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Isoenzyme Diversity and Differentiation of Marsh Scotch pine (*Pinus sylvestris* L.) Populations in The Western Siberia

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Abstract

Genetic diversity and differentiation of Scotch pine (*Pinus sylvestris* L.) populations situated on eutrophic, mesotrophic, oligotrophic swamps and on dry land in south-taiga subzone of Western Siberia lowland (Tomsk region) were studied on the basis of allozyme variation analysis at 20 loci. The vegetative buds collected from 240 trees in eight populations were used as material for study. Horizontal starch gel electrophoresis was applied to separation the isoenzymes of eleven enzyme systems. As a result of the study it was established that *P. sylvestris* populations studied are characterized sufficiently high level of genetic diversity. As a whole 75% of gene loci assayed were revealed to be polymorphic. The mean number of alleles per locus, the mean observed heterozygosity, the mean expected heterozygosity and the effective number of alleles were equal to 2.63, 0.217, 0.212 and 1.37, respectively. The greatest allelic diversity and the highest level of heterozygosity were established in population *P. sylvestris*, situated on mesotrophic swamp 2. More than 97% of total genetic variation was within the population and only 2.3% ($F_{st}=0.023$) was among the populations. The mean genetic distance D (Nei, 1972) between populations studied ranged from 0.004 to 0.016 and averaged 0.007. The most essential distinctions in genetic structure were revealed between populations of Scotch pine growing under contrast conditions of water-mineral nutrition on eutrophic and oligotrophic swamps as well as between populations situated on the various oligotrophic swamps. The obtained results testify to considerable genetic heterogeneity studied in Tomsk region of marsh populations of Scotch pine.

Key words: *P. sylvestris*, The Western Siberia, isoenzyme diversity and differentiation, swamp, dry land

Introduction

Scotch pine (*P. sylvestris* L.) is one of the most wide-spread forest-forming conifers species in Eurasia (Bobrov, 1978). This species occupies a vast territory from 37° to 70° of northern latitude and from Atlantic coast to 120° of eastern longitude (Pravdin, 1964). Within the limits of its area *P. sylvestris* grows in various natural conditions and substantially differentiates one from another on morphological, ecological, physiological features and on forestry characteristics. It has over 20 geographical races and about 100 diversiforms and varieties (Kozubov, Muratova, 1986). The most total information about variability and intraspecific taxonomy of *P. sylvestris* are presented in monograph L.F. Pravdin (1964).

The genetic diversity, structure and differentiation of *P. sylvestris* marsh populations are still poorly studied. The main attention in all investigations carried out earlier was directed at study peculiarities of genetic structure and differentiation of Scotch pine growing on dry lands and on adjacent swamps. For the first time the distinctions in genetic structure of Scotch pine situated on dry land plot and on adjacent water-logged ground were discovered during study of this species in Sweden (Gullberg *et al.*, 1982). The following investigations *P. sylvestris* conducted on Russian Plain (European part of Russia) and Western Siberia showed that pine growing

under contrasting ecological conditions of dry land and swamp are displayed essential differences throughout allelic diversity and alleles frequencies of genes, coding enzymes (Duharev, 1983; Petrova *et al.*, 1989; Petrova, 1994; Petrova, 2002, Semerikov, 1991; Semerikov *et al.*, 1993; Belokon *et al.*, 1998; Dvornik *et al.*, 1998; Sannikov and Petrova, 2003). According to I.V. Petrova data (Petrova, 2002) the most significant genetic differences (at the level of populations) are observed between adjacent dry lands and marsh *P. sylvestris* sites located in southern part of forest zone. In the middle and north taiga the differences between above mentioned sites of pine are expressed to a lesser degree. However it will be noted that only some of local excessively moistening territories were included in these studies. Besides, the comparative analysis of *P. sylvestris* genetic structure was carried out mainly for stands situated on dry lands and on adjacent oligotrophic swamps with extremal conditions of existence of plantations of trees. Genetic structure of Scotch pine stands located on other swamps, in particular eutrophic and mesotrophic swamps, is not analysed to this day.

In present report the materials of comparative study of genetic diversity, structure and differentiation for *P. sylvestris*, growing under various conditions of water-mineral nutrition on swamps (eutrophic,

mesotrophic, oligotrophic swamps) and on dry land in Tomsk region are presented. Earlier such investigations in this region of Western Siberia where high moisture of territory is observed and different types of swamps are existed, not conducted.

Materials and methods

Eight stands of *P. sylvestris* (in this study referred to as populations) situated in south-taiga subzone of West Siberian lowland (Tomsk region) on swamps having different types of water-mineral nutrition (eutrophic, mesotrophic, oligotrophic swamps) and on dry land have been chosen as a object for research. The eutrophic swamps or lowland swamps are characterized by rich water-mineral nutrition, mainly owing to subsoil waters. The oligotrophic swamps or upland swamps are characterized by meager water-mineral nutrition since they are formed only at the expence of atmospheric precipitates containing very little mineral matters. The mesotrophic swamps (transitional swamps) at nature of flora and moderate mineral nutrition are between upland and lowland swamps. The six swamps with various types of water-mineral nutrition and dry land were located nearly one from another on the territory of Timiryazev forestry. The distance between the farthest of them was about 16 kilometres. One oligotrophic swamp was situated far apart remaining (130 km) on the territory of Baktchar forestry. The group of oligotrophic swamps was presented by suffruticous-sphagnous pine forests. At mesotrophic swamps the sedge-sphagnous pine forests were prevailed. The dry land and eutrophic swamp were presented by low sedge-green moss spruce forests. Designations and geographical coordinates of pine populations investigated are shown in Table 1.

In each population 30 trees were included in analysis.

The total number of trees sampled was 240. Buds from each individual tree were used as a material for study. The homogenizing of buds was fulfilled in two or three drops of an extracting buffer (0.05 M Tris-HCl pH 7.7, containing dithiothreitol (0.06%), EDTA-Na₂ (0.02%) and β -mercaptoethanol (0.05%). Electrophoretic separation of extracts was achieved by means of 13-14% starch gel electrophoresis. For electrophoresis used three buffer systems: Morpholine-Citric acid pH 7.0 (Clayton, Tretiak, 1972), Tris-Citric acid, pH 8.5 / Lithium hydroxide-Boric acid, pH 8.1 (Ridgway *et al.*, 1970) and Tris-EDTA-Boric acid, pH 8.6 (Markert and Faulhaber, 1965). Gel and electrode buffers were as recommended for these systems. Eleven enzyme systems were assayed per each tree: malate dehydrogenase (MDH, EC 1.1.1.37), shikimate dehydrogenase (SKDH, EC 1.1.1.25), 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44), isocitrate dehydrogenase (IDH, EC 1.1.1.42), glutamate dehydrogenase (GDH, EC 1.4.1.2), formate dehydrogenase (FDH, EC 1.2.1.2), alcohol dehydrogenase (ADH, EC 1.1.1.1), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1), phosphoenolpiruvate carboxilase (PEPCA, EC 4.1.1.31), phosphoglucomutase (PGM, EC 2.7.5.1) and leucine aminopeptidase (LAP, EC 3.4.11.1). Standard methods of histochemical enzyme staining (Brewer, 1970; Vallejos, 1983; Goncharenko and Padutov, 1988; Manchenko, 1994) were followed with minor modifications. On the basis of allozyme phenotypes revealed the corresponding them genotypes were established and the frequencies of alleles for each of identified loci were determinated. Twenty loci, allozyme variants that well divide in indicated above buffer systems, were included in the study.

To determinate level of genetic diversity in each

Table 1. Locality data for the eight studied populations of *P. sylvestris*

| Population name | Designation | Situation | Geographical coordinates | | Elevation (m) |
|--|-------------|---------------------|--------------------------|-----------|---------------|
| | | | Latitude | Longitude | |
| Oligotrophic swamp I (middle ryam*, top) | O1 | Timiryazev forestry | 56°22' | 84°33' | 125 |
| Oligotrophic swamp II (middle ryam, slope) | O2 | Timiryazev forestry | 56°22' | 84°33' | 125 |
| Oligotrophic swamp III (middle ryam) | O3 | Timiryazev forestry | 56°23' | 84°27' | 131 |
| Oligotrophic swamp IV (lower ryam) | O4 | Baktchar forestry | 56°54' | 82°41' | 118 |
| Mesotrophic swamp | M1 | Timiryazev forestry | 56°23' | 84°32' | 129 |
| Mesotrophic lot of meso-oligotrophic swamp | M2 | Timiryazev forestry | 56°21' | 84°25' | 139 |
| Eutrophic swamp | E | Timiryazev forestry | 56°21' | 84°35' | 125 |
| Dry land | D | Timiryazev forestry | 56°22' | 84°33' | 125 |

* Ryam - local Siberian name of suffruticous-sphagnous phytocenosis differentiating one from another by height of pine vegetation.

population studied the standard parameters such as the percentage of polymorphic loci (P), the mean number of alleles per locus (Na), the observed (Ho) and Hardy-Weinberg expected (He) heterozygosities, and the effective number of alleles (Ne) were calculated. The correspondence between observed and expected genotype frequencies estimated by means of the chi-square tests (χ^2). Chi-square tests of homogeneity for alleles frequencies were also conducted.

For assaying of populations genetic structure the fixation indices (Fis, Fit, Fst) were used (Guriev and Ledig, 1982). Fis and Fit measure the deviation of genotype frequencies from Hardy-Weinberg proportions within populations and in the total population as a whole, respectively, whereas Fst measures the degree of genetic differentiation among populations. The quantitative evaluation of genetic differences degree between populations studied was carried out by calculation of Nei's genetic distances (Nei, 1972). For computation of above named characteristics the computer program GenAlex 6 was used (Peakall and Smouse, 2006). To visualize differences in genetic distances the ordination of populations on plane of two principal coordinates was fulfilled in the packet of program STATISTICA (1998).

Results and discussion

Fifty seven allelic variants at the 20 loci were revealed in the course of the research of the eleven enzyme systems in populations investigated of *P. sylvestris* growing on swamps and on dry land in Tomsk region. Five loci (*Mdh-1*, *Got-1*, *Idh*, *Pgm-2*, *Pepca*) were monomorphic in all populations. The remaining loci were polymorphic. Loci *Mdh-3*, *Mdh-4*, *Skdh-2*, *Lap-1*, *6-Pgd-2*, *Gdh* and *Adh-1* were diallelic. In others loci the number of alleles revealed varied from three to eight. Locus *Skdh-1* had largest number of alleles. The allele frequencies for each population and each gene locus are listed in Table 2.

As seen in Table 2 the thirty alleles are common for *P. sylvestris* populations studied. Eleven alleles (*Mdh-2*⁸³, *Got-2*⁷⁰, *Got-3*⁷²⁰, *Skdh-1*⁸⁵, *Skdh-1*⁹⁰, *Skdh-1*⁹⁸, *Lap-1*¹⁰³, *Lap-2*¹⁰², *Lap-2*¹⁰⁵, *Pgm-1*⁹³, *Pgm-1*⁹⁵) belong to category of rare alleles. Six of them (*Mdh-2*⁸³, *Got-2*⁷⁰, *Got-3*⁷²⁰, *Lap-1*¹⁰³, *Lap-2*¹⁰⁵, *Pgm-1*⁹³) occur only in any single population. Maximum number of rare alleles, including unique alleles (six and three, respectively), was revealed in population of pine situated on mesotrophic lot of meso-oligotrophic swamp (M2). None of rare alleles was showed in population of *P. sylvestris* growing on eutrophic swamp.

For most of polymorphic loci the observed distributions of genotypes in populations were close to those expected according to the law of Hardy-Weinberg. In population O1 the substantial deviations in proportions of genotypes were not revealed none of polymorphic loci. In remaining populations studied at some loci the statistically significant deviations of the observed genotype frequencies from the Hardy-Weinberg expected values were observed. The number of such loci in single populations varied from

one (M1, M2, O4) to three (O3, E, D).

The estimation of main parameters of genetic variability showed that all pine populations investigated in Tomsk region are characterized sufficiently high level of genetic diversity (Table 3). The percentage of polymorphic loci at 100% criterion ranged from 65% to 75%. The mean number of alleles per locus (Na) varied from 2.05 to 2.50. The mean value of observed heterozygosity (Ho) ranged from 0.188 to 0.252 and the mean expected heterozygosity (He) ranged from 0.194 to 0.232. The effective number of alleles (Ne) was from 1.33 to 1.41. On average in each population of *P. sylvestris* the percentage of polymorphic loci composed 68.75 %, the mean number of alleles per locus, the mean observed heterozygosity, the mean expected heterozygosity and the effective number of alleles were equal to 2.26, 0.217, 0.212 and 1.37, respectively. The effective number of alleles was much lower than the actual number of alleles per locus. 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. Population of pine situated on mesotrophic lot of meso-oligotrophic swamp (M2) had the greatest allelic diversity and the highest level of heterozygosity (Na=2.50, Ho=0.252, He=0.232, Ne=1.41). The obtained values of main indices of genetic variability for marsh and dry land populations of *P. sylvestris* from Tomsk region were somewhat higher than those established for populations of this species from different regions of Krasnoyarsk territory, Republic: Khakasia, Tuva and Mountain Altai (P=67.78, Na=2.09, Ho=0.203, He=0.201, Ne=1.35) studied at the identical set of isoenzyme loci (unpublished data).

In populations O3 and D the mean observed heterozygosity was slightly lower than that expected under Hardy-Weinberg equilibrium (Ho<He). In population E observed heterozygosity was equal expected heterozygosity (Ho=He). In remaining populations the insignificant excess heterozygous genotypes (Ho>He) was revealed.

The values of fixation index Fis which estimates inbreeding of individuals relative to the population varied among polymorphic loci from -0.240 for *Skdh-2* to 0.057 for *Pgm-1* with an overall mean of -0.023 (Table 4). Negative value of this index indicative of a 2.3% excess of heterozygotes for each individual within populations. Fit values reflecting inbreeding of individuals relative to the species varied from -0.184 (*Skdh-2*) to 0.088 (*Skdh-1*). The weighted mean of Fit over all loci being equal to zero practically. This means that on the whole in *P. sylvestris* populations studied the observed heterozygosity agrees with expected heterozygosity under Hardy-Weinberg law. Low values of Fis and Fit allow to make the conclusion that *P. sylvestris* growing in Tomsk region on the swamps and on dry land is in position close to equilibrium.

The study of differentiation between populations by means of fixation index Fst showed that above 97% of total genetic variability belong to intrapopulation variability and only 2.3% - to interpopulation

Table 2. Allele frequencies for 15 polymorphic loci in *P. sylvestris* populations studied

| Locus | Allele | Population | | | | | | | |
|----------------|-------------------------------|------------|-------|-------|-------|-------|-------|-------|-------|
| | | O1 | O2 | O3 | O4 | M1 | M2 | E | D |
| <i>Mdh-2</i> | <i>Mdh-2</i> ⁴⁷ | 0.033 | 0.017 | 0.067 | 0.050 | 0.083 | 0.050 | 0.050 | 0.067 |
| | <i>Mdh-2</i> ⁸³ | — | — | — | — | 0.017 | — | — | — |
| | <i>Mdh-2</i> ¹⁰⁰ | 0.967 | 0.983 | 0.933 | 0.950 | 0.900 | 0.950 | 0.950 | 0.933 |
| <i>Mdh-3</i> | <i>Mdh-3</i> ¹⁰⁰ | 0.950 | 1.000 | 0.950 | 0.983 | 1.000 | 0.967 | 0.950 | 1.000 |
| | <i>Mdh-3</i> ¹¹⁴ | 0.050 | — | 0.050 | 0.017 | — | 0.033 | 0.050 | — |
| <i>Mdh-4</i> | <i>Mdh-4</i> ⁵² | 0.133 | 0.217 | 0.067 | 0.150 | 0.017 | 0.233 | 0.267 | 0.183 |
| | <i>Mdh-4</i> ¹⁰⁰ | 0.867 | 0.783 | 0.933 | 0.850 | 0.983 | 0.767 | 0.733 | 0.817 |
| <i>Got-2</i> | <i>Got-2</i> ⁷⁰ | — | — | 0.017 | — | — | — | — | — |
| | <i>Got-2</i> ¹⁰⁰ | 0.733 | 0.783 | 0.650 | 0.617 | 0.717 | 0.733 | 0.684 | 0.734 |
| | <i>Got-2</i> ¹²¹ | 0.100 | 0.017 | 0.050 | 0.250 | 0.033 | 0.067 | 0.100 | 0.067 |
| | <i>Got-2</i> ¹²⁴ | 0.083 | 0.133 | 0.216 | 0.083 | 0.200 | 0.134 | 0.133 | 0.133 |
| | <i>Got-2</i> ¹²⁷ | 0.017 | 0.067 | 0.050 | 0.017 | 0.033 | 0.033 | 0.033 | 0.033 |
| | <i>Got-2</i> ¹⁴⁵ | 0.067 | — | 0.017 | 0.033 | 0.017 | 0.033 | 0.050 | 0.033 |
| <i>Got-3</i> | <i>Got-3</i> ¹⁰⁰ | 0.550 | 0.483 | 0.550 | 0.667 | 0.617 | 0.567 | 0.617 | 0.633 |
| | <i>Got-3</i> ³¹⁰ | 0.067 | 0.150 | 0.033 | 0.017 | 0.067 | 0.050 | 0.017 | 0.050 |
| | <i>Got-3</i> ⁴²⁰ | 0.383 | 0.367 | 0.417 | 0.316 | 0.316 | 0.367 | 0.366 | 0.317 |
| | <i>Got-3</i> ⁷²⁰ | — | — | — | — | — | 0.016 | — | — |
| <i>Skdh-1</i> | <i>Skdh-1</i> ⁸⁵ | 0.033 | 0.017 | — | — | — | 0.017 | — | 0.017 |
| | <i>Skdh-1</i> ⁹⁰ | 0.033 | — | — | — | 0.017 | — | — | — |
| | <i>Skdh-1</i> ⁹⁵ | 0.150 | 0.133 | 0.050 | 0.033 | 0.050 | 0.117 | 0.100 | 0.217 |
| | <i>Skdh-1</i> ⁹⁸ | 0.017 | — | — | — | 0.017 | — | — | — |
| | <i>Skdh-1</i> ¹⁰⁰ | 0.700 | 0.733 | 0.800 | 0.950 | 0.816 | 0.832 | 0.783 | 0.733 |
| | <i>Skdh-1</i> ¹⁰³ | 0.050 | 0.017 | 0.017 | — | 0.017 | — | 0.117 | — |
| | <i>Skdh-1</i> ¹⁰⁵ | 0.017 | 0.033 | 0.133 | — | 0.050 | 0.017 | — | — |
| | <i>Skdh-1</i> ¹⁰⁷ | — | 0.067 | — | 0.017 | 0.033 | 0.017 | — | 0.033 |
| <i>Skdh-2</i> | <i>Skdh-2</i> ¹⁰⁰ | 0.900 | 0.883 | 0.933 | 0.661 | 0.767 | 0.817 | 0.800 | 0.833 |
| | <i>Skdh-2</i> ¹¹⁵ | 0.100 | 0.117 | 0.067 | 0.339 | 0.233 | 0.183 | 0.200 | 0.167 |
| <i>Lap-1</i> | <i>Lap-1</i> ¹⁰⁰ | 1.000 | 1.000 | 1.000 | 0.983 | 1.000 | 1.000 | 1.000 | 1.000 |
| | <i>Lap-1</i> ¹⁰³ | — | — | — | 0.017 | — | — | — | — |
| <i>Lap-2</i> | <i>Lap-2</i> ⁹⁶ | — | 0.017 | — | 0.017 | 0.017 | 0.033 | 0.067 | — |
| | <i>Lap-2</i> ⁹⁸ | 0.017 | 0.017 | 0.067 | 0.017 | 0.033 | 0.017 | — | 0.100 |
| | <i>Lap-2</i> ¹⁰⁰ | 0.967 | 0.933 | 0.933 | 0.950 | 0.950 | 0.916 | 0.933 | 0.900 |
| | <i>Lap-2</i> ¹⁰² | 0.016 | 0.033 | — | 0.016 | — | 0.017 | — | — |
| | <i>Lap-2</i> ¹⁰⁵ | — | — | — | — | — | 0.017 | — | — |
| <i>6-Pgd-2</i> | <i>6-Pgd-2</i> ⁸⁸ | 0.483 | 0.433 | 0.517 | 0.467 | 0.467 | 0.500 | 0.433 | 0.383 |
| | <i>6-Pgd-2</i> ¹⁰⁰ | 0.517 | 0.567 | 0.483 | 0.533 | 0.533 | 0.500 | 0.567 | 0.617 |
| <i>Fdh</i> | <i>Fdh</i> ⁴⁵ | 0.017 | 0.052 | 0.033 | 0.017 | 0.017 | — | — | — |
| | <i>Fdh</i> ¹⁰⁰ | 0.783 | 0.828 | 0.700 | 0.850 | 0.883 | 0.833 | 0.946 | 0.917 |
| | <i>Fdh</i> ¹⁵⁸ | 0.183 | 0.103 | 0.250 | 0.100 | 0.100 | 0.133 | 0.036 | 0.083 |
| | <i>Fdh</i> ²⁰⁶ | 0.017 | 0.017 | 0.017 | 0.033 | — | 0.034 | 0.018 | — |
| <i>Pgm-1</i> | <i>Pgm-1</i> ⁹³ | — | — | — | — | — | 0.016 | — | — |
| | <i>Pgm-1</i> ⁹⁵ | — | — | — | 0.033 | — | 0.017 | — | — |
| | <i>Pgm-1</i> ¹⁰⁰ | 0.933 | 0.917 | 0.967 | 0.967 | 0.967 | 0.900 | 0.983 | 0.933 |
| | <i>Pgm-1</i> ¹⁰³ | 0.067 | 0.083 | 0.033 | — | 0.033 | 0.067 | 0.017 | 0.067 |
| <i>Gdh</i> | <i>Gdh</i> ¹⁰⁰ | 0.783 | 0.717 | 0.883 | 0.717 | 0.800 | 0.733 | 0.617 | 0.700 |
| | <i>Gdh</i> ¹²¹ | 0.217 | 0.283 | 0.117 | 0.283 | 0.200 | 0.267 | 0.383 | 0.300 |
| <i>Adh-1</i> | <i>Adh-1</i> ¹⁰⁰ | 0.517 | 0.667 | 0.550 | 0.550 | 0.611 | 0.583 | 0.625 | 0.550 |
| | <i>Adh-1</i> ¹⁰⁸ | 0.483 | 0.333 | 0.450 | 0.450 | 0.389 | 0.417 | 0.375 | 0.450 |
| <i>Adh-2</i> | <i>Adh-2</i> ¹³ | 0.083 | 0.134 | 0.183 | 0.283 | 0.183 | 0.267 | 0.096 | 0.155 |
| | <i>Adh-2</i> ⁵⁹ | 0.017 | 0.033 | 0.017 | — | — | 0.033 | — | 0.103 |
| | <i>Adh-2</i> ¹⁰⁰ | 0.833 | 0.750 | 0.717 | 0.667 | 0.784 | 0.617 | 0.885 | 0.707 |
| | <i>Adh-2</i> ¹⁵⁶ | 0.067 | 0.083 | 0.083 | 0.050 | 0.033 | 0.083 | 0.019 | 0.035 |

Table 3. Genetic variability at 20 loci in eight populations of *Pinus sylvestris*

| Populations | Percentage of | Mean number of | Effective number | Mean heterozygosity | |
|-------------|------------------|-------------------|------------------|---------------------|------------|
| | polymorphic loci | alleles per locus | of alleles | Observed | Expected |
| | P % | Na | Ne | Ho | He |
| O1 | 70.00 | 2.40±0.35 | 1.37±0.09 | 0.232±0.05 | 0.211±0.04 |
| O2 | 65.00 | 2.30±0.32 | 1.38±0.10 | 0.217±0.05 | 0.215±0.05 |
| O3 | 70.00 | 2.25±0.29 | 1.37±0.09 | 0.188±0.04 | 0.207±0.04 |
| O4 | 75.00 | 2.25±0.25 | 1.38±0.10 | 0.232±0.05 | 0.213±0.05 |
| M1 | 65.00 | 2.30±0.34 | 1.33±0.08 | 0.203±0.05 | 0.194±0.04 |
| M2 | 70.00 | 2.50±0.33 | 1.41±0.09 | 0.252±0.05 | 0.232±0.05 |
| E | 70.00 | 2.05±0.22 | 1.36±0.09 | 0.208±0.05 | 0.208±0.04 |
| D | 65.00 | 2.05±0.26 | 1.37±0.08 | 0.204±0.04 | 0.218±0.04 |
| Mean | 68.75±1.25 | 2.26±0.10 | 1.37±0.03 | 0.217±0.02 | 0.212±0.02 |

Table 4. Values of fixation indices Fis, Fit, Fst for 15 polymorphic loci and chi-square tests (χ^2) of homogeneity of allele frequencies

| Locus | Fis | Fit | Fst | χ^2 | D.F. | P |
|----------------|--------|--------|--------|----------|------|--------|
| <i>Mdh-2</i> | 0.017 | 0.026 | 0.009 | 10.75 | 14 | 0.7053 |
| <i>Mdh-3</i> | -0.047 | -0.026 | 0.020 | 9.57 | 7 | 0.2141 |
| <i>Mdh-4</i> | -0.018 | 0.031 | 0.047 | 22.76 | 7 | 0.0018 |
| <i>Got-2</i> | 0.009 | 0.029 | 0.021 | 50.57 | 35 | 0.0429 |
| <i>Got-3</i> | 0.017 | 0.028 | 0.011 | 24.65 | 21 | 0.2626 |
| <i>Skdh-1</i> | 0.055 | 0.088 | 0.035 | 103.27 | 49 | 0.0000 |
| <i>Skdh-2</i> | -0.240 | -0.184 | 0.045 | 20.89 | 7 | 0.0039 |
| <i>Lap-1</i> | -0.017 | -0.002 | 0.015 | 7.02 | 7 | 0.4274 |
| <i>Lap-2</i> | -0.063 | -0.046 | 0.016 | 36.65 | 28 | 0.1268 |
| <i>6-Pgd-2</i> | -0.072 | -0.065 | 0.006 | 3.08 | 7 | 0.8777 |
| <i>Fdh</i> | 0.031 | 0.065 | 0.035 | 29.46 | 21 | 0.1034 |
| <i>Pgm-1</i> | 0.057 | 0.072 | 0.016 | 25.53 | 21 | 0.2249 |
| <i>Gdh</i> | -0.092 | -0.060 | 0.029 | 13.81 | 7 | 0.0547 |
| <i>Adh-1</i> | -0.018 | -0.009 | 0.009 | 4.12 | 7 | 0.7659 |
| <i>Adh-2</i> | 0.027 | 0.058 | 0.031 | 40.11 | 21 | 0.0072 |
| Mean | -0.023 | 0.000 | 0.023 | 402.23 | 259 | 0.0000 |
| | ±0.017 | ±0.016 | ±0.003 | | | |

variability (Table 4). The value Fst ranged among polymorphic loci from 0.006 to 0.047. The highest contribution to the interpopulation variability was made by locus *Mdh-4*, while the contribution of the *6-Pgd-2* locus was the smallest. The obtained value Fst (Fst=0.023) is indicative of lower on average subdivision of *P. sylvestris* populations in investigated part of area as compared with geographically removed populations of this species (Fst=0.033) studied at the identical set of isoenzyme loci (unpublished data).

The results of χ^2 -tests showed that the statistically

significant interpopulation heterogeneity of allele frequencies was observed only for six loci (*Mdh-4*, *Got-2*, *Skdh-1*, *Skdh-2*, *Gdh*, *Adh-2*). For the remaining loci the observed differences in allele frequencies were insignificant. However for the totality of polymorphic loci as a whole the interpopulation heterogeneity of allele frequencies was highly significant (Table 4).

The mean values of Nei's genetic distance D (Nei, 1972) between populations analyzed of *P. sylvestris* ranged from 0.004 to 0.016 (Table 5) and equalled, on the average, 0.007. The maximum value of D was

found between population located on eutrophic swamp (E) and population situated on oligotrophic swamp O3. The significant differences in genetic structure were revealed also between population from oligotrophic swamp O4 and populations from oligotrophic swamps: O1, O2, O3 ($D=0.012$). The mean genetic distance between populations situated on various oligotrophic swamps was equaled to 0.009. Approximately the same degree of differentiation was observed between population from eutrophic swamp (E) on the one hand and populations from oligotrophic swamps ($D=0.0095$) on the other hand. The degree of the observed differences between above-mentioned pairs of populations corresponds with degree of differences between geographically removed populations *P. sylvestris* studied at the identical set of isoenzyme loci (unpublished data). The differences between populations situated on eutrophic and mesotrophic swamps as well as between populations located on mesotrophic and oligotrophic swamps were less considerable ($D=0.008$ and $D=0.006$, respectively). The mean genetic distances among populations of Scotch pine growing on dry land and on various swamps varied from 0.005 to 0.007. The most essential differences in genetic structure were observed between

population from dry land and populations from oligotrophic swamps. The established level of genetic differentiation visually illustrates arrangement of the studied populations on plane of two principal coordinates (Fig.).

The obtained data testify to the differences in genetic structures are observed both between populations of Scotch pine situated on swamps distinguishing greatly throughout types of water-mineral nutrition (eutrophic and oligotrophic swamps) and between populations situated on various oligotrophic swamps. Furthermore the level of these differences was equal on average ($D=0.010$). Dry land and marsh populations were less differentiated. The mean genetic distance D between them amounted to 0.007. In investigations of others authors (Belokon *et.al.*, 1998; Sannikov and Petrova, 2003) the essential differences in genetic structure of *P. sylvestris* populations located on various oligotrophic swamps were showed also. However the level of these differences was several times as low as level of differences between populations situated on dry lands and populations from adjacent oligotrophic swamps. The comparative analysis populations of Scotch pine growing under conditions of various oligotrophic swamps within Russian Plain and Western Siberia and

Table 5. Genetic distances (Nei, 1972) between studied populations of *P. sylvestris*

| Populations | O1 | O2 | O3 | O4 | M1 | M2 | E |
|-------------|-------|-------|-------|-------|-------|-------|-------|
| O2 | 0.004 | — | | | | | |
| O3 | 0.005 | 0.009 | — | | | | |
| O4 | 0.011 | 0.012 | 0.013 | — | | | |
| M1 | 0.006 | 0.007 | 0.006 | 0.007 | — | | |
| M2 | 0.005 | 0.004 | 0.007 | 0.006 | 0.006 | — | |
| E | 0.007 | 0.006 | 0.016 | 0.009 | 0.009 | 0.007 | — |
| D | 0.005 | 0.004 | 0.011 | 0.008 | 0.006 | 0.004 | 0.005 |

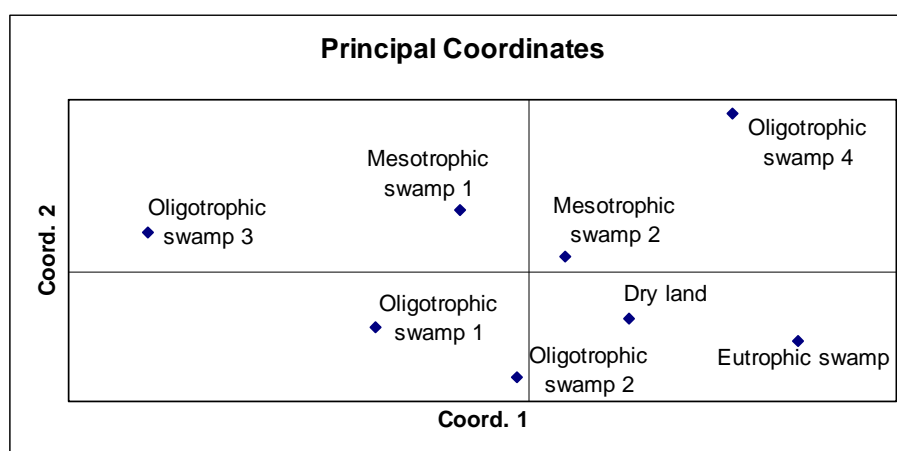


Fig. 1. Disposition of studied populations of *Pinus sylvestris* on the plane of two principal coordinates.

populations from adjacent dry lands biogeocenoses (Sannikov and Petrova, 2003) showed that the degree of populations subdivision on swamps was greatly higher than on dry lands being in more favourable ecological conditions ($F_{st}=0.064$ and $F_{st}=0.028-0.038$, respectively). The significant differences at allelic frequencies of isoenzyme loci tested between populations of Scotch pine growing on swamps and adjacent dry lands sites were revealed V.L. Semerikov (1991). The analogous results were obtained earlier V.A. Ducharev (1983) during investigations of glucose-6-phosphate dehydrogenase of Scotch pine growing in contrasting ecological conditions of dry land and swamp.

One of the reasons of genetic differentiation of *P. sylvestris* populations studied is considerable heterogeneity of conditions of water-mineral nutrition on various swamps and on dry land which owing to long influence microevolutionary factors could lead to genetic heterogeneity of Scotch pine growing in Tomsk region.

Conclusion

As a result of the carried out researches it was established that in spite of low on average differentiation ($F_{st}=0.023$, $D=0.007$) the included in the analysis populations of *P. sylvestris* situated on the various swamps (eutrophic, mesotrophic, oligotrophic) and on the dry land differ at genetic structure. For 13 of 28 compared pairs of populations the revealed differences were statistically significant. The most essential differences throughout the alleles frequencies of 20 tested loci were showed among populations of Scotch pine growing in contrast on conditions of a water-mineral nutrition sites on eutrophic and oligotrophic swamps as well as between populations situated on various oligotrophic swamps. The level of observed differences between these marsh populations corresponded with level of differences between geographically removed populations of *P. sylvestris* studied at the identical set of isoenzyme loci. The differences between marsh populations and population from dry land were expressed to a lesser degree. The obtained data testify to considerable genetic heterogeneity of the investigated in Tomsk region marsh populations of Scotch pine.

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