Genetic structure and postglacial recolonization of *Pinus sibirica* Du Tour in the West Siberian Plain, inferred from nuclear microsatellite markers

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Abstract

The total of 257 trees from the ten natural Siberian stone pine populations of West Siberian Plain, Ural Mountains and Kuznetsk Alatau were studied with the eight nuclear microsatellite loci. Differentiation of population groups indicates the possible existence of separate refugia in the past in the Urals and Kuznetsk Alatau. The northern populations of Western Siberia were characterized by a reduced level of genetic diversity, which could be a consequence of the founder effect in the process of *P. sibirica* migration from the southern regions of Western Siberia and the Urals since the end of the last glacial maximum. The genetic variability distribution among populations shows the uneven dynamics of Siberian stone pine migration from the zones of supposed refugia. The map of the proposed ways of Siberian stone pine migration from the zones of possible refugia was constructed, and it is in a good agreement with the results of palynological investigations.

Keywords: *Siberian stone pine, refugia, genetic diversity, recolonization, phylogeography, last glacial maximum, microsatellites, genetic differentiation, gene flow.*

Introduction

Genetic diversity is one of the key conditions for the stable existence and reproduction of species populations for many generations. To assess and predict the dynamics of genetic variability in populations, it is necessary to have knowledge of their genetic structure and the factors forming it. The current distribution of genetic variability is largely the result of historical processes such as long-term changes in geographic, climatic and ecological conditions. Siberian conifers, like most wind-pollinated tree species with large, continuous populations, are characterized by high genetic variability with low genetic differentiation of populations (Hamrick et al., 1992; Politov, 2007). In case of the boreal species the additional reason of low interpopulation differentiation could be the postglacial recolonization from few refugia in southern regions since the end of the last glacial maximum (LGM) – 18 thousand years ago (Velichko et al., 2009) due to copying of genetic variation of the refugium population over large migration area.

Siberian stone pine is one of the key coniferous species of the boreal zone of Eurasia. The wide ecological amplitude caused a relative diversity of conditions for the species area. The species range extends from the western Urals through the entire West Siberian Plain to Transbaikalia and southern Yakutia. In the north, the range reaches the border of the forest tundra; in the south, the border enters eastern Kazakhstan, Mongolia and China (Goroshkevich, 2000). Diverse climatic and

environmental conditions could well affect the genetic composition of populations. According to the main provisions hypothesis of the „center-periphery", populations on the border of the range grow in less favorable conditions and are characterized by a smaller genetic diversity. Consequently, they are less stable and more susceptible to extinction (Aitken et al., 1994; Ledig, 2000). This situation becomes especially relevant in the light of the problem of rapid climate change.

The current distribution of the genetic diversity and differentiation of *P. sibirica* populations may reflect the pathways of postglacial recolonization since the end of the last glacial maximum. To find out the phylogeographic signal of recolonization events, the use of neutral and highly polymorphic gene markers is required. Nuclear markers, especially microsatellites proved to be useful tools for studying gene flow, phylogeography and demography. (Toth et al., 2017).

Previously, the variability, differentiation and structure of *P. sibirica* populations were studied using allozyme loci across the species' distribution range (Krutovsky et al., 1988; Krutovsky et al., 1989; Krutovsky et al., 1990; Goncharenko et al., 1991; Politov et al., 1992; Goncharenko et al., 1993). Weak differentiation of Siberian stone pine populations was observed in the trans-Urals and Western Siberia, in the populations of the south of Central Siberia and Transbaikalia. Politov (2007) discovered polymorphism in more than 20 isozyme loci in the Siberian stone pine populations.

However, the research on the genetic diversity of *P. sibirica* in the north of Western Siberia was limited. The lack of material from northern *P. sibirica* populations, along with the relatively low variability of isozyme loci, as well as their exposure to a weak selection pressure, did not allow us to confirm or refute the hypothesis of lower genetic diversity of northern populations and its possible causes, and this issue is still debatable.

The aim of the present study was to verify the hypothesis of reduced genetic diversity of *P. sibirica* at the northern border of the post-glacial migration range and to identify in the structure of genetic diversity the signal of recolonization of the West Siberian Plain by Siberian stone pine.

Materials and Methods

257 trees of Siberian stone pine from 10 natural populations in Western Siberia and the Urals were analyzed. The age of the trees was 80-100 years (Table 1). The total DNA was extracted from fresh needles using a standard CTAB-method (Devey et al., 1996). For the purpose of primary screening, we tested 23 nuclear microsatellite loci developed by Belokon (2016). The results of testing the Siberian stone pine loci showed the polymorphism and amplification stability for 8 of them: Ps_80612, Ps_364418, Ps_1375177, Ps_1502048, Ps_31489, Ps_25981, Ps_39709, Ps_718958. The detailed characteristics and amplification protocols of the loci are presented in the literature (Belokon et al., 2016). The amplification products were analyzed by vertical gel electrophoresis (VE-20, Helicon, Russia) in the 6 % non-denaturating polyacrylamide gel in a 1X-TAE buffer at 80

W for 3 hours, followed by the ethidium bromide staining and visualization on a transilluminator (VilberLourmat, France). GenRuler20bp (Fermentas, USA) was used as a standard marker for fragment lengths. Genotyping was performed using the Photo-Capt 12.4 program (VilberLourmat, France).

The genotyping errors were checked using Micro-Checker 2.2.3 (Oosterhout et al., 2004). The presence of null alleles in the loci was checked with FreeNa program (Chapuis et al., 2007) by using the EM algorithm (Expectation and Maximization) (Dempster et al., 1977). Tests for the population deviation from the Hardy-Weinberg equilibrium and linkage disequilibrium were performed in the Genepop on the Web software by default settings (Raymond et al., 1995; Rousset, 2008). The observed and expected heterozygosity (H_O and H_E) was calculated in the GenAlEx 6.5 software (Peakall et al., 2006). The matrix of genetic distances (D_A) (Nei et al., 1983) was used for the analysis of population clustering by the principal coordinate method (Kendall et al., 1976). We used pairwise Hedrick's G''_{ST} implemented in GenAlEx 6.5 from a hierarchical AMOVA. This measure is well suited for inferences on demographic history and migration and it should be used whenever the number of sampled populations is small, especially for pairwise comparisons (Meirmans et al., 2011).

Based on the Bayesian approach with the MCMC (Monte Carlo Markov Chain) algorithm, the Structure 2.3.4 software (Pritchard et al., 2000) evaluated the contribution of genotypes to each population under study. The parameters of the simulation experiment included the Admixture and Locprior model (Hubisz et al., 2009). The number of MCMC simulations was 300.000 with the period length (burn-in) of 50.000. The total was made 30 iterations for each run. The iterations were carried out for K from 1 to 6. The estimation of K ancestral populations was performed using the delta K method (Evanno et al., 2005). Alignment of cluster assignments across replicate analyses was conducted in the CLUMPACK web-program (Kopelman et al., 2015). The graph for the best K value was produced with the Distruct application also realized in the CLUMPACK. A hierarchical analysis of molecular variability (AMOVA) was performed in the Arlequin 3.5.2 software at 10.000 permutations (Excoffier et al., 2010).

The probabilities of recolonization scenarios were analyzed in the DIYABC 2.1.0 software (Cornuet et al., 2014). 10^6 simulations were set up for each scenario. The summary statistics were as follows: the mean number of alleles; the mean genic diversity; the mean size variance; F_{ST}; $(d\mu)^2$ distance. The scenario probabilities were estimated using the direct regression method.

Results

Analysis of microsatellite loci

The analysis of microsatellite loci in the FreeNa program revealed a presence of null alleles in the locus PS_364418 (0.077). The pairwise test F_{ST} values as well with the null alleles as with ENA correction algorithm (Chapuis et al., 2007) did not

show the significant differences (H0 - there is no influence; H1 - there is influence) (P>0.05).

The pairwise test of the loci did not reveal any linkage disequilibrium (P>0.05). HWE-test (without the locus PS_364418) revealed significance disequilibrium only in two the northern populations (Muji and NU) (P<0.01).

Estimation of population heterozygosity

We discovered that the levels of the observed heterozygosity (H_O) in the southern Siberian stone pine populations were higher rather than the northern ones (Table 1). The observed heterozygosity decreased in the south-north direction for the Aiz-Ivd-Muji and Arg-NK-NU.

Table 1
Averaged Ho, He, F_{IS} (over loci) for each population sorted by latitude.

Population name	Sample size	Ho	He	Fis	Latitude	Geographic locations
Aiz	18	0.472	0.507	0.051	57.37	Urals
Ivd	20	0.419	0.478	0.054	60.75	
Muji	17	0.301	0.438	0.265	65.4	
Sur	30	0.483	0.487	-0.017	61.5	West Siberian Plain
NK	24	0.433	0.494	0.147	63.2	
NU	26	0.356	0.500	0.257	66.08	
Mak	32	0.461	0.489	0.011	54.18	Kuznetsk Alatau
Troit	30	0.421	0.473	0.088	55.94	
Arg	30	0.463	0.466	-0.010	57.88	
Chaz	30	0.442	0.484	0.054	58.08	

Cluster analysis

The principal coordinate analysis showed two clearly distinguishable population clusters (Fig. 1).
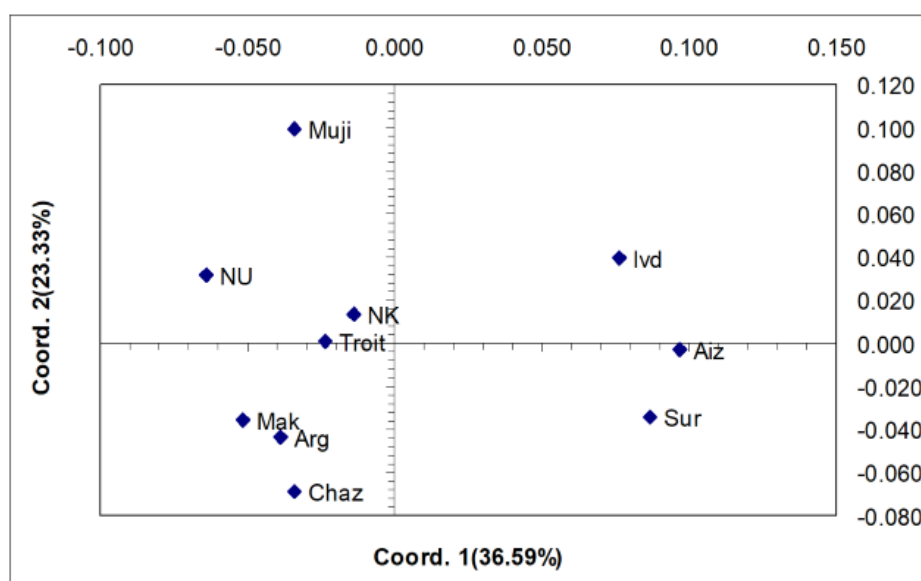

Figure 1
Ordination of *P. sibirica* samplings by the principal coordinate method.

According to the first main coordinate of the population, it can be divided into the Ural (Aiz, Ivd, Sur) and Kuznetsk Alatau-West Siberian Plain (NU, NK, Muji, Mak, Arg, Chaz) groups. According to the second main coordinate, the division occurs by latitude into groups of northern (NU, NK, Muji, Ivd) and southern populations (Mak, Arg, Chaz, Sur, Aiz). The table of pairwise G''_{ST} values also showed that populations significantly divided into two clusters in according with the first main coordinate (Table 2).

Table 2
The pairwise genetic distances (D_A) and Hedrick's G''_{ST} for ten Siberian stone pine populations.

Aiz	Ivd	Muji	Sur	NK	NU	Mak	Arg	Chaz	Troit	
0.00	0.02	0.14*	0.05	0.08*	0.11*	0.11*	0.12*	0.08*	0.06*	Aiz
0.04	0.00	0.14*	0.12*	0.06*	0.11*	0.11*	0.12*	0.14*	0.07*	Ivd
0.11	0.10	0.00	0.15*	0.08	0.03	0.12*	0.10*	0.14*	0.06	Muji
0.05	0.09	0.11	0.00	0.11*	0.16*	0.16*	0.09*	0.10*	0.11*	Sur
0.07	0.06	0.07	0.08	0.00	0.02	0.03	0.05*	0.05	0.02	NK
0.10	0.09	0.04	0.11	0.03	0.00	0.03	0.03	0.04	0.01	NU
0.08	0.08	0.08	0.11	0.04	0.04	0.00	0.01	-0.01	0.01	Mak
0.09	0.09	0.07	0.06	0.04	0.04	0.02	0.00	-0.01	0.02	Arg
0.07	0.10	0.10	0.07	0.05	0.04	0.02	0.01	0.00	0.01	Chaz
0.06	0.06	0.05	0.08	0.03	0.03	0.02	0.03	0.02	0.00	Troit

D_A values below the diagonal. G''_{ST} values based on 999 permutations is shown above diagonal. * – $P<0.01$.

It seems that the longitudinal geographical component was more distinguishable. However, it is well visible the discrepancy of the geographic and genetic distances for Sur population and Muji population (Fig. 2. Table 2).
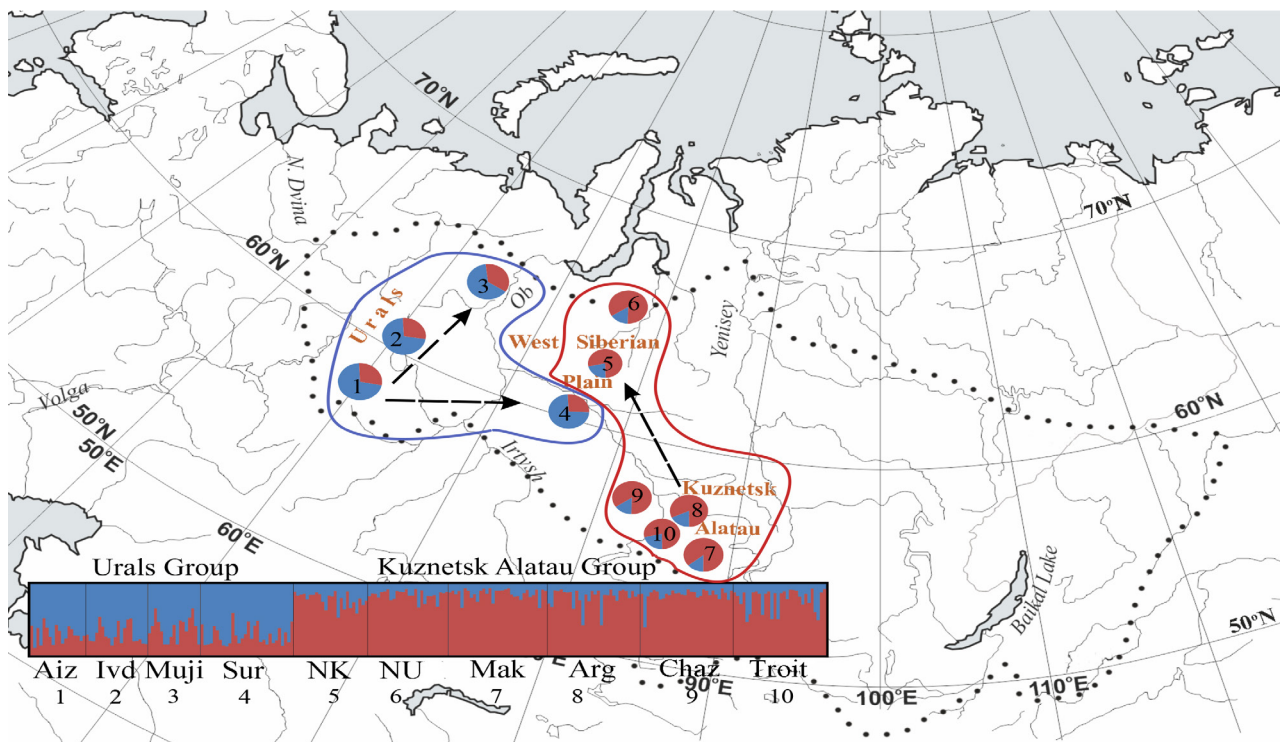

Figure 2
Genetic structure of *Pinus sibirica* populations as inferred by Structure. Individual assignment to two clusters for all 10 populations are visualized as pie charts and summary plot. Each population was partitioned into two colored segments proportionally to its membership in a given cluster; the arrows show the directions of suggested migration ways.

The Bayesian analysis of the population structure and estimation of the number of K populations also showed that the samples were divided into two groups (Fig. 2). Delta K values were as follows: K=2 (12.69), K=3 (0.28), K=4 (0.66), K=5 (0.64).

The assessment of the contribution of genotypes in each population showed that the Muji population contained a higher proportion of genotypes (that is the proportion of that individual's ancestry from population K; further as in the text) by originated from the Kuznetsk Alatau cluster, compared to other samples from the Urals. The Sur population had a smaller share of genotypes from the Kuznetsk Alatau cluster and a highest from Urals group.

The probable reason for these discrepancies could be the uneven ancestral migration from two different refugia (from the southern Urals and the Kuznetsk Alatau). The Muji population included a little more part of gene pool (compared with Urals group) from the Kuznetsk Alatau cluster, likely, through the northern populations (NK, NU) which related to Kuznetsk Alatau group. The Sur population was genetically closest to the Ural group (D_A=0.05) and formed a separate cluster with it, despite its geographic proximity to the northern populations of the West Siberian Plain (NK and NU).

Differentiation of populations

According to the results of clustering, the samples were divided into the groups of Kuznetsk Alatau and north of Western Siberia – the first and the Urals – the second. The differentiation between these groups, in terms of G''_{ST}, was significant (P<0.01) (Table 2). Therefore, the AMOVA variability distribution analysis in *P. sibirica* populations was made for two groups of populations. Most of the variability was concentrated within populations (94.78 %) and only a small part of it was accounted for by the interpopulation (2.22 %) and intergroup (3.01 %) variability components (Table 3).

The estimating of recolonization patterns

The approximate Bayesian computation (ABC) method was used to explore migration ways through the comparing of scenario probabilities. It was prepared the four groups of recolonization scenarios (Fig. 3) based on the cluster analysis results. In order to simplify recolonization models, the total population of the Kuznetsk Alatau was represented by a single population, i.e. Arg.

Table 3

Analysis of molecular variance (AMOVA) data for 10 Siberian stone pine populations

Variability source	Degree of freedom	Sum of squares	Dispersion components	Variation, %	Fixation index
Between 2 groups	1	19.08	0.06	3.01	FCT=0.030*
Among 10 populations	8	34.54	0.05	2.22	FSC=0.023**
Within populations	520	1003.83	1.93	94.78	FST=0.052**
Total	529	1057.45	2.04	100	–

* – P<0.05; ** – P<0.001.

The significant intergroup differentiation indicates a possible existence in the past of separate Siberian stone pine refugia in the southern Urals and the Kuznetsk Alatau.
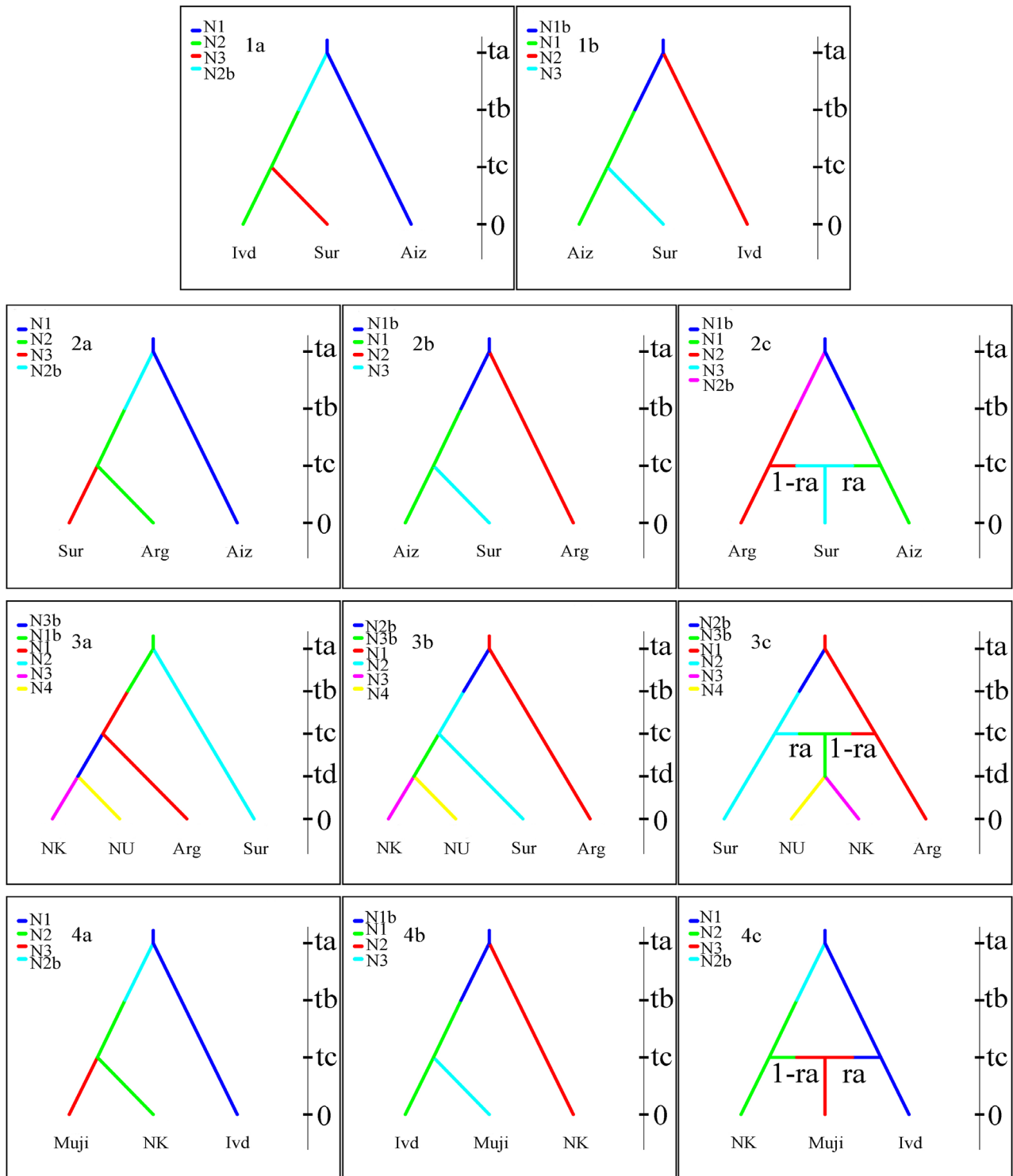
Figure 3
The possibility scenarios of interactions between the ancestral Siberian stone pine populations.

Table 4
Scenarios tested by the ABC approach.

	Probabilities of scenarios	
Scenarios	Probability, %	Possible migration routes (origin)
Testing of migration routes in Urals group		
1a	24.6	Ivd→Sur
1b	75.4	Aiz→Sur
Testing of origin for Sur population between Urals and Kuznetsk Alatau		
2a	29.8	Arg→Sur
2b	70.2	Aiz→Sur
2c	56.0	Aiz*×Arg*→Sur**
Testing of migration routes for the northern populations		
3a	84.8	Arg→NK→NU
3b	15.2	Sur→NK→NU
3c	31.6	Sur*×Arg*→NK**×NU**
Testing of origin for Muji between Urals and the northern populations		
4a	31.4	NK→Muji
4b	68.6	Ivd→Muji
4c	61.8	NK*×Ivd*→Muji**

* – parental population; ** – admixture population; × – admixture event.

The results of scenario probabilities were presented above (Table 4).

The results of scenario probabilities and cluster analysis allowed us to make a few assumptions about the possible migration routes of *Pinus sibirica* (Fig. 2). Firstly, we supposed that the Sur population is the eastern descendant of Urals group of populations. The Sur population also was influenced the gene flow from Kuznetsk Alatau group during to the historical migration of populations from Kuznetsk Alatau along the Ob River into the north and the west. The northern populations (NK and NU) are the descendants of Kuznetsk Alatau group. The Muji population originated from Urals group but underwent the gene flow from the northern populations.

Discussion

The studies of the phylogeographic structure of various species populations showed that the history of their migration leaves a "footprint" on the modern genetic structure (Hewitt, 2000). By identifying specific signals of the modern population-genetic structure with nuclear microsatellite data, we tried to reconstruct the past migration history of Siberian stone pine.

The results of studying the genetic diversity and structure of Siberian stone pine in the West Siberian Plain, the Urals and Kuznetsk Alatau showed that the level genetic diversity of the northern populations was lower compared to southern ones.

No similar pattern was found for the samples from the Kuznetsk Alatau (Mak, Arg, Chaz, and Troit). Since none of the studied populations was isolated, the reduced heterozygosity level of northern Siberian stone pine populations may be a consequence of the founder effect during the migration from the southern regions of Western Siberia and the Urals since the end of the last glacial maximum. The cluster analysis and the results of estimating recolonization scenarios suggest that the *P. sibirica* migration in the West Siberian Plain has been uneven since the end of the last glacial maximum.

According to the cluster analysis results, the Sur population belongs to the Ural group. Consequently, the *P. sibirica* migration from the Urals occurred to the east of the West Siberian Plain. However we found the slightly more proportion of genotypes from the Kuznetsk Alatau cluster for the Sur population. Possibly, there was the historical gene flow from the northern populations (NK and NU) in the Sur population and vice versa. Since the northern populations had a slightly higher proportion of genotypes from the Ural group.

The Muji population, located twice as far from the Kuznetsk Alatau populations as the Sur population, was stronger influenced by the gene flow from the Kuznetsk Alatau group (Fig. 2). Therefore we supposed that the migration of Siberian stone pine from the Kuznetsk Alatau refugia occurred faster than that from the Urals to the north and the east. May be there was a genetic flow from northern populations of West Siberian Plain into the Muji population? We consider that the last assumption is possibly. The assumption was confirmed the finds of the ancient pollen of *Pinus sibirica* in the north of the western Siberia 10 thousand years ago (Blyakharchuk, 2010).

It seems the Siberian stone pine migration from the refugia of the Kuznetsk Alatau was associated with the Ob River. Because of the Muji population included a little more part of gene pool from the Kuznetsk Alatau cluster compared with the more proximately Sur population. Possibly, a some complex of ecological and geographic factors (for example, the more favorable climate and soil conditions) associated with the Ob River valley may have played a important role in the rate of *P. sibirica* migration after the LGM to the northwest. There was noted that Siberian stone pine distributed into the north further 150-200 km along a big rivers (for example the Yenisei River, or the Ob River) and, on the contrary, when big rivers is lacked (Goroshkevich, 2000). According to the most probable scenarios (Table 4. Fig. 3a), the faster migration of ancestral *P. sibirica* populations from the Kuznetsk Alatau occupied the northern watershed of the Ob River. For the Urals the migration spread to the north of the Urals and to the east across the West Siberian Plain along the southern watershed the Ob River (Fig. 2).

Thus, two groups of populations were formed: the first – Urals and the southeast of West Siberian Plain; the second – the Kuznetsk Alatau and the northwest of West Siberian Plain. Also we found evidences for the existence of contact zones along the Ob River, where populations exchanged genes due to a part admixture of genetic groups from Kuznetsk Alatau and Urals.

The recent phylogeographic researches also have shown the complex recolonization history of Siberian conifers. For example, in the Siberian fir, according to allozyme data, alike in Siberian pine, an unexpected similarity was found between the populations of the northwest of Western Siberia and the populations of the south of Siberia (Semerikova et al., 2006; Semerikova et al., 2007; Semerikova et al., 2011). For Siberian larch, based on data on mitochondrial DNA (Semerikov et al., 2013), evidence was found for the presence of secondary refugia in the north of Western Siberia, where larch could persist during LGM and which could participate in the recolonization of the north along with refugia of the south of Siberia and the Urals. However, there are no the paleontological evidences of the presence *Pinus sibirica* at the north of West Siberian Plain during LGM. Therefore, we think that the last scenario poorly applicable to the possible phylogeographic pattern for Siberian stone pine. We believe that the ancient northern populations of Siberian stone pine (10 thousand years ago) were the descendants of rapidly expanded along the Ob River the ancestral population from the Kuznetsk Alatau group. The northern descendants might slowly move into the west along the northern border and interbred with the ancestor of the Muji population. This process might be occurred together with the ongoing migration from the ancestral population the Kuznetsk Alatau along the Ob River to the northwest. Unfortunately, we had no materials from the middle and lower reach of this river in order that to say which the part of the Kuznetsk Alatau group made a greater contribution to the gene pool of the northern Urals: the northern part (NU and NK) or the fast-extending part from the south along the Ob River to the north. Based on the pairwise G''_{ST} values, the NU and the NK populations did not differ from the Muji population (Table 2). Consequently, as it seems to us, the northern contribution in the gene pool the Muji population is a more probable, despite the possible lower efficiency of dissemination and migration in hard conditions at the border of the area.

Generally, our conclusions about the ways of Siberian stone pine migration are consistent with the results obtained in studying the dynamics of its palynoarea (Blyakharchuk, 2010). However, it should be noted that the lack of material from the populations of the West Siberian Plain (east of the Sur sample) does not allow us to make reasonable assumptions about the extreme eastern border of Siberian stone pine migration from the southern Urals. It should also be taken into account that the phylogeographic signal may be hidden by the influence of factors, such as local gene flow, isolation and selection, making it impossible to unambiguously reconstruct the history of species migration.

Acknowledgements

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