0090$; $\theta_w = 0.0050 - 0.0120$) than in other parts of the range of the western race of Siberian larch. A confirmation of this result is the study of *L. sukaczewii* (Araki et al., 2008) where much lower variations of the parameter were revealed in all other parts of the taxon area of distribution ($\pi = 0.0020 - 0.0033$, $\theta_w = 0.0026 - 0.0027$ and $\pi = 0.0033$, $\theta_w = 0.0027$ for the regions of *ITS* and *4CL* genes, respectively). It is possible that the increased genetic diversity of some populations is a consequence of the influence of larch glacial refuges in the Urals (Semerikov and Lascoux, 2003).

The magnitude of the differentiation between pairs of populations in our study ($F_{ST} = -0.021 - 0.260$) is also larger than the limits of pairwise values of the parameter between other larch populations of the western race. So F_{ST} values of populations of *L. sukaczewii* varies from -0.078 to 0.115 (Araki et al., 2008) despite of the much greater distances between pairs of them (according to coordinates of populations in the paper the distances are from 516 to 1346 km). Extremely low differences are found in this study between four stands from all the European part of Russia and the north of the West Siberian Lowland ($F_{ST} = -0.078 - 0.008$ and $F_{ST} = -0.069 - 0.008$ for *ITS* and *4CL* genes, respectively). But F_{ST} values reaches higher levels when we include a population from the Urals into pairwise comparisons ($F_{ST} = -0.001 - 0.115$ and $F_{ST} = 0.087 - 0.238$). These data confirm our results that the race on the Urals has got a relatively high level of differentiation among populations.

Population-genetic studies of Eurasian larch species has been allowed us to establish the uneven spatial distribution of the genetic diversity. Zones with a relatively homogeneous geographic distribution of mitochondrial haplotypes of the Siberian larch were determined although somewhere their distribution can be described in the term of "spatial mosaicism" (Semerikov et al., 2007). Using restriction-site associated DNA (RAD) sequencing, recently a set of newly developed 253 SNP loci of *Larix* spp. was screened on 916 individuals of 52 populations in Russia (Blanc-Jolivet et al., 2018). Well-defined genetic clusters on the East-West transect from the Far East to the Urals were revealed with especially high genetic diversity on borders of the species. Various chloroplast (SSRs, PCR-RFLP), mitochondrial and nuclear markers (isozymes, DNA sequences, AFLP, SSRs) have been demonstrated clearly localized such contact zones among Russian larch species (Semerikov and Lascoux, 1999, 2003; Semerikov et al., 2007, 2013; Araki et al., 2008; Khatab et al., 2008; Polezhaeva et al., 2010; Oreshkova et al., 2013). It is possible that the Middle and Northern Urals are such part of the larch area of distribution, where the gene pool of populations is formed under the influence of both races of *L. sibirica*.

Migration of the larch from the Southern Urals Pleistocene refuges to eastern populations can explain these regularities.

The study of the genetic structure of *L. sibirica* populations from the western (this study) and the eastern parts of the range (Semerikov et al., 2013) did not reveal the relative contribution of the genes of the eastern populations and also points to the isolation of the genotypic composition Ls1 and Ls8 (Figure 6). On the contrary it is suggested (Araki et al., 2008) that the larch of the Eastern European Plain spread also from second refuge, not only from the Southern Urals.

Figure 6

Results of the analysis of main components using the F_{st} values in *Locus 3* (the upper part of the figure). Results of the analysis of the structure of *L. sibirica* populations at *Locus 3* using the STRUCTURE program v. 2.3 (the number of clusters $K = 4$) are given below. Ls1 – Ls9 represent populations of this study, and sLs1 – sLs8 are samples of *L. sibirica* of the Western Siberia (Semerikov et al., 2013).

Our previous studies indicate that in different large geomorphological formations of the Urals the genetic structuring of populations occur in different ways. Thus, while studying *Populus tremula* L. populations in the same region, it was established that the genetic structure in the mixed coniferous-broadleaved forests is formed by the type of "isolation by distance" (Svetlakov et al., 2014) in comparison to the mountainous dark coniferous taiga where genetic spatial mosaicism is a common pattern. While studying the *L. sibirica* of the Urals by using ISSR-analysis (Nechaeva et al., 2014), significant correlations of the genetic parameters within groups of mountain and plain populations with geographical distances ($r = 0.680$, $p = 0.023$ and $r = 0.760$, $p = 0.020$, respectively) and altitudes of stands above sea level ($r = 0.940$, $p = 0.020$). This is consistent with the data of this study, because a significant correlation was detected in the group of mountain populations ($r = 0.761$,

$p = 0.040$). But these patterns disappear in a combined analysis of all the larch populations. A likely cause is the lack of relations between some larch forests within the region. It is known (Svetlakova et al., 2014) that natural or artificial forest fragmentation can lead to genetic changes on the population level. As it was mentioned above, according to the data of the long-term research in the Urals (Putenikhin et al., 2004), the fragmentation of larch stands is clearly expressed on the Urals. The revealed differentiation among populations changed from moderate to high values with participation of more or less significant geographically isolated Ls7, Ls8 and Ls2 in pair population comparisons. Ls8 characterizes a population of the western macro-slope of the Ural Mountains located almost in the center of the "non-larch tongue" (Dylis, 1947). Ls7 is located in a district with a high intensity of economic activity for many centuries. According to the previous our studies (Nechaeva et al., 2014) this population has an altered genetic structure. One of the most obvious reason that cause this degradation of the stand is the partial logging.

Thus, the analysis of the nucleotide DNA polymorphism made it possible to reveal a high level of genetic diversity of *L. sibirica* in the Middle and Northern Urals within and among populations. The obtained data in general are confirmed by the results of studies that were conducted out using other tools, methods and approaches. The uniqueness and diversity of the Urals geomorphological formations, past events in the forest history and anthropogenic influences can determine the regional genetic structure patterns. The revealed larch forests with a unique gene pool and/or high genetic diversity should be objects of population-oriented forestry and conservation.

The reported study was partially supported by the Government of Perm Krai, research project C-26/174.3 31.01.2019.

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